# MEDICAL BIOTECHNOLOGY:
## CURRENT ACHIEVEMENTS AND PROSPECTS. ANOTHER GOLDEN ERA

Albert SASSON


**Publication supported by :**

**Hassan II Academy of Science and Technology, Rabat, Morocco**

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Albert Sasson, M.Sc., D.Sc. (University of Paris), is a founding member of the Hassan II Academy of Science and Technology of the Kingdom of Morocco. He has had a distinguished scientific career as a Professor and Dean of the Faculty of Sciences of Rabat; and thereafter at the United Nations Educational, Scientific and Cultural Organization (UNESCO, Paris). He was Assistant Director-General of UNESCO and Special Adviser to UNESCO Director-General from 1993 to 1999.

He has been Senior Visiting Professor at the United Nations University Institute of Advanced Studies (Yokohama, Japan) and is a senior consultant to United Nations specialized agencies, several governments, national and regional institutions, as well as to the European Commission. Since 2006 he chairs the Association BioEuroLatina which promotes cooperation between Europe and Latin America in biotechnology.

Albert Sasson has been appointed by the King of Morocco as member of the Kingdom's Human Rights Consultative Council (CCDH), the governing board of the Royal Institute of Strategic Studies (IRES), the Economic, Social and Environmental Council, and the Higher Council for Education, Training and Scientific Research.

His research work and science-popularization activities have culminated in over 200 publications, including many books and reviews on biotechnology in developing countries over the last 45 years.

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# PREFACE

The Academy of the Kingdom of Morocco – the first academy of the country – has been created by Royal Decree on 8 April 1977 and it enjoys the tutorship of His Majesty The King of Morocco. In addition to the numerous sessions it has been holding on a wide range of topics and issues since its creation, it publishes an academic journal and books in various areas of scientific knowledge.

The Academy of the Kingdom of Morocco has been recently urged by His Majesty Mohammed VI, King of Morocco and Protector of the Academy, to proceed to several changes in its structure and to give a new impetus to its action at the service of scientific and cultural development across the nation.

The Hassan II Academy of Science and Technology has been created by Royal Decree on 6 October 1993 and enjoys the protection and tutorship of His Majesty The King of Morocco. In 2016, the Academy is celebrating the 10$^{th}$ anniversary of its official installation by His Majesty Mohammed VI, King of Morocco. After ten years of activity, the Academy's missions have been successful in promoting scientific and technological research particularly through assessing, supporting and funding research programmes and projects; in issuing recommendations on the national science and technology policy and priorities (and, since 2015, via the newly created Higher Council for Education, Training and Scientific Research); and in contributing to the advancement of scientific knowledge and culture.

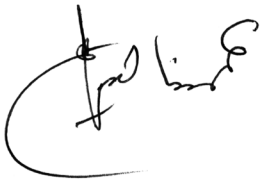
In addition to the *Proceedings* of its sessions, and in particular of its annual plenary session devoted to a specific theme of worldwide and national relevance, the Academy publishes an electronic *Journal*, a *Bulletin* and a *Newsletter*. It also promotes and supports publications and reports dealing with subjects of relevance to its scientific sections.

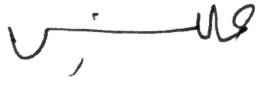
Both national Academies have pooled their resources to publish this book titled ***Medical Biotechnology : Current Achievements and Prospects. Another Golden Era***, authored by Professor Albert Sasson, a founding member of the Hassan II Academy of Science and Technology and director of the Academy's Life Sciences and Biotechnology Section.

Both Academies wanted to wholeheartedly support the publication of this book dedicated to the Late Professor Abdellatif BERBICH, who has been Permanent Secretary of the Academy of the Kingdom of Morocco as well as a founding member of the Hassan II Academy of Science and Technology. By doing so, the Academies and the author want to pay tribute to the outstanding action of Professor A. Berbich, as a renowned physician, scholar and academician, during his whole professional life at the service of his country.

This book highlights the current achievements of medical biotechnology and the very promising prospects in this rapidly-growing area of biotechnology. The huge investments made by the big pharmaceutical companies as well as into very successful startups, the frenzy mergers and acquisitions in pharmaceutical and medical biotechnology, as well as the new strategy and business models of the bioindustry in this field, make many analysts think that we are witnessing another golden era for medical biotechnology.

Like other books authored by A. Sasson and published or co-published by the Hassan II Academy of Science and Technology, such as ***From Green to White Biotechnology: Great Challenges, Urgent Solutions*** (2013) or ***Health Care, Food and Nutrition. Opportunities and Challenges for the Life Sciences and Biotechnology*** (2011), this one also aims to provide the readers with the opportunity to understand the role of biotechnology/bioindustry in today's society and to develop informed opinions about it. In this regard, Professor Albert Sasson enjoys a long-standing reputation for disseminating scientific and technological knowledge, especially with respect to biotechnology in developing countries, over the last 45 years.





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"Everyone by nature desires to know," wrote Aristotle more than 2,000 years ago. But are there limits to what human beings can know? This is the question that Marcus du Sautoy, the British mathematician who succeeded Richard Dawkins as the Simonyi professor for the public understanding of science at Oxford University, explores in *What We Cannot Know*, a book on the limits of scientific knowledge (2016, 4th Estate, 440 pp.).

"As Marcus du Sautoy argues, this is a *golden era* of scientific knowledge: remarkable achievements stretch across the sciences, from the Large Hadron Collider and the sequencing of the human genome to the proof of Fermat's Last Theorem. And the rate of progress is accelerating: the number of scientific publications has doubled every nine years since the second world war. But even bigger challenges await: Can cancer be cured? Ageing beaten? Is there a "theory of Everything" that will include all of physics? Can we know it all?

"Marcus du Sautoy, in the end, has an optimistic message. There may be things people will never know, but they do not know what there are. And ultimately, it is the desire to know the unknown that inspires humankind's search for knowledge in the first place."

# FOREWORD

This book is dedicated to the Late Professor Abdellatif BERBICH (1934-2015), Permanent Secretary of the Academy of the Kingdom of Morocco (April 1982-1 January 2015), member of the Foundation Committee of the Hassan II Academy of Science and Technology, and member of this Academy's Life Sciences and Biotechnology Section for almost a decade.

Both Academies of Morocco have paid tribute to Professor A. BERBICH in due course and highlighted his outstanding professional and human qualities, as well as the remarkable services he rendered to his motherland. "It is every man's duty to give back to the world at least as much as he has received from it," used to say Albert Einstein. Abdellatif BERBICH has largely given to his country as much as he did receive from it.

This book intends to be a modest tribute to the eminent scholar and professor of medicine, but also to the university mate, thereafter to an excellent colleague, and to a very dear friend.

A. Sasson
Resident Member of the Hassan II Academy
of Science and Technology,
Director of the Academy's Life Sciences
and Biotechnology Section

Rabat, July 2016

Professor Abdellatif Berbich
(1934-2015)

# SUMMARIZED CONTENTS

# PART ONE

***WAVES OF INVESTMENTS***
***CREATION OF ROCKETING STARTUPS***
***FRENZY OF MERGERS AND ACQUISITIONS***
***KEY STRATEGIC DEVELOPMENT ISSUES***

# CONTENTS

# UNPRECEDENTED BOOM OF THE BIOINDUSTRY AND BIOECONOMY

While the bioindustry and bioeconomy tends to be overshadowed by the boom of the electronic, communication and information industry and the worldwide-known high-tech companies like Google, Apple, Facebook and Amazon (GAFAs), particularly in the United States, they have been nevertheless experiencing since the late 2000s an almost unprecedented boom of its own. The previous period when the medical biotechnology and health-care industry were so flush was around 2000: companies promised, investors believed that human genomics – particularly deciphering the whole DNA sequence – would revolutionize drug discovery. Stock prices eventually collapsed when human genomics did not yield at that stage the promised novelties. Some life-science venture capitalists had such low returns that they effectively had to close down. For instance Exelixis, a company that went public in 2000 based on the promise of its genomic technology, laid off 70% of its staff in 2014 after its best hope, a drug against prostate cancer, failed in a clinical trial. George A. Scangos who ran Exelixis and thereafter moved to manage Biogen Idec, one of the most successful medical biotechnology companies, stated: "This time is different, in that a lot of the enthusiasm is based on substance" (Pollack, 2015b). It is true that by the end of the first decade of the 21st century funds are flowing into the bioindustry and medical biotechnology as never before. Stock prices are high and in medical biotechnology the number of drug approvals is increasing. And perhaps more important some of the new drugs represent major advances in the treatment of such diseases as hepatitis C, cancers and cystic fibrosis (Pollack, 2015b).

## A boom to stand or a bubble that will burst?

### *Optimism among executives and investors*

Some executives and investors are thus making the bold assertion that the health-care industry and medical biotechnology have turned a corner and that discoveries and new techniques are allowing drug developers to reduce their high failure rate and to take on some diseases for the first time. Others, however, are sceptical and state that the current boom is a bubble that will burst. At the J.P. Morgan Healthcare Conference – the industry's most closely watched investor event – for which 9,000 executives and investors met at the Westin St. Francis hotel in San Francisco, by mid-January 2015, Robert J. Hugin, the executive of Celgene, one of the largest biotechnology companies, stated: "It is an incredibly exciting time for our industry," (in the first presentation at the meeting). The latter last four days and the participants listened to presentations from 400 companies (Pollack, 2015b).

It is true that biotechnology stocks have outperformed the overall market for several years. In 2014 the Nasdaq Biotechnology Index rose 35%, compared with 11% for the Standard Poor's 500-stock index. More than 110 companies in the industry went public in 2014, far more than in a typical year, raising a record US$9 billion, according to *BioCentury*, an industry publication. The amounts raised in other stock and debt offerings were also at or near records. And the US$5.97 billion invested by venture capitalists in private companies in 2014 was up 29% from 2013 and about equal with the record set in 2007, according to Pricewaterhouse Coopers and the National Venture Capital Association (Pollack, 2015b; see also p.19).

One factor lifting stock prices is the fast pace at which small drug developers are being acquired by larger ones. That is in part sustained by the need for big pharmaceutical companies to replenish their pipelines as top-selling drugs lose their patents and become generics. Also the United States Food and Drug Administration (FDA) seems more willing to approve new drugs: it approved 41 new drugs in 2014, the most since 1996. But the increase in approval also seems to reflect the medical bioindustry's success at developing effective drugs. For instance new antihepatitis-C drugs from AbbVie and Gilead Sciences can cure most patients in 12 weeks. Also drugs that help the body's immune system attack cancer cells and tumours have generated great excitement among oncologists (Pollack, 2015b).

"The science has never been better and the pace of progress has never been faster," stated Jeffrey Leiden, the chief executive of Vertex Pharmaceuticals which is selling the first drug that counters a genetic cause of cystic fibrosis. But others counter that human physiology remains extremely complex and more research is needed to understand it in order to develop the most effective drugs. "There are definitely areas and companies that seem to be ahead of themselves, for sure," commented Misha Petkevich, portfolio manager at V2M Capital. For instance the so-called CAR-T therapy, in which a cancer patient's immune system cells are genetically engineered to attack tumours, has shown some dramatic successes in treating leukemia and lymphoma, but the technique is still in early development (Pollack, 2015b). See pp.36 and 314.

Juno Therapeutics, a leading startup in medical biotechnology, raised US$300 million in 2014 in the industry's largest initial public offering (IPO) in memory. It had a market value of almost US$4 billion in 2015. Kite Pharma, another startup that went public in 2014, had a valuation of *ca*. US$3 billion by early 2015. By mid-January 2015 two companies working together agreed to pay the MD Anderson Cancer Center US$100 million in stock for technology that can be used in cancer immunotherapy. They paid an additional US$15 million in stock to persuade the cancer centre to sign the deal in time to be announced at the J.P. Morgan Healthcare Conference in San Francisco by mid-January 2015 (Pollack, 2015b).

A setback in the development of CAR-T or other promising therapies could rapidly change the mood. But the factor investors think could most damp enthusiasm is the increasing pressure from health authorities to rein in what critics say are the exorbitant prices of drugs. For instance biotechnology stocks took a hit in December 2014, when Express Scripts, the largest pharmacy benefit manager in the United

States, stated it would not pay for Gilead Sciences antihepatitis-C drugs for most patients – only for a competing medication from AbbVie which provided a bigger discount. That spurred a sort of a price war as Gilead Sciences and AbbVie were trying to obtain the favour of other payers. Express Scripts next hoped to force such a showdown between drug manufacturers of two powerful new drugs for lowering blood cholesterol (Pollack, 2015b). See pp. 93, 108 and 327.

### *Recognizing the risks*

Some analysts raised the question: "Will the biotechnology bubble be as devastating as that of Internet in 2000?" The reply has been generally: no! The biotechnology boom includes a lesser number of companies. Unlike the "dot.com" bubble, it is not enough to have just an idea in order to create a biotechnology firm. One has to gather a few researchers and to implement rather advanced laboratory work, before heading for a new medical treatment or a diagnostic test of one or another illness. The prerequisites are therefore more constraining and this reduces the number of future biotechnology startups; but the risk is not reduced at all (Jacquin, 2014).

Indeed, success in drug development is not easy: before becoming a possible treatment a drug is systematically studied in humans through clinical trials with three main phases. Phase 1 aims to study the tolerance of the human body to the drug and to identify the secondary or side-effects. It also aims to understand how the drug is transformed and eliminated by the body; and finally to determine the dose of the drug and the ways to administer it, that show efficacy and the least secondary effects. Phase-1 clinical trials are carried out with 50 persons maximum.

Phase 2 consists of determining among *ca*. 100 patients the efficacy of the drug: for which indications the dosage and the uptake of the drug are the most efficient.

Phase 3 of the clinical trials is carried out with hundreds or thousands of patients, divided in two groups (randomized). One group of patients receive the new drug or treatment, while other patients suffering from the same illness receive a placebo or a treatment already known and validated (controlled randomized trials). The health of these patients is closely monitored so that the efficacy of the new treatment or drug is assessed. If it is superior to the current one it will be submitted to the health authorities for approval and commercialization. Also a treatment which is not more efficient but has less secondary effects, will be considered a superior one.

After the commercialization of a drug starts the pharmacovigilance phase (4), i.e. the monitoring of any secondary effect that was not detected during the clinical trials. Depending on the gravity of the secondary effect, the drug commercialization may be suspended or even withdrawn from the market (the drug company having to eventually compensate the damage caused by the secondary effects of its medicine).

Actually even the best research teams could get to a dead end. Whatever the reason – scientific, technological or commercial – the result is the same. For instance the declared bankruptcy of the American biotechnology company Dendreon on 10 November 2014

teaches us some lessons, despite the fact that this company was among the most careful ones before deciding to go public. Dendreon was created in 1992 and was to focus on cancer immunotherapy and cell therapies. It did not approach investors with the promise to launch "one day" or even "soon", a treatment against advanced prostate cancers. In April 2010, after having obtained the authorization by the FDA to commercialize its drug Provenge, Dendreon decided to be listed on the Nasdaq. The medicine was prescribed and in 2013 Dendreon's turnover reached US$284 million (or €229 million). However analysts had predicted that, after the authorization by the FDA, the treatment costing US$93,000 per patient was expected to generate an annual turnover of US$4.3 billion. Consequently the modest sales of Provenge turned out to be a big failure. By the end of 2014 more than half of the company's 1,500 employees were laid off. Creditors could purchase Dendreon at a very low price while this company had been valued at US$7 billion at the stock exchange (Jacquin, 2014).

Dendreon example, among others, underline that the risks of failure exist, even though the following figures illustrate the current boom:

- in 2014 the deals made in the pharmaceutical and biotechnological sector amounted to US$212 billion (or €195.88 billion);
- the British biotechnology company Circassia, when introduced on the London's stock exchange, raised £333 million or €426 million, a record figure in 2014;
- the average amount of funds raised by biotechnology companies in the United States and Europe, when they went public, was US$72 million per company;
- Cellectis – a successful French biotechnology company – stock price rose 350% over a year (from early 2014 to March 2015) [Hecketsweiler, 2015a].

## Flow of venture capital

### *Waves of financing moving through the Silicon Valley*

Analysts underline that the number of startups, each valued at more than US$1 billion, has been growing steadily: more than 85 in 2015. This was due to the hundreds of millions of dollars invested every week by the American venture capitalists. According to the National Venture Capital Association which published a study on this subject on 16 April 2015, US$13.4 billion (or €12.4 billion) had been invested in startups in just three months in the United States: 1,020 operations had been carried out during that quarter, considered the best in 15 years, i.e. since the Internet bubble whose burst caused the collapse of the markets and the bankruptcy of thousands of firms (Belouezzane, 2015).

The new wave of considerable investments concerns mainly the information and communication technology startups or companies. For instance Snapchat that was founded in 2011 by Evan Spiegel, a 23-years-old student, at Stanford University, was valued at US$19 billion in 2015 (it rejected the offer of Facebook that was willing to acquire it for US$3 billion in November 2013). Airbnb or Uber were worth US$13 billion and US$41.2 billion, respectively. *Such an euphoria has also been*

*noted in the case of biotechnology startups or firms*, although the figures were not as high as those of communication-technology startups – these have been christened "unicorns", mythical animals which were considered so rare that one could never see them (Belouezzane, 2015).

Gregori Volokhine, manager of the company Meeschaert Capital Markets, is of the opinion that money flows into the Silicon Valley because the monetary policy is very accommodating: "lower interest rates result in the flow of considerable amounts of money into investment funds, and where can one invest nowadays in a better sector than that of new technologies?" "The phenomenal success and yields of such companies as Facebook has attracted many newcomers in the sector," he added. Despite some warnings, like that of Joe Horowitz, manager of the fund Icon Ventures, specialized in new communication technologies, many experts consider that the new wave of considerable investments will not lead to a bubble. According to Greg Revenu of the bank Bryan Garnier & Co., "these funds invest in enterprises that generate a revolutionary change in many utilization processes and reach dominant positions (e.g. Airbnb or Uber) that would be difficult for a competitor to challenge. Even though the economic models of these enterprises are still to be formulated, it will be the case soon. We are far from the situation that prevailed in 2000 when concepts have been given an economic value without having a real public to use them." And G. Volokhine commented: what is invested in those startups "is not stupid money," although "some enterprises are overvalued." In the opinion of this specialist the real litmus test for these startups or "unicorns" will be their introduction into the stock exchange. "At that time, investors will need to recover their money and at this moment we shall see if there is a bubble or not" (Belouezzane, 2015).

Thus, with waves of financing moving through the Silicon Valley – pushing up valuations for hot startups and allowing talented engineers and scientists to command seven-figure salaries – the question was raised whether the venture-capital market was hot; it was also how and when the cycle would end. That was also debated at Harvard Business School 21st Annual Venture Capital and Private Equity Conference, on 1 February 2015 (Alden, 2015). "My own view is we still have one, two or three very good years ahead. But then we should be very cautious, because there is no example in history where the cycle does not come to an end and drag us through a very painful period," stated Adam Valkin, a partner at the venture-capital firm General Catalyst Partners. Kate Mitchell, a co-founder and partner of Scale Venture Partners, said there was a "real war for talent" in technology. One startup in her portfolio, she mentioned, lost its chief technology officer when Oracle recruited the individual for "well over US$1 million in salary." "I mean, a start-up is not going to be able to compete with that," K. Mitchell added. Discussions like these are hardly unusual in technology circles nowadays. The magazine *Fortune* called the current moment "The Age of Unicorns". In her presentation K. Mitchell noted that until recently technology investors did not expect to be competing with giant asset managers like T. Rowe Price and BlackRock. But those firms and others are now regular participants in startup financing rounds (Alden, 2015). Despite these concerns the gears of venture capital appear to be running smoothly, with institutional investors eager to commit to venture-capital funds.

## *Opportunities for the health-care industry*

In July 2014 when the United States Federal Reserve's chairperson, Janet Yellen, spurred a sell-off in health-care stocks by stating that valuations in shares of biotechnology companies looked "stretched", portfolio manager Graham Tanaka saw an opportunity. After a yearlong buying spree, he owned in June 2015 more than a quarter of his US$17 million Tanaka Growth Fund portfolio in health-care companies such as Gilead Sciences, up from just 5% at the beginning of 2015. His fund is beating the Standard & Poor's 500-stock index by *ca*. 13 percentage points since the beginning of 2015, putting Tanaka in the top 1% of equity-fund managers tracked by Morningstar. Even with a big bet on health-care, he planned to add more (Randall, 2015).

"With ageing demographies in the United States and the developed world, health-care needs are going to grow dramatically faster than the growth domestic product (GDP)," G. Tanaka stated. In fact health-care is expected to rise to 19.9% of the American GDP by 2022 from 17.7% in 2013, according to the Office of the Actuary at the Centers for Medicare and Medicaid Services. GDP itself was expected to grow 2.4% in 2015, according to a Reuters poll of economists. In the 11 months since J. Yellen's warning, other fund managers have benefited by shaking off concerns about high valuations and increasing their holdings of health-care companies. Yet some market strategists and analysts commented that the move to health-care after its significant outperformance reminded them of the tech bubble of the late 1990s, when stock funds piled into the hot sector just before the "dot.com" crash. "Being negative on pharmaceuticals and biotechnology has not been the right move thus far in 2015, but almost all the objective data suggests that underperformance is likely in the months ahead," Citigroup's chief US equity strategist Tobias Levkovich stated in a note to clients on Tuesday 2 June 2015, citing a declining number of upward earnings revisions (Randall, 2015).

Still, because health spending is becoming a larger part of the economy, a broad sell-off in health-care companies akin to the popping of the tech bubble is unlikely, stated Randy Gwirtzman, co-portfolio manager of the US$95 million Baron Discovery Fund, who more than doubled his holdings of health-care companies over 2014. R. Gwirtzman is looking for companies that can help cut costs, rather than biotechnology firms that offer the promise of new drugs, he said. His top holding, Foundation Medicine Inc., offers a form of cancer testing that allows physicians to prescribe targeted therapy more effectively. Shares of the company were up nearly 60% by mid-2015, after the Swiss pharma group Roche took a majority interest in the company in January 2015 (Randall, 2015).

Health-care companies overall in the Standard & Poor's 500-stock index were expected to post an average earnings growth of 11.4% in 2015, compared with just a 0.6% gain among the index overall, according to S&P Capital IQ. For instance shares of Gilead Sciences were up 19% for up to mid-2015 and nearly 40% over the 12-month period, May 2014 - April 2015 (Randall, 2015).

Venture-capital funding of biotechnology startups in France

In France, Sofinnova, a venture-capital fund specialized in the life sciences, led by Denis Lucquin, is investing large amounts of money in very successful biotechnology companies, and the return on investment is very good. During the five-year period, 2010-2014, the fund had recovered *ca*. €1.5 billion from companies whose total value was estimated at €6 billion. Sofinnova was created in 1972 and it funded American biotechnology startups such as Genentech (now a subsidiary of the Swiss pharmaceutical group Roche) or Biogen whose value is 2015 was estimated at more than US$100 billion (Hecketsweiler, 2015g).

Later on the focus of Sofinnova was not any more on the United States, but on Europe and France. But the approach remains the same. The model is that of Actelion, a biotechnology company which commercialized a molecule against lung hypertension. "Children affected by this disease used to spend most of the day in bed with a transfusion syringe in their arm. Now, they take two pills a day," stated Denis Lucquin. The biotechnology firm, valued at more than €12 billion in 2015, has enabled Sofinnova to recover 34 times its investment! Other examples are: Bernard Gilly, the co-founder of Pixium whose bionic eye may enable blind people to recover their vision, and who earlier on had created Fovea, a biotechnology company which was sold to the French pharmaceutical group Sanofi for €370 million; Jacques Seguin, a heart surgeon who created the companies Stentys and CoreValve, that was purchased by a big American group for US$850 million (or €783.86 million ); Pierre-Henri Behamou, the co-founder of DBV which developed a patch for the treatment of allergies caused by eating peanuts. DBV has been listed on the American stock exchange and was valued at more than US$800 million in 2015 (Hecketsweiler, 2015g).

On 25 March 2015 the biotechnology company Cerenis announced that it had raised *ca*. €55 million after being listed on Paris Euronext. Stock pricing was to start on 30 March 2015 and this was considered the largest operation in the sector since 2000. The investors have been attracted by a revolutionary treatment of cardiovascular diseases: a drug that increases the blood concentration of high-density lipoproteins (HDL or the so-called "good" cholesterol) and reduces the impact of low-density lipoproteins (LDL or "bad" cholesterol) on coronary arteries. While the development of such a drug is not as easy as it might seem the operation was a good deal for Sofinnova that had bet since 2005 on Cerenis and invested more than €20 million in the company over ten years (Hecketsweiler, 2015g).

Sofinnova recovers about five times its investment, as a general average, according to Antoine Papiernik, one of the fund's associates. Sofinnova tries to recruit the best coaching experts, such as Jean-Jacques Garaud, who had worked for Novartis and Roche, or Henri Termeer, the former executive officer of the American biotechnology company, Genzyme, purchased by Sanofi for a high price. Sofinnova also tries to pool its efforts with other funds. Hervé Ronin of the bank Bryan Garnier & Co. – an investment bank operating in health-care – considers that Sofinnova "is the best French

venture-capital fund because of its size (*ca*. €250 million) and of the competence of its staff." Its main rivals in France – and sometimes partners – are the investment funds of Edmond de Rothschild's bank and Public Investment Bank (Bpi-france). "They are only those which can invest €15-20 million in a startup," stated H. Ronin (Hecketsweiler, 2015g).

## Biotechnology companies: an increasingly attractive market in Europe

American investors have thrown more than US$1 billion into the European biotechnology sector during the first half of 2015, i.e. much more than the US$794 million invested in 2014, US$153 million in 2013, and zero dollar during the three years earlier, according to the data supplied by the New York Mellon Corp. bank. In addition to the high increase in initial public offerings (IPOs) of small European startups in the United States, the above-mentioned investments showed that the value of biotechnology companies seemed to be relentless. However investors did not agree whether this was a bubble that might burst (Roland, 2015).

For instance GW Pharmaceuticals plc, a London-based firm that develops drugs from raw cannabis, has raised US$481 million on the Nasdaq stock market, in just two years, through four distinct transactions. The main quotation of GW Pharmaceuticals was on the Alternative Investment Market (AIM) of the London stock exchange. But the company's shares are much more traded in the United States. Justin Gover, GW Pharmaceuticals' director-general, stated that the United States are "the most attractive market", and that the transformation of GW Pharmaceuticals from a startup into a pharmaceutical company "would be impossible without the access to this kind of funding," (Roland, 2015).

In this regard, one should remember that the "first wave" of biotechnology firms, such as Amgen, Gilead Sciences and Celgene, went public by the late 1980s and early 1990s, and they became large biotechnology companies. Their success led to the birth of a large community of specialized investors and of funds devoted to biotechnology which continue to support this sector. And such a trend is not unique to the United States. For instance Verseon Corp. – a company that develops drugs designed through the utilization of computers in California – has raised £65.8 million or US$101.2 million on the Alternative Investment Market (AIM) at the beginning of May 2015, with the support of several investors, including Neil Woodford – the manager of British funds. Adityo Prakash, the CEO of Verseon, stated that he was "impressed by the long-term vision of institutional investors in the United Kingdom." Also, in 2014, Allied Minds plc – a Boston-based firm specialized in the commercialization of results of university and government research centres – raised £124.4 million on the London market. The United Kingdom has an advantageous position with respect to the United States in the "space of technology transfer," because it is already the key IPOs centre for many firms belonging to this sector, including, for instance, IPGroup plc and Imperial Innovations plc. Another technology transfer enterprise based in Boston, PureTech Health, was also to be listed on the London market (Roland, 2015).

European biotechnology companies had another reason to go to the United States in 2012, when President Barack Obama signed the Jumpstart Our Business Startups (JOBS) Act. This bill aimed to enhance the growth of small businesses in the United States thanks to the introduction of more flexibility in the regulation of firms whose annual gross revenue was less than US$1 billion and which wanted to raise funds on public markets. The JOBS Act has created an "unprecedented attraction" for small-sized enterprises which could save "thousands, and even hundreds of thousands dollars, relating to the costs of regulatory guidelines and internal checks" (Roland, 2015).

Most foreign firms have access to American investors through the American Depository Receipt (ADR) which enables these firms to go public on the American markets. For instance at the beginning of May 2015 the Belgian biotechnology firm, Galápagos NV, announced it went public with *ca*. US$242 million; this was the most important introduction to the Nasdaq for a European biotechnology company. Also the French biotechnology firm Cellectis and the British Adaptimmune Therapeutics plc raised more than US$100 million on the Nasdaq by the end of the first quarter of 2015 (Roland, 2015).

Davis Pinniger, manager of biotechnology funds at Polar Capital, London, stressed that European biotechnology companies were heading for the United States because there, the financial markets are more "sensitive" to their growth histories, and because investors are "ready to take risks." The sector's performance during recent years was drawn by the growth, higher than foreseen, of the profits of large companies, such as Gilead Sciences, rather than by the long-term speculation of small-equity firms that were not making profits. D. Pinniger added that there had been a profound change in the productivity of bioindustry which attracted the investors' interest for the sector. He talked of a profound mutation induced by the new powerful tools resulting from the combination of informatics and communication technologies with genomics (Roland, 2015).

# CREATION OF ROCKETING MEDICAL BIOTECHNOLOGY STARTUPS

## The clue to technological innovation

The Silicon Valley in California and similar campuses in the United States and other countries are clusters devoted to advancing knowledge and research in order to give rise to technological innovation and commercialization of novel applications through startups and enterprises. Biotechnology companies are illustrative examples of this chain of added value, from the university to the market.

### *Cornell Tech*

The example of Cornell Tech, the campus of Cornell University, Ithaca, New York, devoted to technologies and entrepreneurship, is an excellent illustration of this concept. Its objective is to support the development of the high-technology sector of New York City through the training of its future engineers. This US$-2-billion project over 25 years is funded by private investors, philanthropists who are former alumni of Cornell University. In 2017 the new campus will be partially opened on Roosevelt Island, ubicated on the East River, between Manhattan and Queen boroughs. However courses have started in 2014 at Google's headquarters in New York. Cornell Tech is the unique outcome of a close cooperation between the city, the academic world and the entrepreneurial sector, as underlined by its dean, Dan Huttenlocher. With a PhD from the Massachusetts Institute of Technology (MIT), this 56-years-old technologist knows the private sector: he had been working for 11 years at the Xerox group's PARC laboratory, as well as at the technical directorate of the software editor Intelligent Markets. He had been teaching informatics at Cornell University before taking the lead of Cornell Tech (Guédel, 2014a).

On 15 May 2014, in an interview with the French newspaper *L'Opinion*, D. Huttenlocher explained how Cornell Tech had been created. In 2011 Bloomberg, who was New York's mayor, and his administration opened a contest in which universities worldwide could participate, with a view to submitting a project on a new campus, located in New York City – Cornell University, Ithaca, is at a 370-kilometer distance from Manhattan – and devoted to bringing together advanced studies and research and new enterprises. New York was considered a very appropriate location for such a project. Cornell University focused its proposal on digital and information technologies. Despite several formidable competitors like New York University, Columbia University and Stanford University, Cornell won the contest, in particular thanks to an operational plan that included the immediate beginning of courses without waiting for the construction of the new campus. Google's facilities in Manhattan served for that purpose – a Master and a PhD were already being prepared. The project started rapidly and several enterprises, in addition

to Google, brought in their support and collaboration: Qualcomm, a mobile telephone company, the medias group Hearst and the incubator Betaworks. Students are engaged with these enterprises, not just as interns, but by working on projects, with a university professor as research director and a member of the enterprise's staff as mentor. Students must have an immersion in the enterprise and the whole of Cornell Tech campus is oriented towards the interaction with the entrepreneurial world (Guédel, 2014a).

Cornell Tech started with the preparation of a Master degree in informatics, an MBA in informatics and a Master degree in connective media – the latter was accredited by Cornell University and the Israeli Institute of Technology (Technion). The MBA is focused on business, while the Master degree on informatics is technology-oriented and the Master in connective media is focused on business, technologies and design. The collaboration with Israel's Technion which leads to a double degree, recognized in both countries, is rather unique. It was the first time a foreign higher education and learning institution was installed on an American University's campus. This could be compared with the Broad Institute – a partnership between Harvard University and MIT, devoted to biomedical and genomic research, but both are American institutions. The partnership with the Technion was materialized as the Jacobs Institute, that bears the names of Joan and Irwin Jacobs, co-founders of Qualcomm and renowned philanthropists. The courses started during the fall of 2014 (Guédel, 2014a).

Dan Huttenlocher underlined in that interview that a crucial goal of Cornell Tech is to offer new opportunities to young graduates, others than a good job in a big corporation or in an administration as civil servant. Graduates should be trained in order to start their professional life via the entrepreneurship. This is a risk to run, but it is the best moment to take such a risk, when one leaves the university and has not yet built a family (Guédel, 2014a).

### *Israel*

With a population of 8 million inhabitants, Israel, dubbed the "startup" nation, is one of the most dynamic countries in terms of creation of startups – more than 200 of them had been created between the beginning of 2013 and mid-2014. Adam Schwartz, a professor of engineering at the Haifa-based Technion – the Israeli Institute of Technology – explains that there is in Israel a long tradition of entrepreneurship, as well as a mindset with regard to success and failure. In Israel failure is not considered a big problem; by the way, most startups fail. There is a real tolerance vis-à-vis the person who has not succeeded, while he/she was trying to do something. And who fails accepts the criticism, listens to advice and tries again, but differently in order to win. Israeli youth's icons are not the rockstars, but those entrepreneurs who have become wealthy thanks to their hard work and through creating value. These examples give the young entrepreneurs the basic tools that are the key to success, said A. Schwartz in an interview with the French newspaper *L'Opinion*, published on 15 May 2014 (Guédel, 2014b).

There are two advanced-technology centres in Israel, in Haifa and Tel Aviv, a rather similar situation to that existing in the United States: the Silicon Valley and Silicon Alley. A. Schwartz, who is the director of the Jacobs Institute in New York City, recalled that in the 1980s the creation of startups was initiated by several groups. Some of

them who have been trained at the Technion – at that time, the only national institute of technology – left Israel and worked for foreign companies such as Intel. Back to Israel they founded research-and-development enterprises and that was followed by a surge in the demand for engineers whom the universities had to train. At the same time, in both the United States and Israel, more and more private funds were invested in small technology startups. Business angels and venture capitalists appeared. Finally, in the 1990s, further to a strong wave of immigration, Israel's population increased by more than 10%. Most of these immigrants were Russian engineers. Those who did not find work upon their arrival, created their own enterprise, with the government's impulse. There has been therefore an enormous change in the country, with a very favourable environment for small high-technology startups (Guédel, 2014b).

The Israeli government promotes the creation of startups through subsidies, setting up favourable regulation, and also through the higher education system and partnerships between the entrepreneurial sector and the leaders of academic institutions. In fact, at secondary school level, there are already programmes and contests oriented towards entrepreneurship. Adam Schwartz, however, reckons that the relationships between the universities and the business sector are generally not easy to build up and consolidate; universities are often quite conservative and the business sector is not familiar with their ways of functioning. There are from time to time successful examples. That is the purpose of the alliance between the Technion and Cornell Tech: a closer relation between an advanced-knowledge and academic institution and the world of enterprises (Guédel, 2014b).

In the overall favourable context prevailing in Israel, the big American Internet groups have been showing an increasing interest in investing in this country. A few days before the spectacular acquisition of Whatsapp by Facebook, another purchase illustrated the international fame of Israeli startups: the Japanese group Rakuten bought Viber application for US$900 million. That was a peculiar deal because the startup, created and led by a former officer of the Israel Defence Forces, Talmon Marco, had never raised significant funds. That was not the case of the communitary GPS Waze which Google acquired for more than US$1 billion in June 2013; in 2011 two American venture capitalists, Horizon Ventures and Kleiner Perkins Caufield & Byers, had invested *ca*. US$30 million in that Israeli startup (Sedouramane, 2014).

This example among many others shows the large presence of American investors in Israel. In fact Israeli entrepreneurs, once their domestic market conquered (this is a testing ground for them), look at the American market. According to Tel Aviv IVC Research Center – a research institution that monitors the new technology market in Israel – venture-capital investments provided by American funds made up more than 50% of the amounts of money invested in Israeli startups in 2013. During this year 83 foreign funds had made at least one investment in Israel, a figure that was 11% higher than that of 2012 and 223% higher than in 2009. The rising amount of foreign investments in Israel can be explained by the nature of operations, increasingly carried out by foreign investors as starters, and certainly because the size of funds forces the Americans to carry out more small-sized operations; and also because they want above all to become part of the capital of the most profitable startups and very soon (Sedouramane, 2014).

Israel is a unique case in the world: while the United States and China have the highest number of startups in the sector of the Internet, Israel has the highest number per capita. According to a study made by Bowei Gai and devoted to digital ecosystems worldwide, Israel had *ca*. 375 startups per million inhabitants in 2014, that is *ca*. 2.5 times more than the United States. It is therefore impossible for any American big group not to seize the innovation opportunities offered by Israel. For instance, in April 2014, IBM opened in Israel an accelerator called AlphaZone, while in May 2013 eBay announced that it would launch a similar structure specialized in e-commerce, social networks and big data (Sedouramane, 2014).

The relationship between the American big companies and Israeli startups is a rather old one. For instance, according to the *Times of Israel*, Cisco had invested *ca*. US$1.5 billion in Israeli startups over the period 2000-2014, in addition to the US$5 billion purchase of NDS in 2012; this firm was created in 1988 at the Weizmann Institute of Science in Rehovot. The American telecommunication giant was also expected to pursue its investments; some of them would be made indirectly by an Israeli venture-capital fund, the Jerusalem Venture Partners (JVP). Cisco objective is to target innovative startups specialized in cybersecurity – a very relevant goal, as JVP opened in 2014 an incubator specialized in this area, in partnership with Ben Gurion University of the Negev in Beersheva. Israel is the second foreign country present on the United States Nasdaq, just behind China. In 2013 there were *ca*. 90 Israeli companies on the Nasdaq and, according to the press agency Reuters, the number of Israeli companies to be listed on the New York stock exchange was expected to increase swiftly in 2014 and beyond (Sedouramane, 2014).

Another striking example of startup development in the area of pharmacology and biotechnology is the investment currently made in the culture and production of cannabis for medical purposes. It also illustrates the know-how of several Israeli startups, often financed by American funds and which are willing to transfer this know-how and innovation strategies overseas. In Israel, cannabis (marijuana) is an illegal and dangerous drug. However, its medical use, which is strictly regulated, has been authorized since the early 2000s. Nowadays an estimated 23,500 persons suffering from cancer, epilepsy, chronic pain and some neurological diseases are authorized to consume cannabis with a view to alleviating their ailments. This number was expected to increase while a reform project aimed at making the whole system more liberal by the summer of 2016: further to the wish of Israel's health minister, Yaakov Litzman – an ultraorthodox Jew -, cannabis prescription will become easier to obtain and the sale of the drug will be facilitated through a broad network of authorized pharmacies (De Vergès, 2016).

Cannabis is not new to Israeli scientists. In 1964, an Israeli chemist, Raphael Mechoulam, was the first to isolate and thereafter synthesize the THC or tetrahydrocannabinol – the psychotropic molecule of cannabis. This discovery opened up the therapeutic use of this substance and at the same time provoked discrepancies among physicians about its use. Many doctors are worried about addiction or dependence risks as well as behavioural disorders, and they add that studies carried out until now on the subject involve very low numbers of patients. In Israel clinical trials aimed at knowing more about the therapeutic properties of cannabis ("ganja") are being encouraged and they involve both producers of the drug and advanced-research cannabis at the renowned Technion Institute (university) in Haifa (De Vergès, 2016).

One of the eight cannabis producers in Israel is BOL Pharma which has received an authorization from the government. It has been growing marijuana since 2008 in its greenhouses under natural light, with computer-controlled humidity as well as the biochemical parameters of the inflorescence of each plant. Some 200,000 plants are produced annually: only the inflorescence are used, the rest of the plants is destroyed. BOL Pharma has launched an overseas programme with a view to building greenhouses across several countries with a total area of 130 hectares (De Vergès, 2016).

In Israel it is forbidden to export cannabis inflorescences. As Michael Dror, chief physician at the Official Agency for Medical Cannabis, explained, "some members of the government are afraid of the fact that Israel could be depicted as an exporter of weapons and drugs." That is why "cannabusiness" people have set another goal: to commercialize their medical, technological and agrobiological know-how outside the country. Two cannabis varieties are currently used in Israel: Erez with a high content of THC, and Avidekel, with a high content of cannabidiol but without relaxing effects. Both varieties were developed by Tikun Olam (in Hebrew "fix the world") which is the main producer of medical cannabis and supplier of one-fourth of patients through its authorized pharmacies. The patients receive their monthly dose of the drug (in the form of oil, pills or joints) at a fixed price of 370 shekels, or €86, per month (De Vergès, 2016).

In 2015, Tikun Olam signed an agreement with the Canadian cannabis producer MedReleaf, in order to allow the latter to cultivate the varieties developed in Israel. Tikun Olam also struck a deal with an American investment group. "Like a brand, we have sold it our rights so that he can make business on our behalf," explained Ma'ayan Weisberg, a spokesperson of the company. In fact, the American market is growing rapidly and can be a lucrative one for the Israeli cannabis producers. While marijuana is still illegal at the federal level, 24 States have made legal its medical use. According to New Frontier – a specialized analyst – the annual turnover of therapeutic cannabis in the United States reached US$4.2 billion (or €3.7 billion) in 2014 and it was expected to jump to US$10.7 billion by 2020. This is therefore a very coveted market by Israel where the local market was valued at between US$20 million and US$25 million (De Vergès, 2016).

"Israel indeed is targeted by international investors in this sector," explained Jeffrey Friedland, the head of the American company Friedland Global Capital that made investments in two agritech and one pharmaceutical Israeli enterprises. "In this country, you have the science of the plant, agrotechnology, medical research and innovation in the devices needed for administering the drug," he added. He was negotiating the conditions of investment in another four Israeli companies. Since 2014 American investments in the Israeli market have reached *ca*. US$50 million, according to the data provided by Saul Kaye, the organizer of CannaTech – a conference on marijuana – in which have participated by mid-March 2016 in Tel Aviv investors and entrepreneurs from *ca*. 30 countries. American investments comprise the purchase of patents as well as the funding of startups. "They would amount to more than US$100 million in 2017," S. Kaye stated. "We are developing an industry as prosperous and renowned as we have done with high-tech," claimed the pharmacist who made an alliance with BOL Pharma with a view to creating the first Israeli incubator of startups specialized in medical cannabis (De Vergès, 2016).

In fact several startups are already well known. Among them Syqe Medical has developed a marijuana inhaler. This devise enables the patient to consume a precise dose of cannabis. By the end of January 2016 the American tobacco company Philip Morris invested *ca*. US$20 million in that startup. Another one called Eybna is specialized in the identification and production of terpenes – the organic compounds that give to cannabis its aroma and also have several therapeutic properties. "We have isolated more than 300 terpenes, while only 150 were known before," proudly stated Aviv Junno, the spokesman of the company, which works with the best research laboratories in the country. Its objective is to produce a purified drug, otherwise, a "standardized" substance that would target specific pathologies and would be devoided of harmful secondary effects. Eybna has already successfully developed cannabis essential oils without psychotropic ingredients. The startup that received several million US dollars from American and Israeli funds by the end of the first quarter of 2016, sells its legally authorized product to its clients across the world: manufacturers of electronic cigarettes, candies, candles and even shoes, who wish to give to their products the heady fragrance of marijuana. What is at stake for the Israeli startups is to grow bigger and bigger, which A. Junno summarized by boasting: "What we are doing here is to build up the Apple of the cannabis industry" (De Vergès, 2016).

## *Japan's challenges in research and innovation*

The 2014 Nobel Prize in Physics was particularly rewarding for the Japanese industry and academia. The prize which was awarded to three scientists who developed blue light-emitting diodes (LEDs) came at a time of growing concern about the future of physics in the country. The development of blue LEDs is a glaring case of university research leading to commercialization of products; and in this case the Nobel Prize rewarded a technological innovation rather than a discovery, in conformity with the approach of Alfred Nobel who wanted to do so through awarding the annual prizes. Amid Japan's prolonged economic stagnation, the cooperation between academia and the industry has been crucial for companies wary of investing in risky endeavours with no guarantee of success. The government is supportive of academia-industry collaborations. In 2014 the government's Council for Science, Technology and Innovation launched a programme called ImPACT, which stands for Impulsing Paradigm Change through Disruptive Technologies. The council selected 12 research themes and assembled researchers from academic institutions and the corporate sector (Taki, 2014).

The government also established the Japan Agency for Medical Research and Development in April 2015 to support breakthroughs in pharmaceuticals and medical equipment. Research budgets related to the life sciences, currently handled by multiple government offices, will be consolidated. Japan has few ventures of the same calibre as American startups which frequently discover drug prototypes before passing them to the big pharmas. The new agency may help fill this gap by developing drugs using leads from university laboratories. Japan is a global leader in stem-cell research and therapies (see p. 389). In the case of blue LEDs Isamu Akasaki and Hiroshi Amano came up with the basic technology at Nagoya University in the mid-1980s; thereafter they launched a joint research project with Toyoda Gosei, an autoparts maker. The Japan Science & Technology Agency supported the project which aimed to put the innovation to practical use. Separately Shuji Nakamura devised a mass-production method for a high-brightness blue LED at Nichia Chemical Industries (Taki, 2014).

When Japan's economy was booming scientists could drum up funds for all sorts of research seen as having commercial potential, on top of what they received from universities or other institutions. But as corporate and government funding has been tightened financial, support has become far less available. Nowadays the Japanese government emphasizes research that promises significant benefits to industry and society. This policy tends to guide public budget allotments to national universities. While considerable amounts of money are devoted to endeavours expected to bear such fruit, there is not much left over for research regarded as less crucial. This orients scientists into a few select fields where they are likely to produce practical results (Taki, 2014).

"More researchers now waver when they feel they lack a concrete purpose," stated Yuichiro Anzai, head of the Japan Society for the Promotion of Science and a former president of Keio University. There is, he added, a worrying tendency to change the direction of research for the express purpose of obtaining more state funding. If researchers lose their sense of mission and feel compelled to change course midstream, they will be less likely to produce true innovations. According to Taki (2014) the number of researchers is already on the decline and Japan is also falling behind in terms of its volume of scientific papers. Some scholars warned against taking a single-track view of science – the idea that basic research is followed by applied research, which in turn is followed by commercialization of technological innovation. Akiyoshi Wada, professor emeritus at the University of Tokyo, makes a distinction between "pure research aimed at elucidating the essence of nature and society, and applied research that leads to solutions to specific problems." Research aimed at understanding nature can produce practical innovations, just as seeking solutions to specific problems may raise new scientific questions relating to a broader understanding of the world. The crux of Japan's challenge is to find ways to revitalize basic research. By the end of 2014 indeed the government council started discussing a new five-year plan for science and technology, to take effect in 2016 (Taki, 2014).

## France investments in medical biotechnology startups

### *Volume of investments*

In France, in 2013, €147 million were allocated to research in biotechnology firms that had 18 drugs in development including five of them in phase-3 clinical trials. Comparatively, the French pharmaceutical group Ipsen had spent €349 million, but had only 19 molecules that were being tested including six of them in phase-3 clinical trials – the last stage before approval by health authorities and commercialization – according to the data published on 18 December 2014 by the association France Biotech – the federation of French biotechnology companies. The big pharma Sanofi invested €6.3 billion in research and development on 111 candidate drugs including 20 of them in phase-3 clinical trials. The wave of funding startups is a reality in France that will continue, as the French government has decided, despite the economic crisis, to support R&D and innovation, in particular in the life sciences and biotechnology (Hecketsweiler, 2015e).

### *Building alliances with American investors*

Bearing in mind several examples, Gilles Nobécourt, a partner in the venture capital Biodiscovery, specialized in the life sciences, of Edmond de Rothschild Investment Partners – one of the most important venture capitals in the sector – analyzed in an

interview with the French daily newspaper *Le Monde* the reasons why an increasing number of French biotechnology startups are trying to build alliances with, and find support of, American investors. The first reason is that Europe has not enough funds available: we are dealing with national markets; the funds available to support a startup amount to €50 million-€70 million, compared with several hundred millions of US dollars in the United States. Although as mentioned above, funding of startups can be significant in Europe (see pp. 21-23), going overseas allows the successful startups to find more important funding. In Europe furthermore there are not enough biotechnology companies that are listed on the stock exchange, so as to justify the existence of specific funds. And generalist funds which are cautious, hesitate to trust, and invest in, companies whose activity is not always easy to understand. Conversely, in the United States, biotechnology startups are coached by experienced investors; and big banks such as JP Morgan or Goldman Sachs have teams devoted to life sciences; as they have more experience they are less risk-averse (Hecketsweiler, 2015a).

However all chief executive officers of companies that have set foot in the United States have underlined the constraint to manage a double listing on the stock exchange in Europe and in the United States. It indeed means twice more constraints and twice more time to devote to the investors. This explains why some biotechnology startups are making a backstep, especially when more funds become available in Europe, e.g. Circassia in the United Kingdom (p. 18) or Molecular Partners that rose more than €84.8 million or US$100 million in November 2014 on Zurich's market place (Hecketsweiler, 2015a).

Gilles Nobécourt underlined that since 2012 Paris stock exchange had widely opened its door to biotechnology startups. According to this expert it is the most active in Europe. He nevertheless pointed out that after the creation of these startups, the industrial follow-up is often lacking and the innovation process is incomplete. Furthermore despite a little more advantageous fiscal system, the fact that the head of a startup must keep at least 10% of his company's equity is not appropriate for biotechnology firms. The amounts of funds raised are such that the firms' founders are very quickly diluted below this threshold. Consequently entrepreneurs prefer to develop their new startups elsewhere (Hecketsweiler, 2015a). In his interview with *Le Monde* Gilles Nobécourt underlined that he firmly believed in the bright future of several French medical biotechnology startups.

### *Examples of success stories*

DBV Technologies

DVB Technologies was the first French biotechnology company to be introduced on New York stock exchange in 2015, after already having been listed at Paris stock exchange in 2012; on 15 July 2015 DBV announced it had been able to raise an additional US$240 million (or €218 million) from American investors. "This is a record for a French company," said Pierre-Henri Benhamou, co-founder with Bertrand Dupont, both pediatricians at Paris Saint-Vincent-de-Paul hospital, of DBV, who ended its road show in San Francisco by mid-July 2015. At this date DBV was valued at more than €1.4 billion and its share increased 700% in three years (Hecketsweiler, 2015m). This startup deserved it status of "unicorn."

The innovation that made DBV a "star" among biotechnology companies in the United States is a skin patch, named Viaskin Peanut, that may become the first treatment in the world of allergenicity to peanuts. This disease affects *ca*. one American out of 100. P.H. Benhamou explained that "peanut or groundnut is an 'atomic bomb' for persons who are allergic to it. Minute quantities of this legume species induce a reaction that can be lethal; consequently conventional techniques which try orally or via injection to reduce the allergenicity cannot be used effectively." The French physician added that he was shocked by the impact of this allergy on the daily life of the hundreds of thousands affected schoolboys and girls; "the consumption of peanuts is so widespread that the life of these pupils is threatened each time they accept a biscuit from their schoolmates; and the anxiety is so high among parents that peanut-free schools have been created" (Hecketsweiler, 2015m). That allergy caused 150-200 deaths and 125,000 urgent medical consultations annually (Hecketsweiler, 2016d).

DBV patch is simply put on the skin and it is expected to achieve a risk-free "education" of the immune system thanks to the activation of certain cells of the epidermis, but without overcoming this barrier; this is the main difference with the classical desensibilization techniques (injections, drops or pills). Children are the priority target and the patch has been qualified as a breakthrough by the United States Food and Drug Administration (FDA) in April 2015. The FDA had already granted in 2012 a fast track approach to test the patch, as it generally does for a limited number of drugs whose therapeutic value may be outstanding. By the end of December 2015 DBV initiated clinical trials among children between four and 11 years of age. If everything goes well the launching of the Viaskin Peanut patch will occur in 2018 in the United States and in 2019 in Europe (Hecketsweiler, 2015m; 2016d).

Analysts predict that the sales of the patch could reach US$2 billion between 2015 and 2020. That is why large investment funds have shown great interest in supporting DBV. For instance Baker Bros, one of the most widely known investment funds in biotechnology and which was one of the first to bet on DBV, decided to bring in US$75 million in July 2015. "Such support for a French startup is exceptional," stated David Schilansky, the deputy director-general of DBV. "We are moving from the status of a small biotechnology company to that of an international pharmaceutical laboratory" (Hecketsweiler, 2015m). DBV or "Peanut company" as it is called in America, is installing its headquarters in Summit, New Jersey : "it's there where the big pharmas are headquartered; it's there that we have to be," stated enthusiastically P.H. Benhamou (Hecketsweiler, 2016d).

With more than €300 million in cash DBV can fund the clinical trials of the Viaskin Peanut patch and also recruit the salespersons to sell it in the United States. "This is a new concept, and there will be a lot of education work to carry out," explained P.H. Benhamou. The latter intends to pursue the development of DBV technology with a view to treating other allergies, in particular the allergy to cow milk; the efficiency of a Viaskin Milk patch was to be demonstrated at the end of a study that had been authorized on 8 July 2015. In the longer term this kind of patch could be used as a vaccination substitute (Hecketsweiler, 2015m).

On 31 May 2016 the startup announced an agreement with the giant agrifood Nestlé who made in 2010 (Peter Brabeck who still the CEO) the decision to invest in the prevention of ageing, in nutrition and nutritional therapies. It was for these reasons that NestléHealthSciences was created. DBV, while signing such an agreement, joined the startups working for NestléHealthSciences. This deal could yield up to US$100 million (or €89.82 million) to DBV – of which an initial instalment of US$10 million – as well as two-figure royalties on world sales (Hecketsweiler, 2016d).

Both co-founders of DBV Technologies wanted with the help of NestléHealthSciences to develop their test for allergy against cow milk. This was designed in the beginning of 2000, before that of Viaskin Peanut, and it was authorized by the French Agency for Drug Safety (ANSM) for commercialization. But by the end of 2014 the ANSM changed its mind and asked for supplementary clinical details. And finally it authorized DBV to commercialize its Diallertest and without any advertisement. "This patch was at the origin of DBV … After having invested so much energy and time, to see our test to be developed again was a true satisfaction!", stated P.M. Benhamou (Hecketsweiler, 2016d). About 2% to 3% of infants would be affected by this allergy to cow milk, but pediatricians have no means yet to identify them. To do so presents an interest for public health but also a commercial stake. NestléHealthSciences, specialized in "nutritional therapies," had developed a range of milks for the infants allergic to cow milk, but it counted on the DBV patch to boost its marketing. This patch christened MAG1C would lead to clinical trials before the end of 2016 and if the latter are concluding ones this patch could be commercialized in 2021. The market of this patch is considered to be huge: one-third of babies present during their lifetime symptoms that could evoke an allergy to milk (regurgitation, vomiting and loss of weight), DBV stated; detecting among them those who are really allergic would have a public-health interest (Hecketsweiler, 2016d).

Having signed an agreement with Nestlé would be considered, according to P.M. Benhamou, as a solid partnership with a group that "could enable DBV to be present in Asia." "The pediatrician announced a Viaskin Egg to be commercialized in about two years after the Viaskin Milk and he was thinking of a fourth kind of Viaskin patch" (Hecketsweiler, 2016d).

Adocia

The Lyon-based startup Adocia is specialized in antidiabetes drugs. By the end of 2014 it signed an agreement with the American big pharma Eli Lilly in order to develop an ultra-fast insulin. Adocia went public in 2012. After the announcement of the cooperation agreement with Eli Lilly Adocia's share jumped 37%. According to this agreement Adocia was expected to receive up to US$570 million (or €466 million), further to an initial payment of US$50 million. Adocia was also to cash royalties on the sales of the newly developed insulin. "It is a true recognition of the quality of our drugs and of our know-how," stated Gérard Soula, the founder of Adocia, who leads the startup with his two sons (Hecketsweiler, 2014 l).

Gérard Soula is a typical biotechnology entrepreneur. Before Adocia he had created Flamel Technologies, another startup specialized in pharmaceutical formulation. This company was among the very few to be listed on the Nasdaq, and it was valued at US$700 million by the end of 2014. G. Soula recalled that he presented his technology several times to Sanofi, but there was no follow-up, or response, from the French big pharmaceutical group. Conversely the Indianapolis-based Eli Lilly welcomed Adocia's know-how. An earlier alliance between them had been set up in 2011, but it came to an end in 2013. Eli Lilly's come back in 2014 should be put in the context of the race for innovation in the area of antidiabetes drugs, where the sector's leaders – Eli Lilly, Novo Nordisk and Sanofi – are all involved, because they are losing the patents on their blockbuster drugs (Hecketsweiler, 2014 l).

These big pharmas have been developing more sophisticated versions of human insulin, called "analogues". There are two types on the market: the so-called "slow" insulins which regulate the concentration of glucose in the bloodstream or glycemia in a resting condition (e.g. Sanofi's Lantus); and the "fast" insulins which must be injected to the patient during the meals (e.g. Novo Nordisk's NovoLog – sales of US$3 billion in 2013 – and Eli Lilly's Humalog – sales of US$2.6 billion in 2013). Patients must follow a precise schedule for their insulin injections; they must also anticipate the weight and timing of their meals with great precision, so as to evaluate the appropriate dose of insulin and estimate the right time to take it. "If this is done too early the patient runs the risk of hypoglycemia; if it is done too late insulin cannot act properly and the concentration of glucose in the bloodstream rises rapidly," explained G. Soula. The ideal drug is therefore a "just on time" which is injected when it is needed by the patient. Adocia's insulin, christened BioChaperone, seems to be close to that kind of drug. The results of clinical trials carried out with BioChaperone have been presented in June 2014 at the annual conference of the American Diabetes Association. They showed that with this new drug the speed of action of the hormone is accelerated, with a peak reached in 42 minutes, compared with 69 minutes with Eli Lilly's Humalog. "The key opinion leaders – i.e. the physicians-experts who are very influential in the United States – were amazed by the results," said proudly G. Soula (Hecketsweiler, 2014 l).

Adocia's know-how aims to customize the insulin bought from Eli Lilly in bulk; it is encapsulated in polymers which accelerate the hormone's diffusion into the bloodstream, before they are dissolved. The composition of these polymers is an industrial secret and Adocia has filed more than one hundred very coveted patent requests. Adocia's insulin could be commercialized as soon as 2017 or 2018 – a record in the pharmaceutical industry where the development of a drug generally takes a dozen of years. G. Soula explained: "Because we use existing products, we shall not have to repeat all the tests." He also hoped that his company's innovation will be adopted by emerging countries where human insulin is the basic antidiabetes treatment because of its low cost. But its action starts 15 minutes later than that of insulin analogues. G. Soula claimed that "his technology would correct that and thus align the performance of human insulin with that of analogues, and at a much lower cost" (Hecketsweiler, 2014 l).

The deal made by Adocia with Eli Lilly by the end of 2014 can make a dent into the global insulin market. The sales of the hormone were close to US$25 billion and their annual growth rate was at least 10%. The number of diabetics would increase from 382 million in 2013 to *ca*. 592 million in 2035, according to the data provided by Adocia. One-fourth of these diabetics are treated with insulin that is the only option for type-1 diabetics – an autoimmune disease where the patient destroys its own beta-cells of the Langerhans islets, which synthesize the pancreatic hormone; and it is also the last recourse for type-2 diabetics whose pancreas endocrine function is altered (Hecketsweiler, 2014 l). See also pp. 76 and 335.

Cellectis

Cellectis story starts in the 1990s when André Choulika, co-founder and executive officer of the company, was working at the Pasteur Institute in Paris and discovered molecular tools (meganucleases) or DNA "scissors" that can modify specific DNA sequences and thereby transform the cell's genome. In 1999 he founded Cellectis with another researcher of the Pasteur Institute, David Sourdive. Ten years later they were the first to sell "ready-to-work" kits which made that technology available to cell-biology researchers. Thanks to these DNA "scissors" they can engineer human cells, for instance, to test the efficacy of new drug molecules. In 2009 Cellectis signed an agreement with Monsanto which wanted to develop new genetically modified crop varieties, as well as the French seed cooperative Limagrain (Hecketsweiler, 2015a).

After the sequencing of the human genome in 2003 and the subsequent plummeting costs of DNA sequencing Cellectis entered another stage of its development. In 2011 it started selling a new kit of DNA "scissors", called Talen, at just a price of US$5,000. The French startup beefed up its commercial team in order to conquer the market. But in June 2012 another technique, also used to edit a genome sequence and named CRISPR-Cas9, was described in *Science*. In less than a year this technique spread rapidly and at a cheaper cost than Cellectis Talen. As a result of this unexpected competition and further to a collapse of Cellectis sales, A. Choulika had to lay off employees: from 240 to 90. The DNA "scissors" business was sold and the company kept only the licenses for its technology it uses for its own needs. But by the end of 2013 Cellectis had *ca*. €7 million in cash and its shares plummeted to just €2. Maïlys Ferrère, director of the fund Large Venture of Bpi-france (Public Investment Bank) which is the main shareholder of Cellectis, stated: "As it occurs in breakthrough technologies, what we have predicted did not materialize (Hecketsweiler, 2015a).

In January 2014 A. Choulika, who had not given up, attended the JP Morgan Healthcare Conference in San Francisco, and he brought with him several scientific papers on a new biotechnology tool: genetically modified T-lymphocytes that could target tumour cells and kill them. These so-called CART immune cells were the research focus of several teams in the United States, since Carl June, a researcher at the University of Pennsylvania, had successfully used them in a patient suffering from a leukemia. A. Choulika became an actor in the development and utilization of chimeric antigen receptor T-lymphocytes or CART-cells (Hecketsweiler, 2015a).

In March 2014 American investors were therefore interested in Cellectis and they contributed to a €20-million increase in the company's equity. The venture capitalists made a good deal since Cellectis shares that were bought at a price of €5 each rose to €20. While European investors did not show great interest American ones rose their share in the company's equity up to 40% in 2015. In June 2014 Cellectis made a historical deal with Pfizer – the American big pharma and a global top leader – which owned 10% of Cellectis equity. Thanks to Pfizer Cellectis had more than €100 million in its cash flow and could therefore fund its own research projects in addition to those carried out in collaboration with the big pharma. A. Choulika asserted: "Our technology can engineer T-lymphocytes from healthy donors and inject them to any patient, whereas competitors do not yet know how to do it." "In order to avoid the risk for the patient's cells to be attacked by the engineered T-lymphocytes, or a reject like in the case of a graft, the researchers reinject to the patient its own cells after having engineered them. This approach is very costly: from US$500,000 to US$1 million per patient and the process cannot be scaled up," added A. Choulika (Hecketsweiler, 2015a; see also pp. 314-315).

The commercialization of these new therapies cannot be expected before 2020 and A. Choulika hoped to launch a clinical trial in 2015. In fact Pfizer wanted to test the efficacy of CART-cells in treating 15 distinct cancers. To that end Pfizer had already allocated US$80 million to Cellectis in 2014 and it expected to make additional payments that could reach US$185 million per drug tested. On January 2015 Cellectis announced its forthcoming introduction into New York stock exchange. On 5 November 2015 it announced that one of its experimental immunotherapy treatments of cancer had been successful, when used to treat an 11-months-old British child, suffering from leukemia that was resistant to all existing conventional treatments. The very young girl, Layla Richards, had been hospitalized at London's Great Ormond Street Hospital (GOSH) in the Bone-Marrow Transplant Unit, led by Paul Veys (Hecketsweiler, 2015a,w).

Since 2010 several pharmaceutical companies such as the Swiss big pharma Novartis, as well as two American biotechnology companies, Kite and Juno, have been trying to develop these therapies which seemed to be very efficient (more than 90% of the patients were cured). Unfortunately only 200 patients worldwide could benefit from these therapies till November 2015. This is due to the fact that currently CART-cells are engineered from the patient's own immune cells so as to avoid their rejection. Each dose of transformed cells being unique, production is done at a very small scale and consequently the cost of the treatment is very high. "Demand is extremely high, but because of the lack of a bioindustrial production scheme, the waiting lists of patients are endless," stated A. Choulika (Hecketsweiler, 2015a; see also pp. 314-315).

Cellectis main rival, Juno, founded by the end of 2013, reached a stock value of US$4.7 billion in 2014. The other rival, Kite, was listed on the Nasdaq in June 2014 and it was valued at US$3 billion. Compared with both of them Cellectis US$670 million showed a big gap, which is not justified, said Gilles Nobécourt of Edmond de Rothschild Investment Partners. These companies are scientifically and technologically at the same stage of advancement in their research work, and the

gap in terms of assets between the French startup and the American firms will be reduced, asserted G. Nobécourt. Such a diagnosis was shared by Maïlys Ferrère who concluded that "in order to increase its assets, a biotechnology company must be renowned in the United States." That is the case of Cellectis which had become an important actor in cancer immunotherapy while by early 2014 it was almost bankrupt (Hecketsweiler, 2015a).

Cellectis approach to cancer immunotherapy is different: "Our technology consists of suppressing a very precise receptor on the surface of immune cells which are thereafter inoculated to patients so as not to be perceived as foreign bodies," explained A. Choulika. If this approach is efficient it will be possible to standardize the manufacture of drugs and to commercialize them at an affordable cost. The British infant had received in June 2015 a treatment called "UCART19", exceptionally. Clinical trials will be initiated at the end of 2015 with 12 British patients and commercialization of this immunotherapy was not foreseen before at least 2020. The impact of Cellectis successful treatment of Layla Richards was very positive for the company's share price: it jumped 11%. The company, listed in Paris and New York stock exchanges, was valued in 2015 at US$1.4 billion, behind its rivals, Kite (US$3.1 billion) and Juno (US$5.1 billion). The French pharmaceutical group Servier signed an agreement with Cellectis in order to develop, and possibly commercialize, five other drugs, including UCART19 (Hecketsweiler, 2015a). Cellectis is developing CART, equipped with other molecules and that are able to destroy a variety of tissues. It is of course very important that the genetically modified T-lymphocytes do not attack the body's organs. It is also important to administer them to patients who are rather healthy, because they generate a flu-type reaction that could be lethal (Hecketsweiler, 2015a).

Abivax

A fourth example of success stories among French biotechnology startups and enterprises is that of Abivax whose president is Philippe Pouletty, director of the investment fund Truffle Capital. On 1 April 2015 Abivax announced the creation of a "SiliCuban Valley Advisory Board" at the Cuba Opportunity Summit, held in New York with a view to reviewing the economic opportunities that would accompany the new diplomatic situation between the United States and Cuba. P. Pouletty was the only French participant in this summit. The creation of a SiliCuban Valley Advisory Board, focused on medical biotechnology and health-care, followed a close collaboration between Abivax and the Cuban biotechnology community. Abivax that is developing two vaccines, one against hepatitis B (to be commercialized in 2018) and the other one against AIDS/HIV, started to be interested in the Cuban medical and health-care sector in 2010-2011. This collaboration led to the creation in 2013 of BioCubaFarma which comprises 38 biotechnology companies in addition to the Center for Genetic Engineering and Biotechnology (CIGB, Spanish acronym). BioCubaFarma and Abivax started to work on the production of new drugs, e.g. antihepatitis-B (Lauer, 2015).

Cuban researchers contribute efficiently to this cooperation scheme thanks to their low-cost research tools, while Abivax facilitates the approval of products by the important international regulatory agencies, as well as the international exchanges regarding these products. It should be underlined that Cuba has given priority to health-care since the 1960s and in 1983 it created one of the most important biotechnology centers in the developing world: the CIGB where 1,400 researchers were working in 2015 and which had been able to own more than 1,200 patents. The health-care sector is nowadays the second-largest exporting one, with US$600 million (€555 million) or 10% of the value of total exports, just behind nickel and twice the value of cigars and rhum exports. Thanks to a perseverant state policy medical and pharmaceutical biotechnologies have received a high priority in terms of investments and training of very competent research teams. P. Pouletty stated: "Cuban researchers, despite their low salaries – some US$50 per month – are proud of their work and they are willing to collaborate if patronizing them is avoided" (Lauer, 2015).

The SiliCuban Valley Advisory Board comprises P. Pouletty, the director-general of the Pasteur Institute (Paris), Christian Bréchot, the president of Havana University's scientific council, Luis Montero-Cabrera, the CIGB research-and-development director, Gerardo Guillen, and Paul Tomasic of the Royal Bank of Canada. It was also expected that Luc Teyton of the Scripps Institute in La Jolla, California, will also join the Board. The objectives are to work on projects of joint companies (Cuban and foreign ones), to set up exchanges of researchers and to study new economic models in the health-care sector. "Cuba has an outstanding health-care system," recalled P. Pouletty. "It trains three times more physicians per inhabitant than France." In a context of globalization of health-care private clinics can be set up in order to welcome and treat patients that have increasing difficulties to get access to their health systems in developed countries, because of the soaring costs," he added (Lauer, 2015).

According to P. Pouletty France can play a significant role in the international cooperation with Cuba, further to the opening up of the country and to the normalization of its diplomatic relations with the United States. BioCubaFarma is considered an important step in this direction: on 11 May 2015 it received the visit of the French president, François Hollande, during his official journey to Cuba. It seems obvious that when the United States embargo is lifted the competition with American biotechnology companies and big pharmas will become harsher. But P. Pouletty thinks that the French initiatives will give an edge to national biotechnology companies such as Abivax (Lauer, 2015).

# ROLE OF NEW ACTORS IN MEDICAL BIOTECHNOLOGY AND HEALTH-CARE

## Bill and Melinda Gates Foundation

*The world's wealthiest foundation and a key partner in global health improvement*

In 2015 Bill and Melinda Gates celebrated the 15th anniversary of their foundation, with the installation of a sculpture by the American artist Janet Echelman, that is suspended between the buildings of the foundation headquarters in Seattle, Washington State. The amazing artist's work has been christened Impatient Optimists, an expression that is dear to both philanthropists and billionaires. In these buildings were working in 2015 *ca*. 1,500 physicians, engineers, economists and humanitarians, with a view to designing the most efficient strategies for struggling against diseases and poverty. The means and resources available to this staff are considerable: Bill Gates, the founder of Microsoft, and Warren Buffet – one of the world's wealthiest persons – have transferred a large part of their fortune to the foundation. The latter was in 2015 the wealthiest in the world, with a capital of US$43.5 billion (or €37.2 billion). It is also the most influential foundation (Hecketsweiler, 2015 l).

In a little more than 10 years the Gates foundation has become a key partner of NGOs, the large international organizations such as the World Health Organization (WHO), the Global Fund and even governments. Through its own initiatives with appropriate funding the foundation can orient the states' strategies, even though it does not intervene on the field. For instance, in April 2015, Bill Gates, in the *New England Journal of Medicine* (*NEJM*), criticized the countries which looked disorganized and lacking the means to struggle against an epidemic such as that of Ebola hemorrhagic fever (Gates, 2015). The mantra of the foundation is: "Here, we do not decide on the basis of emotions, but of facts." When defining its priorities the foundation relies on very strict evaluations. "Every dollar is an opportunity: before investing money, we review all the available data in order to optimize our impact," underlines Christopher Elias, one of the most prominent executive officers of the foundation. The analysis of these data relies on the work of the Institute for Health Metrics and Evaluation (IHME), a research institution associated with the University of Washington, Seattle, and funded by the Gates Foundation. In the 1990s the data used for designing public-health policies were not vey reliable, recalled Chris Murray, the IHME director. With the adoption in 2000 of the Millennium Development Goals (MDGs) the situation changed markedly. "In order to follow the progress made in the field, everybody had to use accurate means of collecting and analyzing data. Even the least democratic states had to publish these data," added C. Murray. As a result it is now possible to evaluate with

great precision the return-on-investment of various tools or strategies of public health. From this viewpoint Chris Elias hammered that "vaccination is the most effective tool or strategy" (Hecketsweiler, 2015 l).

### *Global Vaccination Initiative (GAVI) and Program for Appropriate Technology in Health (PATH)*

Consequently the first outstanding initiative of the foundation has been to invest US$750 million in the creation of the Global Vaccination Initiative (GAVI), that helps the poorest countries buy vaccines. The Bill and Melinda Gates initial funding has drawn other donors, including the United Kingdom, United States, France or Norway. Altogether they have spent US$10.2 billion, and according to GAVI this has helped vaccinate more than 500 million children and 8 million lives have been saved (Hecketsweiler, 2015 l).

An indication of the technological orientation of the foundation has been the key role it played in the growth of the Program for Appropriate Technology in Health (PATH). This Seattle-based organization has been created in the mid-1970s and it has become the "laboratory" of the Gates. With an annual budget of US$300 million the PATH allocates funds to the development of vaccines, drugs and technologies aimed at improving the health conditions of the poorest. One of the outstanding projects of PATH is the contribution to the development of a vaccine against malaria that still kills 600,000 people annually, most of them children. Thus the foundation has allocated US$456 million to the Malaria Vaccine Initiative (MVI). Behind this action there is a new approach of sharing risks, which led to promoting the allocation of funds to the development of a product whose economic profitability is not guaranteed, indicated Emmanuel Hanon, who is in charge of the research on vaccines at GlaxoSmithKline (GSK). The clinical trials of a candidate vaccine, RTS, S, the first to be developed against malaria by GSK, have been terminated in 2014, but the results were not much promising. But Emmanuel Hanon commented that "what has been learnt during these trials has been extremely useful" (Hecketsweiler, 2015 l; see also p. 273). It is true that Bill Gates does believe in vaccines, even though some results have been disappointing. According to Steve Davis, the PATH director-general, the foundation's commitments in the area of vaccine development distinguish the Bill and Melinda Gates Foundation from large international organizations such as the WHO. The foundation accepts to take risks and even suffer setbacks.

Regarding the development by Doctors without Frontiers of new treatments for neglected diseases, e.g. trypanosomiasis or sleeping sickness, and river blindness or onchocerciasis, Bernard Pécoul, its director, is of the opinion that "due to its enthusiasm, the Bill and Melinda Gates Foundation does not sometimes make a difference between progress and revolution; for instance they speak of eradicating certain diseases, while elimination of those is already an ambitious objective." This statement by B. Pécoul referred to a new drug against leishmaniasis, a parasitic disease; the foundation has been initially very enthusiastic about this drug, but it had later on to withdraw it. B. Pécoul who has been given US$60 million over five years

in November 2014 added: "We do not always agree with the foundation's staff, but we must reckon that we often have profound scientific discussions with groups of competent people (Hecketsweiler, 2015 l).

### *Reconciling the NGOs with the pharmaceutical corporations*

Even though the bets taken or made by the foundation can be debated, they had nevertheless the merit to reconcile the NGOs with the pharmaceutical corporations. In 2012, during its CEO roundtable, attended by all the CEOs of the big pharmas, Bill Gates had convinced them to invest in the control of ten neglected diseases and to lower the prices of their drugs. As a counterpart the billionaire philanthropist made the commitment to open a lucrative market for them: "We guarantee a volume of sales," explained Julie Sunderland, the foundation's negotiator. "This enables our partners such as GAVI to save millions of dollars," she added. For instance the price of contraceptive implants produced by Bayer and Merck has been halved. Furthermore the foundation is willing to invest money in startups in order to encourage the emergence of new technologies (Hecketsweiler, 2015 l). Also, academic researchers are invited to participate in the foundation's Grand Challenges in Global Health programme (see p. 182).

### *The figures that support the foundation global aid to public health*

To sum up, after 15 years of existence, the Bill and Melinda Gates Foundation has become closer to the field where action takes place and has therefore entered an era of pragmatism. With an amount of US$32.9 billion allocated as subsidies since the creation of the foundation in 2000, this represents 6% of the global aid to public health. The annual expenses of the foundation have increased from US$1.3 billion in 2004 to US$3.9 billion in 2014 and US$5 billion in 2015, while the annual budget of the WHO was *ca*. US$2 billion in 2015. Regarding the funds allocated to PATH and GAVI in 2014 they amounted to US$278 million for PATH and to US$222 million for GAVI; these are the funds that aim to develop vaccines and health technologies through research and development, and innovation. Between 2007 and 2013 the control of the following diseases received US$0.3 billion for diarrheic diseases, US$0.83 billion for tuberculosis, US$0.92 billion for HIV/AIDS, US$1.05 billion for malaria and US$3.82 billion for neglected diseases, the Bill and Melinda Gates Foundation is the world's leading investor in the research on these diseases, ahead of the pharmaceutical industry (US$2.65 billion between 2007 and 2013). In terms of lives saved between 2001 and 2013 in the case of malaria, the Bill and Melinda Gates Foundation estimated them at 4.3 million; and the number of deaths caused by malaria fell down from *ca*. 50 per 1,000 persons at risk in 2000 to *ca*. 25 per 1,000 in 2014. Regarding vaccination the foundation estimated that its subsidized programmes had saved a total of 3.9 million lives between 2011 and June 2015 (Hecketsweiler, 2015 l).

### *A widening range of action*

The foundation which has started its activities in the research area is now guided by the need to respond to pragmatic issues coming from the grassroots level. Thus Mariam Claeson, who had been working for the World Bank for a long time before joining

the foundation, stated: "Two-thirds of maternal and infant mortality could be avoided thanks to the knowledge we already have, but which is not enough disseminated." The project that is dear to her heart is Mama Ye!, a communication campaign aiming to give concrete advise to African women, e.g.: Are blood donations in their countries sufficient to treat hemorrhages occurring during deliveries? Are there enough middlewives? Is contraception making progress? Is the state investing enough resources in health-care? According to M. Claeson the campaign is sending a strong message to African women: "You have the right to a good health-care," (Hecketsweiler, 2015 l).

Similarly the foundation has been trying to solve such concrete problems as the distribution of drugs through pharmacies. For instance, in Tanzania, a programme of drug certification has been initiated in order to struggle against the production and sale of counterfeit drugs and also to make sure that the patients are correctly advised. Also Bill and Melinda Gates have adopted a more global approach to health. This was illustrated by a US$77-million investment announced by Melinda Gates at the beginning of June 2015, in order to combat malnutrition. "According to an editorial of *The Lancet* (2013) malnutrition causes 45% of the deaths among children under the age of five years; but less than 1% of the assistance for development is devoted to control this rife," explained Shawn Baker who is in charge of this new initiative of the foundation. The latter was expected to fund pilot programmes in a limited number of countries before extending them to whole regions. This will allow the collection of new data on the economic implications of malnutrition. This kind of reasoning has convinced a finance minister to allocate funds to combat malnutrition (*The Lancet*, 2013; Hecketsweiler, 2015 l).

Another World Health Organization?

The widening range of action of the foundation makes it "look like another WHO, but more efficient, with Bill Gates as a global health head of state," commented Robert Sebbag, vice-president of Sanofi department of access to medicines. "But what would be the status of public health without this new actor?," he added. In fact the foundation is filling the "enormous gap" due to the lack of sufficient commitment by the governments. In 2011 according to the data compiled by the Institute for Health Metrics and Evaluation (IHME) the Bill and Melinda Gates Foundation had spent US$2.2 billion in order to fund health-care projects in poor countries. This is much less than the United States, the global leader with US$11.2 billion for health-care assistance, but as much as the United Kingdom and much more than Canada (US$1.2 billion), Germany (US$1.1 billion) or France (US$870 million). In 2015 the foundation was expected to spend US$5 billion, i.e. almost twice the amount allocated by the WHO to this kind of assistance. When she has been interviewed about the "rivalry" between the foundation and WHO, Susan Desmond-Hellmann, the foundation's director, stated: "We stay away from politics and we respect the states' sovereignty." This is not easy and the frontier is sometimes blurred by the very nature of American philanthropy (Hecketsweiler, 2015 l).

## *The Bill and Melinda Gates Foundation and American philanthropy*

According to the Foundation Giving USA American citizens' gifts to charities amounted to *ca*. US$360 billion in 2014. Such kind of generosity, considered nique in the world, is partly channelled through foundations which received US$55 billion. These foundations which are the legacy of the 19th century play a key role in American political life. Olivier Zunz, a historian at the University of Virginia and author of a book on philanthropy in America, explains the role of these foundations when he has been interviewed in June 2015 by the French daily newspaper, *Le Monde*. He recalls that after the Secession War the United States federal government invested a lot of money in the creation of schools in order to promote literacy among the former slaves. But the representatives and senators of the South States did everything possible to oppose such policy. In order to overcome this obstacle John D. Rockefeller, one of the fathers of the great American philanthropy, first created many Baptist schools. With other industry magnates the Rockefeller family financed in 1902 the General Council of Education, with the hope that education would finally eradicate segregation. They also allocated funds to public health and the upgrading of agriculture. Finally the American philanthropists have broadened their action across the world, e.g. to Brazil, Mexico and Ceylon (Sri Lanka) [Hecketsweiler, 2015 l].

Olivier Zunz explains that American philanthropists drew their inspiration from a publication by Andrew Carnegie, the steel magnate. This *Gospel of Wealth* was published in 1889. They nevertheless considered that charity was not to be thrown away and it should be considered as an investment. To that end they have created universities (like Johns Hopkins in Baltimore, Maryland) and launched ambitious research programmes related with tuberculosis, malaria and yellow fever. Later on the Ford Foundation started to use loans (rather than donations) as a leverage of philanthropy and the Rockefeller Foundation had the same approach. The Bill and Melinda Gates Foundation is the heir of such tradition (Hecketsweiler, 2015 l).

In 1913 the income tax was not so high and consequently the tax exemption offered to the foundations did not really matter for the wealthy industry magnates or tycoons who were acting for "the well-being of humankind." By contrast the establishment in 1936 of succession rights that could be as high as 70% has been a key factor in the creation of the Ford Foundation – the wealthiest American foundation before the creation of the Bill and Melinda Gates Foundation. That was the only way for the family to control the corporation while at the same time escaping the succession rights, estimated at more than US$320 million. The heirs of the motor-car magnate played the game fairly and this money was invested in humanitarian causes. O. Zunz also mentioned that foundations had been created just in order to evade taxes. This was severely criticized to the point that some politicians thought of eliminating the foundations in the 1960s. However a middle way has been found: the tax code demands that the foundations should annually spend at least 5% of their capital in charities, otherwise they would lose their status. Regarding their involvement in politics O. Zunz explains that theoretically and by law the foundations must not mingle with politics; this is a counterpart of the tax exemption they enjoy. But in fact American philanthropists do intervene in public affairs. In 1966 the first African American mayor of a big city, Cleveland, had been

elected thanks to a campaign funded by the Ford Foundation. Nowadays the foundations cannot support a candidate, using the same approach, but their "education" campaigns influence public opinion, as also does the research carried out by their think-tanks. This is a kind of lobbying. As mentioned before the Bill and Melinda Gates Foundation is very careful with respect to its independence from the states or governments but it does not hesitate to criticize them or even oppose large international organizations like the WHO (Hecketsweiler, 2015 l).

## Global communication-technology giant corporations: new key players in life sciences, medical biotechnology and health-care

While the information and communication technologies are introducing revolutionary changes in medicine and pharmaceutical research, Google (now Alphabet), Apple, Facebook and Amazon (GAFAs), Microsoft and IBM are increasingly investing in the health-care sector – considered a new Eldorado with a global market valued at US$9,600 billion. For instance Google Life Sciences, a division of Google, has been developing since 2013 technologies for improving disease diagnosis, the coaching of patients and the improvement of their health. "Our principle is to support collaborative work between scientists with different profiles and background. And we are not afraid of the complexity and the huge volumes of data that must be analysed in order to reach our objectives," stated Andy Conrad, the director of Google Life Sciences. Regarding the collaboration between the big pharmas and Google Life Sciences, the complementarity of approaches has been underlined: "Each partner has to do its job. Our strength is above all the knowledge of the diseases and to focus on the patient," said Pascale Witz, in charge of the diabetes and cardiovascular diseases division at the French big pharma Sanofi (Hecketsweiler, 2015r).

In this context it is worth recalling the very relevant comment made by J. Craig Venter in 2007: "For the past 15 years at even faster rates we have been digitizing biology. By that I mean going from the analog world of biology through DNA sequencing into the digital world of the computer. The human genome is perhaps the best example of digitizing biology. Our computer databases are growing faster per day than during the first 10 years of DNA sequencing" (see Sasson, 2011, p.48).

### *Google Life Sciences, Mountain View, California*

In 2014 Google's annual global sales amounted to US$66 billion or €61.3 billion. During the first quarter of 2015 Google's turnover grew only 12%, reaching US$17.3 billion (or €15.8 billion). Despite a strong dollar the profits of the group rose 4%. Google is confronted with a harsher competition from Facebook in the area of mobiles. Furthermore its market shares in mobile advertisement fell down to 38% from 42% between 2013 and 2014 (Hecketsweiler, 2015r).

Objectives of the new venture

After having played the key role of pioneer in the development of Google Car (car without a driver), Google Glass (connected glasses), delivery drones ("Wing" project), or in designing stratospheric balloons in order to connect with Internet the planet's most remote regions ("Loon" project), in 2013 Google stepped into the life sciences

and related biotechnologies. Serguey Brin and Larry Page, Google's founders, consider this is a very promising area and business where they have already invested significant amounts of capital. Moreover S. Brin and L. Page share the utopia of the so-called transhumanists that are influential in California: a human being freed from its biological constraints thanks to new technologies. Their investment fund, Google Ventures, launched in 2008, was managing in 2015 more than US$2 billion, including one-third of this total in health sciences and biotechnologies, with such startups as 23andMe (genetic tests), Foundation Medicine (cancer genetic profiling) and DNAnexus (exchange and analysis of genetic data). Google recruited the geneticist Andrew Conrad as the manager of the group's life-sciences sector. A. Conrad who became a millionaire after selling the start-up he created in the area of blood testing, was leading a team of 150 engineers, biologists, geneticists and physicians within the so-called Google X, an experimental structure of the company (Hecketsweiler, 2015r).

It is above all a unique observatory for identifying the most promising techniques and the most talented scientists. Bill Maris who leads Google Ventures is part of the brain trust of Calico, the very secret California Life Company, created in 2013 by Google. Its research work is led by two "stars" of biotechnology: Art Levinson and Hal Barron who have been working for a long time for Genentech and whose objective is to extend human life expectancy "up to 500 years" (Hecketsweiler, 2015j,r). In an interview with the French daily newspaper *Le Monde* (25 April 2015) Andrew Conrad stated that the main objective of his team is to find the ways and means of detecting the diseases before they are diagnosed. The medicine of the future, in his view, will be based on the continuous follow-up of parameters that we measure today from time to time. "Our objective is to develop measuring tools that are simple, useful and not costly; for instance contact lenses which help measure the concentration of glucose in the blood during the whole day, while at the same time correcting the vision; nanocaptors that help identify cancer cells in the bloodstream; or a spoon that reduces the tremor in people suffering from Parkinson's disease and also enables the physician to follow the evolution of the disease" (Hecketsweiler, 2015k).

Contact lenses measuring glucose in tears

Brian Otis, former professor at the University of Washington, Seattle, joined Google at its Mountain View, California, campus in 2012; he initially expected to spend a sabbatical year, but he stayed there. A specialist of integrated circuits, B. Otis has developed contact lenses that continuously measure the amount of sugar in tears. These lenses might change the life of diabetics who have now to test several times a day blood glucose through punctures into the skin. These lenses are manufactured using a very flexible material and they have a rim containing three tiny squares: a captor, a chip and a battery to supply energy to these extremely miniaturized components for a whole day. A thin golden circle completes the whole set: this is the antenna that transmits the data in real time to a smartphone. B. Otis has been able to develop its lenses in a very short time thanks to a dream team where several scientific, medical and technological disciplines have been combined: from ophthalmology to electrochemistry and through mechanics. "These lenses have been manufactured using a machine-tool entirely created in our laboratory, but as soon as the technology is fully working satisfactorily the production of the lenses will be passed on to an industrialist," stated B. Otis, who also ensured that their price will not exceed a few cents. In fact the industrial partner

has already been found: it is Novartis, the world's biggest pharmaceutical group, who signed a licensing agreement with Google in July 2014, i.e. six months after the first public presentation of the new lens. Google's and Novartis' scientists are now working hand in hand in order to evaluate the reliability of the measures made by the contact lenses – a requisite for obtaining the approval by the FDA (Hecketsweiler, 2015k).

Lift Labo: inventing a "smart spoon" for Parkinson's disease patients

Anupam Pathak is another charismatic researcher of Google Life Sciences. His company, Lift Labo, was acquired by Google in September 2014. He invented an "intelligent" spoon that compensates the tremor of persons suffering from Parkinson's disease. The technology used by this engineer is close to that working in digital cameras and which corrects the natural movement of the operator. This intelligent spoon has been launched at Christmas 2014 and, thanks to Google's marketing force, it was very well received. A. Pathak who emphasized the creativity and the high competence of the scientists working with him within a small team on the Mountain View campus, stated that "they are interested in other handicaps and they have the ambition to correct them and even to increase the capacity of the patients concerned" (Hecketsweiler, 2015k).

Nanoparticles tracking cancer cells

Vicky Delmas who also works at the Mountain View campus was recruited by Google in June 2014, when she was carrying out research at a startup based in Boston. In her laboratory nanoparticles are developed in order to track cancer cells, long before the disease symptoms appear or are diagnosed. "One of the major challenges is to make these nanoparticles invisible, because otherwise the patient's immune system will destroy them," explained V. Delmas. Once their targets are detected these nanoparticles emit a fluorescent signal that is captured by a bracelet. Thereafter another kind of nanoparticles could be targeted at malignant cells and destroy them. On 5 March 2015 Google was granted a patent for this invention by the World Intellectual Property Organization (WIPO). Human trials should follow and to that end Vicky Delmas' laboratory is developing an artificial skin, "whose thickness, grain and colour may interfere with the signal emitted by the nanoparticles," indicated the researcher. She thinks that this technology could be applied to other pathologies than cancer. It could also be used in the Baseline Study – another Google's project aimed at establishing the genetic and medical profile of 10,000 persons (Hecketsweiler, 2015k).

Baseline Study

In his interview with *Le Monde* Andrew Conrad explained that the goal of the Baseline Study, which he is leading, is to follow 10,000 volunteers over a long period, through sequencing their genome, but also collecting all the relevant data on their health status (biological and biochemical tests, clinical data, imagery, heart rhythm, blood sugar concentration, recorded in real time thanks to connected objects). "This is the first time we follow in a comprehensive way what is going on in the human body," indicated A. Conrad. The pilot stage of the project involves 200 volunteers and is aimed at refining the methodology. Thereafter the objective is to recruit 10,000 persons and to associate with partners in order to give a full scale to this study. The first patient was recruited

in February 2015 and three sites for recruitment were opened in the United States and others were planned in Europe, Africa and Asia. From the ethical viewpoint the data collected by Google Life Sciences will not be communicated. The clinical trials are carried out in the associated (partners) universities of Stanford, California, and Duke, Durham, North Carolina. Each patient signs a consent form; he/she will have access to a web site where he/she will be able to share information, talk with a physician, but all this will be done on a voluntary basis. A. Conrad recalled that Google Life Sciences is not risk-averse and is not afraid of making errors from which a lot can be learnt. In Google Life Sciences laboratories a wide range of expertise exists and scientists and engineers with distinct careers are working together, which is not often the case in the more conventional companies or groups belonging to the health sector. Finally Google should cope with the enormous volume of collected data that must be deciphered in order to achieve the goals of the group regarding the early detection, prediction, prevention and therapy of diseases (Hecketsweiler, 2015k).

Partnerships with big pharmas

The partnerships with Novartis and Biogen – an American biotechnology giant – are unavoidable, explained A. Conrad, because Google Life Sciences vocation is not to develop new drugs or manufacture millions of contact lenses. Both Novartis and Biogen have the expertise needed for conducting clinical trials and fulfilling all the regulatory requisites. The tools developed by Google Life Sciences (now called Verily Life Sciences) will bring them important and solid information on diseases and will help them find out new approaches to treatments (Hecketsweiler, 2015k).

Laurent Alexandre, founder of the French start-up DNA Vision, specialized in the sequencing of human genomes and their analysis, stated that "Google's leaders have well understood that the future of medicine relies on the mastering of data and algorithms, and they intend to recoup the power lost by the big pharmas and physicians." Industrialists seem to be puzzled. "It is intriguing, we do not really know what they (Google's leaders) intend to do, and at the same time we raise the question: are not we losing something?" commented Cyril Schiever, the chief executive officer of Merck's French subsidiary. In July 2014, after signing the cooperative agreement with Google, Joseph Jiménez, the CEO of Novartis, said that "this first step beyond the conventional limits of our job is a key stage for us." "One thing is sure," wrote Chloé Hecketsweiler, "Google has not finished to shake the skittles play of industrialists" (Hecketsweiler, 2015k). Thus, on 1 August 2016 was announced the creation of Galvani Bioelectronics – a joint venture between Verily Life Sciences (45%) and the British pharma GlaxoSmithKline (GSK, 55%) – with a view to researching, developing and commercializing bioelectronic treatments, with a €640-million budget over seven years, and a staff of *ca* 30 scientists, physicians and engineers. GSK with an annual turnover of €32.7 billion in 2015, a €4-billion budget devoted to research and development and a staff of 11,000 employees across the world, has been involved since 2012 in bioelectronic implants. The diseases targeted are those which can be treated or mitigated with electric impulses through the nervous system using implants (e.g. asthma, arthritis and even diabetes). The creation of Galvani Bioelectronics confirms that in life sciences and related biotechnologies Google has become an important actor in the research, development and innovation process.

## *Google-Sanofi alliance*

On Monday 31 August 2015 Sanofi announced that it had reached an alliance agreement with Google Life Sciences (Verily Life Siences) on diabetes. Through this alliance Sanofi is trying to master the analysis of medical data drawn from the real life of patients, that has become a vital stake for the big pharmas involved in the treatment of diabetes. By so doing Sanofi hopes to outpace its rivals. In the United States diabetes treatment and care make up *ca.* 10% of all health-care expenses; consequently any solution that may optimize the treatment of the disease will be a competitive advantage. What is at stake is not only to improve the life of patients, but also the management of the health-care systems (Hecketsweiler, 2015q).

The market of antidiabetes drugs is one of the most lucrative and competitive of the pharmaceutical sector. Evaluate Pharma, a consulting firm, estimated that in 2014 the sales of these drugs had brought in a revenue of more than US$41 billion (or €36.3 billion) to the pharmaceutical companies. These sales could reach US$60 billion (or €53 billion) in 2020 for the whole world. Novo Nordisk – the Danish big pharma – is the global leader and its world's market share was 30% in 2015, ahead of Sanofi whose share was 23%. Sanofi's insulin, Lantus, with US$6billion sales in 2014, has lost its patent protection in 2015. Lantus's replacement (newly patented), Toujeo, is not expected to be as successful and it will face the competition of similar drugs developed by Sanofi's rivals (Hecketsweiler, 2015q).

That is why Sanofi wants to rely on the Internet giant in order to improve its competitiveness. The analysis of patients' data can play a key role in this respect. Andy Conrad underlined that "with the emergence of new technologies that allow the continuous follow-up, and in real time, of the patients' health conditions, we can propose more efficient and proactive methods for controlling diabetes." Pascale Witz, who is leading Sanofi's new diabetes and cardiovascular diseases division, explained that, beyond the medicines, the company's challenge is to help patients to better follow their treatment: "Nowadays one patient out of two abandons the treatment during the first year and, among those who carry on, half of them do not reach meaningful results. It is useless to develop new drugs if the patients are not taking their current medicines correctly." John L. Brooks, head of the Joslin Diabetes Center and who is part of the collaboration between Sanofi and Google Life Sciences, explained: "Technology, sensors, digital analyses and solutions are going to drastically change the ways glycemia data are managed. This will result in a better quality of life, reduce the risk of complications, lower the costs and overcome obstacles associated with diabetes care" (Hecketsweiler, 2015q). Sanofi had already acquired some experience in connected or e-health. For instance it designed a glycemia reader that can be connected to an Apple iPhone, as well as different smartphone applications, like the Glucometer. "But technologies are evolving very rapidly and we are not the best developers of them," insisted Pascale Witz. That will be the task of Google Life Sciences within the framework of its alliance with Sanofi (Hecketsweiler, 2015q).

### *Apple*

Next to Google's campus Apple is also becoming a key actor in medical biotechnology and health-care. Launched in 2007 its iPhone is one of the cornerstones of 3.0 medicine. Without the smartphone revolution One Medical – a company created in 2005 in San Francisco – would not have existed. It employs 200 physicians with 35 outlets in San Francisco, Boston, New York and Chicago. "The use of new communication technologies enable the physician to optimize the time spent with their patients and to have a real interaction with them," stated Leah Rothman, a 33-years-old general practitioner who joined One Medical in 2011 and who was tired of the tedious work that is the rule in most physicians' cabinets. Thanks to its success One Medical was able to raise US$117 million (or €105 million), partly from Google (which was renamed Alphabet). In October 2014 Google launched with One Medical an application which allows the patient to chat with a medical doctor. In the Silicon Valley this kind of joint venture is not anymore a surprise (Hecketsweiler, 2015r).

Similarly FitBit – a startup listed on the stock exchange since June 2015 and valued at US$7 billion – is the leader of connected bracelets, with 85% of the American market. The data collected and transmitted by this bracelet include the number of steps made during the day, the calories spent, the sleeping time, the heart rhythm, etc. Linked to an application this electronic coach is used by *ca*. 20 million people (in 2015), and Apple is expecting to be as successful with its iWatch, launched in April 2015. This huge cohort is to be a gold mine for academic researchers and pharmaceutical firms, because Apple iWatch will provide data on the follow-up of patients in "real life." Apple had in fact realized that this challenge could be met and, in June 2014, it launched its HealthApp – a keyboard that centralizes the data collected by FitBit and other connected objects (e.g. balances, tensiometers, glycemia trackers, inhaler machine), as well as by the the iPhone itself. This was not considered another gadget because six months after its launching, in 2015, the Apple Researchkit was presented as a tool aiming at transforming the iPhone into a powerful means of medical research. Scientists could therefore create customized applications for their studies and connect them to the HealthApp in order to collect the data they need (Hecketsweiler, 2015r).

Among the studies already being carried out with the help of Apple Researchkit, "Share the Journey" aims to understand why some patients who have survived after a breast cancer, recover more rapidly than others; "mPower" aims to be the world's largest study on Parkinson's disease. "With the help of the advanced sensors of iPhone we can make a better simulation of a patient's asthma and consequently prescribe a more precised and personalized treatment," underlined Eric Schadt of the Icahn School of Medicine at Mount Sinai Hospital in New York. This kind of prospect is also of great interest to pharmaceutical companies which are strongly requested by the health authorities to precisely demonstrate the efficacy of their drugs (Hecketsweiler, 2015r).

### *Facebook, Microsoft and management of health data*

Facebook is working on the creation of communities of patients as well as on health applications. In 2012 it met with an immediate success when it dealt with donors of transplants. Microsoft has similar tools as Apple: an application christened Microsoft

Health (2014) and a connected bracelet, Microsoft Band. Both of them collect, transmit and store medical data. In 2012 Microsoft and General Electric created Caradigm, a company specialized in the management of health data concerning 175 million patients and 1,400 hospitals across the world. Eric Horvitz, a pioneer of artificial intelligence, is the head of Microsoft Research main centre in Richmond, near Seattle, Washington State. His teams have developed models for the health sector (Hecketsweiler, 2015r).

In an interview with the French daily newspaper *Le Monde*, published on 8 September 2015, E. Horvitz explained how artificial intelligence can help physicians in their daily work. For instance, in order to identify the patients who may be infected by *Clostridium difficile* in hospitals all over the world, Microsoft Research's teams analyzed the data concerning almost 35,000 admissions in a big American hospital, with more than 10,000 parameters related with the data collected at the entry of the patient and during his/her stay in the hospital, and also taking into account informations on the location of the patient's room or the itinerary followed by the patient on his/her way to the hospital. The analysis and crosschecking of all these data led to setting up recurrent schemes and to assigning to every patient a risk factor. This score is a very valuable information for the medical doctor who may decide to apply a specific treatment to the most vulnerable patients (in the United States hospitals, 5% of the patients are infected during their stay with pathogens causing nosocomial diseases). In this respect E. Horvitz made the following statement: "In the United States more than 100,000 deaths per year are caused by errors made in the hospitals, and they can be avoided. Many of these errors could be identified by artificial intelligence systems before they provoke the death of patients," (Hecketsweiler, 2015r).

Therefore the adoption of targeted preventive measures, based on objective criteria, would result in the decrease in the number of deaths and in the savings of billions of dollars. For instance the back-and-forth displacements of some patients, from and to the emergency departments of hospitals, are a good illustration of this approach. A study, published in 2009, showed that 20% of patients affiliated to the Medicare system – the disease insurance system in the United States – returned to the hospital within 30 days after their first admission; this figure rose to 35% during the following three months. The cost for the American taxpayers estimated at US$17 billion (or €15 billion). E. Horvitz explained in the interview with *Le Monde* that in order to understand this phenomenon and even prevent it, his team analyzed the data concerning thousands of patients: more than 30,000 different variables relating to diagnoses, prescribed medicines, results of biochemical analyses, physicians' reports, time spent in the emergency departments, etc. This analysis led to the identification of these variables or data that are correlated with readmissions and to the design of a model which alerts the physicians about the patients at risk. In the case of patients suffering from heart failure E. Horvitz and his colleagues demonstrated that targeted preventive measures lowered the number of readmissions by 30% and total costs by almost 15% (Hecketsweiler, 2015r).

### *Microsoft Research approach to pharmacovigilance*

On the other hand in a study carried out in coordination with the FDA, Microsoft Research scientists in Richmond showed it was possible to develop pharmacovigilance warnings through comparing the requests made by patients on the Internet regarding

the disease symptoms with those about medicines. The scientists chose two drugs that are commonly prescribed: paroxetine, an antidepressant, and pravastatin, an anticholesterol drug. When taken together they can provoke an increase in the concentration of blood sugar. This negative interaction was not known in 2010; but the analysis of the information requests, made by Americans about those drugs, and of the symptoms, have since then shown a clear-cut relationship. In the future these models could complete the FDA analyses in order to anticipate the secondary effects of drugs more rapidly and to make the appropriate recommendations. Another example is that of the study of cooking recipes that are downloaded by Americans from the Internet: it has enabled the scientists to establish a correlation between the quantity of salt consumed and the number of patients suffering from heart failure and admitted in hospitals' emergency departments. Both curves show a good correlation, with peaks during the end-of-the-year festive periods (Hecketsweiler, 2015r).

To sum up, the predictive models, based on the analysis of a huge volume of data, such as the model concerning the evaluation of the risk of readmission into a hospital's emergency department, help the medical doctors to make the best decisions, and they consequently avoid unnecessary expenses in hospitals. Some of these models, such as that on the risk of readmission, are integrated into Microsoft Research software and are working in many hospitals across the world. Other models are still at a pilot stage and they may be added to Caradigm's powerful tools for the management of health-care data. All these models, according to E. Horvitz, will be part of Microsoft's strategy in the health-care sector, as well as in other areas where predictive models can be used (Hecketsweiler, 2015r).

### *IBM Watson artificial intelligence system*

IBM Watson artificial intelligence system is even more ambitious than that of Microsoft Research. IBM Watson group, set up as a separate business in January 2014, has its East Coast headquarters in downtown Manhattan. The company will open a second headquarters for Watson in San Francisco in 2016 and eventually employ several hundred people. At first the company focused on demonstration projects with big companies and institutions, especially in medicine and health-care. But the plan was broadened, especially in 2014, to move beyond custom work for major clients to creating a growing collection of services, so that software developers at startups and elsewhere can easily use them in applications. IBM calls its approach to artificial intelligence "cognitive computing." In 2015 IBM said it had 350 company partners using Watson to make products, with *ca*. 50 services on the market. Some 70,000 software developers, IBM stated, were using Watson technology in some way. Many are in large organizations like ANZ Bank, Johnson & Johnson, the Department of Veteran Affairs, the Mayo Clinic and the University of Texas MD Anderson Cancer Center (Lohr, 2015 ). Watson is also being tested at Memorial Sloan Kettering, a renowned cancer hospital and research centre in New York City. It aims to help physicians to choose the best cocktail of anticancer drugs for each patient.

### *Cloud computing, connected health-care*

A harsh competition is also expected among the information-and-communication technology giants (GAFAs) with regard to cloud computing. Without these data storage capacities and remote-accessible services, it would be useless to sequence

a whole human genome for US$1,000. Because the researchers would be unable to analyze and share the data that can be derived from the sequencing work. For instance J. Craig Venter decided to rely on Amazon's Cloud which has been involved since 2006 in the storage and analysis of data, using very powerful servers that are remotely accessible. He is not the only geneticist to do that: according to analysts the cloud market associated with genomics would reach a value of US$1 billion in 2018, and Amazon and Google would compete for the global leadership. Their servers are already helping scientists to carry out vast studies on cancers, autism and Alzheimer's disease. Some startups are also part of this competition. This is the case of DNA Nexus, created in 2009 by scientists of Stanford University and specialized in the analysis of genetic data. Google is one of the stakeholders of the startup which is also a client of Amazon. David Shaywitz, DNA Nexus medical director, stated that "thanks to Google's or Amazon's servers we can compare genomes, identify variants, detect aberrant sequences or also sort out the patients according to certain characteristics." The estimated market value of connected healthcare or e-health was US$30 billion in 2015: US$19 billion for telemedicine, US$8 billion for mobile applications and US$3 billion for portable objects (Hecketsweiler, 2015r). See p. 113.

## *Conclusion*

The value of the global health-care market is estimated at US$9,600 billion. As mentioned above, Google is ahead of its main competitors in conquering this new and very promising market: the investments of Google Ventures in health have increased from 9% of the total in 2013 to 36% in 2014. "And this is just a small wave, but we are heading for tsunamis," said Laurent Alexandre, a French geneticist who authored a book titled *La mort de la mort* (The Death of Death, JC Lattès ed., Paris, 2011) and founded DNA Vision. According to him "this downpour of data is going to upheave the balance of forces between the big pharmas and the information-and-communication technology giants (GAFAs) in favour of the latter, while physicians may not be able to master the complexity of data. Oncologists will lose their freedom of prescription in about ten years and they will rely on algorithms" (Hecketsweiler, 2015r). Such grim future for the big pharmas may explain the alliance between Novartis and Google in July 2014, followed by similar agreements with the American pharmaceutical company Johnson & Johnson, and with the French corporation Sanofi (see p. 48).

A further step could be the hybridization between humans and machines. This ambition – that of transhumanists – is largely shared by the high-tech giants, even though some of them like Bill Gates have publicly expressed their concerns regarding such likelihood. Laurent Alexandre, who is not a transhumanist, nevertheless stated that "we should prepare ourselves for a real change in civilization. Unfortunately our elites are completely overwhelmed and there is no real debate on these issues" (Hecketsweiler, 2015r).

# FRENZY OF MERGERS AND ACQUISITIONS (M&As) IN THE PHARMACEUTICAL SECTOR AND MEDICAL BIOTECHNOLOGY

## 2015: the year of a big wave of mergers and/or acquisitions in a wide range of industrial sectors

The number and dollar volume of deals (mergers and/or acquisitions) announced in the first half of 2015 have not only surpassed those of 2014, which was a healthy one for corporate transactions by any standard, but they were also on pace to catch up with 2007, the last year of unbridled merger optimism before the financial crisis occurred. Nearly 20,000 deals worth US$2.2 trillion have been announced as of 29 June 2015, according to data from Thomson Reuters and the consulting firm Mergermarket. That was *ca*. 40% higher, in terms of dollar value, than the first half of 2014. And it approached US$2.3-trillion worth of deals announced in the first half of 2007, still remembered as one of the most buoyant times for mergers. Driving much of the activity is the continued consolidation among huge swaths of the industry. The biggest merger announced by mid-2015 was Royal Dutch Shell's US$70-billion takeover of the BG group, a deal that had been rumoured for years. Much of the consolidation in 2015 arose within the worlds of telecommunications and health-care, where many of the big players have felt compelled to become even bigger by swallowing up competitors (De La Merced, 2015).

Investors rewarded many acquirers by pushing up their stock prices after a merger transaction was announced. "Confidence is king, and we are getting closer to normal," analysts at Goldman Sachs wrote in a report on merger activity. Buyers appeared to be more disciplined about the prices they were willing to pay. The price multiple – the ratio of total enterprise value to earnings before interest, taxes, depreciation and amortization – of deals struck in the first half of 2015 was lower on average than at the same time in 2014, at 16.3 times compared with 19.9 times according to an analysis by Standard and Poor's Capital IQ. The lone exception is the health-care industry where the average price multiple was 20.6 times. Among financial advisers Goldman Sachs was leading the group in worldwide activity, with 213 deals worth US$672 billion, as of early 2015. The firm was followed by Morgan Stanley, with US$569 billion worth of activity, and JP Morgan, with US$476 billion (De La Merced, 2015).

Commenting these figures, and particularly the US$2.2-trillion worth of mergers and acquisitions (M&As) by mid-2015, Arnaud d'Aligny, an asset manager at Sycomore Asset Management, stated: "One may think that this is an explosion, but this is above all a period of catching up after several anemic years." After the bankruptcy of Lehman Brothers in 2008 the global economic crisis stopped all mergers of companies.

However, since 2014, the machine has started again, and, as mentioned above, the record figure for M&As in 2007 was almost reached (Cosnard, 2015).

*Examples of big mergers and acquisitions*

The following examples illustrate the size of mergers and acquisitions in 2014-2015, excluding the pharmaceutical and health-care sector:

- Comcast / Time Warner (media/entertainment) US$70.7 billion
- AT&T / Direct TV (media/entertainment) US$67.2 billion
- Kinder Morgan / Kinder Morgan Energy Partners (energy) US$58.6 billion
- City Pacific / Citic (financials) US$ 42.2 billion
- Holcim / Lafarge (cement, construction materials) US$39.5 billion
- Halliburton / Baker Hughes (energy) US$38.5 billion
- Berkshire Hathaway (Warren Buffett) / Precision Castparts (equipment for the energy industry and aeronautics) US$37.2 billion
- Reynolds American / Lorillard (tobacco) US$27.7 billion

(Cosnard, 2015 ).

On 10 August 2015 Warren Buffett's company Berkshire Hathaway announced the acquisition of Precision Castparts – an American equipment manufacturer for the energy industry and aeronautics – for US$37.2 billion (or €34 billion), paid cash. At Wall Street some analysts thought that Warren Buffet overbought Precision Castparts, while disbursing 22 times the net result of a company – a big supplier of Boeing – that was not in its best shape. In fact this purchase by Berkshire Hathaway confirmed that W. Buffet was betting on the American industry. At almost 85 years the most respected American investor – the "Oracle of Omaha" –, considered a model even at Google, triggered through this purchase the feverish trend of mergers and acquisitions for the second half of 2015 (Cosnard, 2015).

In addition to the purchase of Precision Castparts the first days of August 2015 were marked – after a peak in July 2015 with eight operations totalling more than US$10 billion – by the announcement of the acquisition of the American company Baxalta by the Irish laboratory Shire; of an enormous fusion of three Coca-Cola bottling companies in Europe; and the likely merger of two main Italian telecommunications operators. The most obvious facilitating factor of M&As was the availability of money; interest rates have plummeted at extremely low levels. Banks were struggling to lend funds to companies – at least the bigger ones – in order to finance their acquisitions. Investment funds were also waiting for good opportunities and did supply money. The second factor, as mentioned above, was the wish or the necessity to grow for companies and their CEOs. In Europe part of the M&As was related to the lack of economic growth, which induced the consolidation in conventional sectors, such as that of the cement industry. Thus, after the laborious acquisition of the French cement manufacturer Lafarge by its Swiss competitor, Holcim, their German rival Heidelberg Cement was ready to acquire ItalCementi (Cosnard, 2015).

Proactive investors like Bill Ackman or Nelson Peltz were enticing CEOs to participate in the current trend of M&As. And they were indeed more and more powerful: their funds amounted to US$126 billion in 2014, compared with US$32 billion in 2008. Furthermore the prices of shares rose without nevertheless reaching unreasonable levels, so that buyers and sellers could in general find a common ground of understanding (Cosnard, 2015). Coming back to Warren Buffet he described the multiple activities of his conglomerate: "From lollypops to aircrafts." Even though he is present in the banking sector with Wells Fargo or in the insurance business with Geico, the American businessman is increasingly focusing his investments on the industry. Thus the "Oracle of Omaha" had bought the American equipment manufacturer Marmon, the Israeli Iscar and the chemical industry firm Lubrizol. Based in Portland, Oregon, Precision Castparts manufactures several kinds of spare parts and equipments, mainly for the aeronautical industry; the group employed 30,000 persons in 2015 (Cosnard, 2015).

The decisive factor behind the M&As was that companies were once again believing in the future. Despite Greece's economic breakdown and the slump of the Chinese economy (including the devaluation of the yuan in August 2015), the companies' executives wanted to firmly believe that global growth will remain strong and that they will be able to draw profits from the firms they were buying. The overall result was a feverish bust, mainly in the United States and Canada which totalled 42% of the transactions. In Asia (excluding Japan) the activity was also growing, while in Europe the "merger mania" was less important. During the first half of 2015 the M&As amounts even decreased by 8%, compared with the same period in 2014, according to Mergermarket. For instance, in France, they fell down by 44%, showing that the necessary trust was not yet back. The transactions worth mentioning were the purchase of Alcatel by Nokia (Finland), that of Norbert Dentressangle by an American group and the bailout of Areva – the renowned French nuclear firm – by Electricité de France – EDF, the national public producer and supplier of electricity (Cosnard, 2015).

## *Agrifood business*

In November 2014 Joseph Safra, Brazil's richest banker, confirmed he had won the bidding war to acquire the Norman Foster-designed "Gherkin" – the City's of London second-tallest at that time and most recognizable building, resembling a giant pickled cucumber. At a price of £726 million the deal ranked as one of Brazil's 20 largest overseas acquisitions of all time (Pearson, 2014).

### Chiquita Brands Int

Two weeks before the Gherkin's acquisition, and this time in the agribusiness, the Safra Group sealed a joint deal with Jose Luis Cutrale – another Brazilian billionaire – to buy the American banana company Chiquita Brands Int., outpacing the Irish company Fyffes that also coveted the banana firm. The Brazilians disbursed US$681 million and

also took the burden of the company's debt; thus Chiquita Brands' value was estimated at US$1.3 billion – the biggest United States purchase by a company from a Latin American country in four years. This acquisition was considered emblematic because Chiquita Brands, Fresh Delmonte and Dole were the iconic American trio that had dominated the global banana market for a long time (Pearson, 2014; Girard, 2015b).

AB InBev

Brazil's richest man, in 2015, Jorge Paulo Lemann who has been ranked by Forbes magazine as the 26th richest person in the world, has orchestrated in 2008 the US$52-billion takeover of Anheuser-Bush (Budweiser) with the Belgian/Brazilian brewer Interbrew. This was done after buying such brands of beer as Brahma and founding the Brazilian company Ambev. J.P. Lemann who started his career as a banker operated with two Brazilian associates, Marcel Telles and Carlos Alberto Sicupira, ranked by Forbes as the 101st and 122nd richest persons in the world. The three billionaires participated in the creation in 2008 of the global leader in beer production and sales, AB InBev, whose chief executive officer is the Brazilian Carlos Brito – a former employee of Brahma and thereafter of AmBev (Girard, 2015b).

Fast food; meat market

In 2010 J.P. Lemann and his two associates bought Burger King for US$3.25 billion. In October 2014 they used the fast-food business to launch the US$11.4-billion takeover of Canada's baked-goods market leader, Tim Hortons. A year before (2013) the private equity firm 3G Capital Investimentos belonging to J.P. Lemann, M. Telles and C.A. Sicupira, made its most headline-grabbing deal to that date: teaming up with Warren Buffet's Berkshire Hathaway to buy Heinz – the American food company – for US$ 28 billion (Pearson, 2014).

Another example of the Brazilian spirit of purchasing agrifood companies was that of JBS – a firm that bears the initials of its founder, José Batista Sobrinho. The butcher enterprise, created in 1953, became a leader on the global meat market. Led by Wesley Batista, the son of the founder of JBS, the value of the company was estimated at US$37 billion in 2015 and it was among the top 10 agrifood companies. It was just ahead of its American rival, Tyson Foods, and it has grown rapidly thanks to several purchases, supported by Brazil's public banks: e.g. the Brazilian activities of the French chicken producer Doux; the chicken-production branch of the Brazilian Marfrig; and above all the United States leader of chicken production Pilgrim's Pride. All these purchases amounted to US$17 billion, with an increase in the company's overall debt. In the meantime Tyson Foods acquired in 2014 the hot-dogs manufacturer, Hillshire Brands, for US$8.5 billion. Similarly, in 2013, the Chinese investment fund Shuanghui International Holdings bought the American cooked-meats company Smithfield Foods for US$7.1 billion (Girard, 2015b).

### Heinz / Kraft merger

All these deals that have shaken up the global agrifood industry were expected to continue. Thus on Wednesday 25 March 2015 was announced the merger of Heinz and Kraft – two giants of the food industry. This merger will give birth to the world's fifth-largest food group. This US$70-billion combination of Kraft and Heinz was considered a very big deal, behind which were Heinz's owners, Berkshire Hathaway (Warren Buffet's fund) and the Brazilian private investment group 3G Capital Investimentos (J.P. Lemann, Marcel Telles and C.A. Sicupira). The deal was another evidence of Brazilian investors' appetite for global agrifood companies (Girard, 2015b).

Kraft's stock surged 36% on the day the merger was announced. The Heinz-Kraft merger is notable in that it is specifically structured to keep Berkshire Hathaway and 3G in control. That is the reason for the added US$10 billion, or US$16.50 a share, dividend, financed by Berkshire and 3G. This kept 3G and Berkshire combined ownership of the new company at 51%. After the end of the transaction 3G and Berkshire will have control over the new company, christened Kraft Heinz Company. According to reports W. Buffet was expected to appoint three directors and 3G another three. Five other directors were expected to be appointed by Kraft. The shareholders of Heinz proposed that the current chief executive officer of Heinz, the Brazilian Bernardo Hees, former CEO of Burger King, be appointed as the CEO of Kraft Heinz Company. His main objective was to make huge cost cuts, so as to reach the US$1.5 billion in annual cost savings that Heinz expected. Numerous lay-offs were foreseen and that was one of the reasons why W. Buffet accepted to partner with his Brazilian associates. The parties agreed that the headquarters for the combined company were both Pittsburgh and Chicago for the time being. Both companies are in the packaged food business, so antitrust issues may arise in this transaction. Heinz (which is private and whose shares do not trade in the market) agreed to take all steps to clear the transaction with the antitrust authorities (Solomon, 2015).

### Agrochemicals and seeds

Another big acquisition at the beginning of 2016 was that of the Swiss agrochemical and seed company Syngenta by the Chinese industrial group ChemChina. The latter, a public company, was offering US$43 billion (or €40 billion) for this transaction and the offer was unanimously approved by Syngenta's governing board. The announcement was officially made on 3 February 2016. If the acquisition becomes a reality it would be the biggest acquisition ever made by a Chinese company overseas. ChemChina, valued at US$39 billion in 2014, was not a newcomer on the European market. In 2006 it bought Adisseo, a feed company and a subsidiary of the French chemical group Rhône-Poulenc, as well as the silicon activity of Rhodia, also a French company. In 2015 ChemChina purchased the Italian tyre manufacturer Pirelli and later on it acquired with other investors a renowned German machine manufacturer, KraussMaffei, for €925 million. Also, ChemChina had a participation in the Swiss trader Mercuria. In 2011 ChemChina's agrochemical subsidiary merged with the Israeli company Makhteshim Agan Industries (phytosanitary products) and it disbursed for that purpose €2.2 billion (Girard, 2016).

To acquire Syngenta ChemChina proposed to the Swiss company's shareholders a price of US$465 per share in cash, which meant a 20% premium over the closing stock-exchange rate on Tuesday 2 February 2016. The Chinese group also committed itself to keep the management team of Syngenta, as well as four members of the governing board. This acquisition highlights the strategic importance of agriculture in China: feeding the world's largest population is a crucial challenge. This issue is a daunting one, not only because enough food must be provided, but also because eating habits are changing among Chinese middle classes (e.g. more dairy foods and meat have to be supplied). China has become the biggest importer of agrifood products and consequently it plays a key role in their price fluctuations (Girard, 2016).

China also acquired several food or agrifood companies and tried to be involved in the international trade of agricultural products. Thus, in 2013, the Chinese WG Group, a company that produces and commercializes pork, acquired its American rival, Smithfield Group, for US$4.7 billion. The Chinese large public conglomerates are mostly involved. For instance Cofco, the largest agrifood group, acquired 49% of the equity of the agricultural activity of the trader Noble, for US$750 million. Cofco's ambition is to compete with such big traders as Cargill or ADM: this was the aim of combining Noble Agri – its acquisition – with another acquisition, that of Nidera, a Dutch seed corporation. Cofco is also very active in the areas of dairy product and children's nutrition. Cofco signed a partnership agreement with Danone which owns 9.9% of Cofco's subsidiary Mengniu – a dairy product company – and 25% of that of Yashili, specialized in foodstuffs for young children. Danone gave its trademark Dumex to Yashili (Girard, 2016).

Western companies operating in China are often confronted with the measures taken by government authorities to protect national interests. That was, for instance, the case of the French seed cooperative Vilmorin – the world's fourth biggest seed firm – which had to face China's protectionism. Implanted in China since 1997, Vilmorin had to give up its shareholding in the Chinese leading firm in hybrid rice, LongPing, in 2009. This firm was considered a strategic one and should remain in the exclusive hands of Chinese companies. Since then Vilmorin has tried to set up collaboration with another company in order to be able to produce wheat and maize varieties (Girard, 2016).

With the acquisition of Syngenta ChemChina will become one of the most powerful global suppliers of seeds, pesticides and fertilizers. "This might also open the GM-seed market to China," stated Emmanuel Rougier, deputy director-general of Vilmorin. Syngenta is indeed very present on this market. Furthermore this acquisition occurs when the agrochemical sector, as a whole, is being consolidated. Two giant chemical manufacturers in the United States, DuPont and Dow Chemical, announced their merger in December 2015. After the merger the new company was expected to split into three entities, one of them being specialized in agrochemistry. This consolidation of the sector has to do with the plummeting prices of cereals that will subsequently have an impact on the costs of seeds, pesticides and fertilizers (Girard, 2016).

According to the data published on Wednesday 3 February 2016 Syngenta's annual turnover decreased by 11% in 2015, reaching €12 billion; and the company's net profit fell down to €1.2 billion (-17%). The Swiss group was therefore going through a difficult period and it was very coveted. In 2015 Monsanto tried several times to acquire its competitor but failed to do so. Monsanto had also to face hardships and it had to reduce the size of its staff by 16%. ChemChina was therefore more successful than the global leader of GM-seeds in acquiring Syngenta (Girard, 2016). But Monsanto, after four months of negotiations, was acquired by Bayer – the German pharmaceutical and agrochemical company – for US$66 billion on 14 September 2016, pending the approval of the deal by authorities in 30 different country jurisdictions.

## *Mergers and acquisitions in the pharmaceutical sector and health-care*

Health-care companies of all kinds – drug makers, medical biotechnology firms and big pharmas, hospital groups and insurers – have been frantically circling to be sure they are not left out of the frenzy of merger-and-acquisition deals since 2013. A study by Ernst and Young – a consultancy firm – estimated at US$200 billion (or €186 billion) the amount of the mergers/acquisitions in 2014 in the pharmaceutical sector. In the case of generic drugs the amount of M&As was estimated at US$100 billion, according to Bloomberg. This figure was five times higher than the annual figure recorded for this sector since 2005 (De Vergès, 2015). The activity has been dizzying among pharmaceutical companies. Also the biggest American health insurers have been circling one another, eager to find a merger partner to help cut costs and bolster their presence in lucrative areas like Medicare. Thus Anthem has proposed buying Cigna for US$47 billion; Humana has been weighing takeover approaches from the likes of Aetna and Cigna. Adding special urgency to their deliberations was the possibility of being left out, since government regulators were likely to allow only some, not all, efforts among health insurers to combine (De La Merced, 2015).

As explained in more detail later on (see p. 63) some M&As among pharmaceutical companies are worth mentioning. In November 2014 Actavis tried to acquire Allergan, the Botox manufacturer and to sell its generic business to Teva Pharmaceuticals (Israel) for *ca*. US$45 billion (see p. 64). Then there were the mergers that were not: AbbVie, a drug maker, dropped its deal with Shire of Ireland, at least temporarily ending the flurry of "tax inversion" mergers that had occurred as a way to cut tax obligations by merging with an overseas company. However talks between Pfizer and Allergan came to fruition by the end of November 2015 and became the year's biggest merger. Pfizer's acquisition of Allergan represented a return to American drug makers' strategy of finding base overseas. Thus Pfizer would move its headquarters to Dublin where are located those of Allergan, so as to pay less taxes in the United States (see p. 63) [Abelson, 2015].

The reasons and factors behind the M&As in the pharmaceutical sector

Low interest rates and cheap capital are fueling merger activity across many industries, as described above, but health-care is especially devoted to the mantra that bigger is always better. And there are both the short-term goal of increasing revenue and the longer-term need to restructure in response to changes in the health-care landscape in

the United States under the Affordable Care Act (President B. Obama's reform of the health-care system in the United States). Unlike other areas, like telecommunications, where a tremendous volume of consolidation had already occurred, health-care was fragmented with many small players (Abelson, 2015).

All of the parties are under pressure to reduce costs and consolidation is seen as necessary to do so, especially among small entities like hospitals. Politics aside, the United States Affordable Care Act is credited with accelerating the pace of change. It has forced health insurers and hospitals to try to reduce costs. On the other hand consumer advocates and others worry that some of the big deals, including those proposed by large for-profit health insurers, will not benefit consumers. They say competition could decline significantly in some businesses, like private Medicare plans, in some local markets where they may already be powerful players. Consumers may pay more because, without competition, insurers could raise premiums or hospitals could raise prices. While the overall state of the economy and other factors could curb the enthusiasm for deals in the United States, health-care companies are likely to pursue them: "Our view from the ground is that the pace will continue," stated Torrey McClary, a lawyer for Hogan Lovells who specializes in mergers (Abelson, 2015).

With respect to the pharmaceutical industry the M&As reflect the difficult conditions to which big pharmas and medical biotechnology companies are confronted worldwide. Firstly the development of new drugs demands very large financial investments: the big pharmas invest 10% to 12% of their annual turnover into research and development (R&D); in addition longer periods are necessary for developing these drugs, from seven to ten years, and the failure rate is high. R&D activities should be carried out in close cooperation with public hospitals and research centres that are involved in the clinical trials aimed at demonstrating the innocuity and efficacy of the drugs being tested. The last issue has to do with intellectual property rights: new drugs are protected for a limited period beyond which the patent is no more valid, and consequently generic drugs can be produced by competitors. Henceforth the need for the big pharmas to steadily feed their innovation pipeline. Furthermore, as the stakeholders of big pharmas request high and regular dividents, these companies should either reduce their R&D activities, select the most profitable products in the short term and buy innovative drugs from other companies or acquire biotechnology startups; or, conversely, intensify and broaden their R&D activities, as well as optimize their management policies (Hatchuel, 2014).

For instance Pfizer and AstraZeneca, each, for several years, have been adopting one or the other of both strategies. Pfizer reduced its R&D activities and made big acquisitions. The company lost the patents protecting some of its blockbuster drugs: Lipitor, a cholesterol-lowering drug, the annual earnings of which could reach up to US$13 billion; Viagra, Lyrica (an antiepileptic drug) and Celebre (a painkiller), with sales amounting to US$2.9 billion in 2013. The British AstraZeneca devoted up to 18% of its annual turnover to R&D activities. For instance its cholesterol-lowering drug Crestor – the largest-selling drug of this category in the world – brought in

US$5.6 billion in 2013, while patent protection was expected to last till 2016 in the United States. Moreover AstraZeneca had several innovative drugs in the pipeline, particularly against respiratory diseases and cancers. Pfizer was interested in acquiring AstraZeneca for US$118 billion by mid-2014. Through such a purchase the American big pharma wanted to broaden its innovative drug portfolio, reduce its R&D spending and optimize its tax bill by moving its headquarters to the United Kingdom, thereby using funds that would be taxed if the company remained in the United States. Pfizer offer was rejected in England and Sweden – the countries of origin of AstraZeneca – because the merger might lead to a dramatic decrease in the British company R&D activities, with the layoff of highly qualified staff and a negative impact on medical research in both European countries. After rebuking Pfizer AstraZeneca's leadership had to promise much higher profits to its stakeholders in the forthcoming years. That was a bet on the future which would depend on the commercialization of innovative drugs. Or would AstraZeneca have to reduce its R&D endeavours and finally adopt a development model close to that of Pfizer, i.e. based on short-term profits (Hatchuel, 2014).

Thinking beyond the example of a possible merger between Pfizer and AstraZeneca, Hatchuel (2014) raises the issue whether enterprises still bound to a high level of R&D might become an easy and attractive prey for those which are not so much interested in investing in R&D. He also wonders whether these big pharmas should adopt a distinct governance that aims to protect investment into research as a priority commitment which much be respected by any new stakeholder. Nowadays there exist, like the flexible corporation or benefit corporation in the United States, several legal forms of corporation that aim to protect a mission or values approved by the stakeholders against any takeover. According to A. Hatchuel their possible adaptation to the pharmaceutical sector should be studied because what is at stake is health-care for all (Hatchuel, 2014).

## Outstanding mergers and acquisitions

"Clearly, you have an 'up' year in (mergers and acquisitions – M&As –), but in particular the vertical trend in the pharmaceutical sector has been extraordinarily robust," stated Phillip Torrena, a partner with Honigman, Miller, Schwartz and Cohn, who specializes in mergers and acquisitions within the medical device and life sciences sector. "I think there is a lot of issues that many companies are facing with patents rolling off and finding ways to increase their pipeline," he added. "And the only way to do that is to continue to get bigger and bigger," he concluded. Actually a wave of tie ups among companies occurred in 2014-2015. For instance the German chemical and pharmaceutical Bayer announced in May 2014 that it was buying Merck's consumer-care business for US$14.2 billion. On 26 February 2015 the company announced that its turnover in 2014 had reached another peak to €42.2 billion (+5.2% compared with 2013 turnover); its net profit also climbed 7.4% to reach €3.4 billion. In April 2014 Novartis, GlaxoSmithKline (GSK) and Eli Lilly joined forces in a US$28.5-billion deal. Pfizer had acquired Wyeth in a US$68-billion deal in 2008, and Hospira, a maker of generic treatments, for *ca*. US$17 billion in 2015 (Bray, 2015). Thereafter, followed the Pfizer-Allergan merger on 23 November 2015.

## *The Botox story*

Botox has been known for a long time: it is the botulin toxin produced by anaerobic bacteria belonging to the species *Clostridium botulinum*. It provokes the paralysis of muscles. First used to treat eye illnesses such as strabism, it has become since the 2000s a widely used product in esthetic or plastic surgery, e.g. to eliminate wrinkles. Botox was approved for cosmetic use in 2002 by the FDA. It has been advertised by Hollywood stars such as Nicole Kidman who was nicknamed "Frozen Face", further to multiple injections of Botox. In 2013 the American actress acknowledged that she used too much of the Botox treatment. The global market of Botox kept growing, mainly thanks to the consumption in the United States: according to Mathieu Chabert, analyst at the bank Bryan Garnier, "the value of the botulin toxin market is estimated at US$3 billion and it should expand at an annual rate of 10% due to new indications or treatments in both medicine and cosmetics (Hecketsweiler, 2014b).

Alan Scott – Botox inventor – sold it to the American pharmaceutical company Allergan in 1991 for only US$4.5 million. In 2012, in an interview with the *Times of India*, he confessed that he deeply regretted the deal: "If I had kept my intellectual property rights, I should have become a billionaire. When I developed the product, I knew that it could have outstanding applications in the treatment of neurological disorders. But I had no idea whatsoever about its use in esthetic surgery and cosmetics". With global sales of *ca*. US$2 billion Allergan's Botox is the unrivalled blockbuster, but it is challenged by several competitors. The most important threat comes from the French laboratory Ipsen which sells a product called Dysport whose sales amounted to a modest €240 million in 2014. But Ipsen is working hard to catch up thanks to developing other promising medical applications of the drug, for which it has filed eight patent requests. It should be recalled that the botulin toxin, in addition to its medical and cosmetic applications, is a formidable biological weapon: tiny amounts can cause lethal neurological disorders. That is why Ipsen's factory located in the United Kingdom is under the permanent surveillance of the army. The acquisition by Ipsen of the British company Syntaxin in a deal amounting to €28 million may give the French laboratory a competitive advantage, because Syntaxin has a very rich portfolio of toxins; the latter, produced by genetic engineering, could be patented, while natural botulin toxins cannot; only their applications can be protected by patents (Hecketsweiler, 2014b).

The American big pharma Johnson & Johnson had also participated in the "Botox war" when it acquired in December 2008 the laboratory Mentor for US$1.1 billion. Mentor was developing a product called Purtox, considered as the future competitor of Allergan's Botox. But the investments needed for manufacturing the product discouraged Johnson & Johnson and in April 2014 it withdrew from the race and decided to focus on another booming market, that of breast implants, where it was already competing with Allergan (Hecketsweiler, 2014b). Bill Ackman, the American billionaire who owned 10% of Allergan capital, has been trying to facilitate a deal through which the Canadian company Valeant Pharmaceuticals could acquire Allergan for US$53.5 billion (or *ca*. €39 billion). Valeant was acting with the help of Ackman's

Pershing Square Capital Management Fund. On 22 August 2014 the American businessman requested a meeting of Allergan shareholders before 20 December 2014 in order to make a decision regarding Valeant's offer. On 27 October 2014 Valeant raised its offer for Allergan to US$200 or more per share. Valeant wanted to acquire the blockbuster Botox in order to treble its size in 2016 from a stock value estimated at US$50 billion in 2014 (Hecketsweiler, 2014b).

On Monday 17 November 2014 the American pharmaceutical company Actavis announced that it will acquire Allergan in a US$66-billion deal that thwarted Valeant's endeavours to purchase the Botox manufacturer through a hostile takeover. The acquisition was valued at US$219 per share paid in cash and with Actavis shares. "We will create a top ten pharmaceutical company, generating more than US$23 billion annually," stated Brent Saunders, Allergan's chief executive officer, in a conference call with investors. Company officials spent the following months seeking various regulatory approvals. The acquisition was expected to be finalized during the second quarter of 2015. The new company would have annual synergies estimated at US$1.8 billion or more starting in 2016, with a continued US$1.7 billion investment in research and development (Snider and Shell, 2014).

### *Pfizer-Allergan merger*

On Monday 23 November 2015 Pfizer stated that it had struck a US$160-billion deal to merge with Allergan, the manufacturer of Botox based in Dublin. This was one of the biggest takeovers in the latest and largest deal aimed at helping an American company lower its taxes by reincorporating overseas (in addition to increasing its innovation rate, better facing competition from rivals and reducing costs). The agreement was also considered the biggest deal in what has been a banner year for mergers, driven in part by consolidation in the health-care and pharmaceutical sectors. It should be underlined that merger and acquisition activity *worldwide* surpassed US$4 trillion by mid-November 2015. In the United States, according to a tally by Mergermarket, mergers and acquisitions worth *ca*. US$270 billion were announced in the first nine months of 2015 (Abelson, 2015 ; Bray, 2015).

The Pfizer-Allergan transaction which includes debt, would be structured as a so-called reverse merger, in which Allergan, the smaller of the two companies, would technically be the buyer. Allergan has its headquarters in Dublin but the bulk of its operations are based in Parsipanny, New Jersey, allowing the deal to sidestep rules introduced in 2014 to deter some companies from pursuing such deals, called "inversions" or "tax-inversion" mergers. President Barack Obama called these inversions "unpatriotic". His administration tried to crack down on the strategy in 2015, with the Treasury Department and the Internal Revenue Service announcing additional rules by mid-November 2015 meant to further restrict the practice. The United States government had already lost billions of dollars in tax revenue from inversions. Treasury Secretary Jacob J. Lew stated by mid-November 2015 that the department would make every

effort to end the use of inversions – and planned further rule changes in the coming months -, but he also urged Congress to act. In 2014 Pfizer's tax rate was *ca*. 26.5% and it was expected to be *ca*. 25% in 2015. By comparison Allergan reported a tax rate of 4.8% for 2014 and was expected to have a tax rate of *ca*. 15% in 2015 (Bray, 2015).

Pfizer will lead the combined company which would have US$60 billion in combined sales and a product portfolio that includes Viagra, Celebrex, Botox and the cosmetic treatment Juvéderm. The combined company would be named Pfizer and be domiciled in Ireland. Its global operating headquarters would be in New York and its main executive offices would be in Ireland. Jan Read, the Pfizer's chief executive, would be the chief of the combined company, while Brent Saunders, Allergan's chief executive, would serve as president and chief operating officer. The combined company's board would comprise 15 directors with Pfizer's 11 current directors and four directors from Allergan. The transaction, which requires shareholder and regulatory approval, was expected to close in the second half of 2016, but could face stiff opposition from lawmakers in the United States. Under the terms of the all-share deal, Pfizer would pay US$363.63 for each Allergan share, representing a more than 30% premium to Allergan's share price in late October 2015 before news emerged that they were in talks. Allergan's shareholders would receive 11.3 shares of Pfizer for each share of Allergan they hold. Pfizer's shareholders would receive one share in the combined company for each share they hold, but have the option to take up to US$12 billion in cash for some or all of their shares instead. After the transaction Pfizer's shareholders were expected to own *ca*. 56% of the combined company, with the remaining 44% owned by Allergan's shareholders (Bray, 2015).

The transaction is contingent in part on the completion of Allergan's pending divesture of its generics business to Teva Pharmaceuticals (Israel), which was expected to be completed in the first quarter of 2016, in a *ca*. US$45-billion deal. The companies stated that they expected to make more than US$2 billion in annual cost savings over the first three years after the deal closes. By acquiring Allergan Pfizer was expected not only to save on its overall tax rate, but it would also be better able to use earnings from its international operations for additional acquisitions or other activities. Under current rules Pfizer must pay American corporate taxes on the billions of dollars in earnings from international operations if it ever tries to bring the money back to the United States, restricting its ability to use that money for certain corporate functions. The company kept US$74 billion in earnings offshore in 2014 to avoid that bill (Bray, 2015).

The Pfizer-Allergan deal could be a precursor to Pfizer eventually being split in two. Pfizer has discussed whether to become two companies, one dedicated to higher-growth, brand name treatments, and one focused on slower-growing mature drugs that face pressure from generic counterparts (Bray, 2015). Robert Cyran, while acknowledging that the Pfizer-Allergan merger could pay off in terms of tax benefit and cost savings, considered that it could carry adverse effects. Firing lots of workers, as a great part of savings, can dishearten those who remain. Worse, laboratory productivity may not improve. The result is often a disappointing pipeline of new therapies – and stagnating

share price. Splitting Pfizer in two companies could help reduce bureaucracy in the new firm but permanent change may not help employees get on with their jobs (Cyran, 2015). *The Pfizer-Allergan deal was suspended in April 2016* further to the federal government's reaction regarding the tax inversion scale of the proposed merger.

### *Other acquisitions in the United States*

On 4 March 2015 the American pharmaceutical company **AbbVie** announced it had agreed to buy the California-based small-sized pharmaceutical firm Pharmacyclics, making a cancer drug that some analysts predict will eventually become one of the best-selling treatments for the disease, for *ca*. 21 billion. The deal was the first by AbbVie since its aborted attempt in 2014 to acquire Shire, an Ireland-based drug manufacturer. Under the terms of the deal AbbVie was to pay US$261.25 per share in cash and stock. AbbVie, a medium-size pharmaceutical firm with 26,000 employees at the time of the deal, needed to diversify because more than 60% of its annual turnover (US$20 billion in 2014) came from the sales of the drug Humira, a treatment for rheumatoid polyarthritis and of various autoimmune diseases and one of the world's best-selling medicines. But Humira was to lose patent protection in 2016 in the United States and in 2018 in Europe, and was expected therefore to face competition from biosimilars. AbbVie was developing a drug called ABT-199 with Roche's Genentech that would treat some types of blood cancer (leukemias). But instead of massively investing in research in order to find new medicines AbbVie's strategy rather focused on buying young pharmaceutical or biotechnology firms that had or expected to have in the short term new drugs (De La Merced and Pollack, 2015).

That was the case of Pharmacyclics. Based in Sunnyvale, California, Pharmacyclics – a small-sized firm with 600 employees in 2015 – focuses on anticancer drugs. Its best-selling product, Imbruvica, authorized in 2013, reached net sales of US$548 million in 2014, with US$492 million of that in the United States, Pharmacyclics announced. The company's chief executive, Robert W. Duggan, had no experience in pharmaceuticals when he took over the company in 2008 (a year in which the stock plummeted below US$1 a share), but he had become an investor in Pharmacyclics in 2004 because he had a son with a brain tumour. He predicted that sales of Imbruvica – used to treat leukemias and not brain cancers – in the United States were to reach US$1 billion in 2015. And some analysts predicted that the drug would eventually reach US$3.5 billion in annual rates in 2018. A one-month treatment with Imbruvica could cost US$9,000 or more. Pharmacyclics revenue was US$370 million in 2014, compared with US$260 million the previous year. Johnson & Johnson has co-developed Imbruvica with Pharmacyclics and co-markets it in the United States and sells it abroad. It was considered the lead bidder for Pharmacyclics, but AbbVie was finally the buyer of the company. In a statement on 4 March 2015 Johnson & Johnson said: "We are looking forward to continuing our collaboration with the team at AbbVie to further develop and commercialize this important therapy for patients and their health-care teams." There was also some early evidence that the drug co-developed by AbbVie and Genentech – ABT-199 – could be used together with Imbruvica (De La Merced and Pollack, 2015).

The deal made by AbbVie in buying Pharmacyclics for US$21 billion raises the question: Why investing such a huge amount of money in just one molecule (Imbruvica)? In addition to the inflationist impact of this kind of strategy on the cost of the marketed drugs, one may raise the issue of the lack of risk distribution. Outstanding research in order to benefit from the best innovations can be justified; but if it triggers a rocketing speculation this strategy appears less meaningful. The molecule that was initially developed into Imbruvica had been acquired by Pharmacyclics more than ten years ago for US$6.6 million. This strategy indicated there was an ongoing transformation of the so-called pharmaceutical laboratories into pharmaceutical groups. That was the case of AbbVie's deal (Jacquin, 2015).

In November 2014 Bill Ackman's Pershing Square Capital Management took an 8.5% stake in **Zoetis**, the animal vaccines and medications business spun off from Pfizer in 2012. Another hedge fund, Sachem Head Capital Management, run by former Ackman's employee, Scott Ferguson, acquired a further 1.6%. Both investors were leading a US$2.2-billion raid on Zoetis so that a bigger company's management could strip out costs. Zoetis' chief financial officer, Paul Herendeen, stated: "We are a public company. We are for sale every day. And to the extent someone were to come in and be interested they could do a better job with our collection of assets; there is certainly the opportunity for them to express that." Shares of Zoetis rose nearly 9% to close at US$43.72, valuing the New Jersey-based company at *ca*. US$22 billion on 12 November 2014. Zoetis sells more than 300 lines of products to livestock producers and veterinarians across 70 countries (Foley and Platt, 2014).

**Salix Pharmaceuticals**, based in Raleigh, North Carolina, develops and markets treatments for gastrointestinal diseases, including the drugs Apriso, Fulyzag and Giazo. The company reported a net income of US$143 million in 2013. It struck a deal in July 2014 to acquire the Irish arm of an Italian drug manufacturer, becoming one of the American companies to seek to move its headquarters abroad for tax purposes. Under the deal Salix Pharmaceuticals merges with Cosmo Technologies, the Irish unit of Cosmo Pharmaceuticals of Italy, in exchange for a stake of more than 20% in the combined merger. Salix Pharmaceuticals was therefore expected to reincorporate in Ireland which will allow it to save millions of dollars in corporate taxes. The combined company was also expected to seek to be listed on the Nasdaq (Bray, 2014).

### *Roche*

A very successful big pharma

The Swiss pharmaceutical group Roche, at the third rank on the list of the top ten big pharmas in the world in 2014, has been and is experiencing the strategies of mergers and acquisitions of successful biotechnology companies and start-ups. For many years it owned a majority stake in Genentech, the San Francisco Bay Area-based American biotechnology firm, that developed Roche's "blockbuster" drugs: the anticancer medicines Avastin, Herceptin and Rituxan. These have contributed to make Roche the most "expensive" pharmaceutical firm in recent history, with an estimated stock value of more than €200 billion in 2014 – on equal footing with the other Swiss big

pharma, Novartis. These medicines, produced by genetically engineered bacteria or by hamster cells, and targeted against several types of cancer, made the fortune of Roche whose 2014 turnover, published on 28 January 2015, increased 1% to reach the figure of 47.5 billion Swiss Francs. Roche whose chief executive officer has been since 2008, Severin Schwan, a 47-years-old Austrian executive, decided to acquire in 2000 all of Genentech, a move that was resisted by Genentech's management at that time and led to departures of some top executives. Genentech was founded in 1976 by the American biochemist Herb Boyer and the business angel Bob Swanson. Forty years later Genentech and Roche are in the heart of the process that is leading to the medicines of the future; they are asking themselves: what is next? In a very competitive environment, at the crossroads of scientific and technological innovation, and big business, they must find the clues for even more targeted diagnostic tools and therapies (Hecketsweiler, 2015d; Pollack, 2015a).

Acquisitions and partnerships

"About one-third of our products are derived from partnerships and acquisitions, and 99% of the research is carried out outside our campus," stated S. Schwan. And in 2014 Roche announced several deals aimed at broadening its drug portfolio. By the middle of the year the group acquired Genia, a Californian startup specialized in DNA sequencing and based in Mountain View, where Google is also headquartered. Genia is developing a technology that aims at simplifying and lowering the cost of genetic tests. In this area the global leading company is Illumina, based in San Diego, California, which Roche could not acquire in 2013 (Hecketsweiler, 2015d; p. 124).

Also by August 2014 Roche was willing to acquire 100% of the equity of the American firm InterMune, specialized in the treatment of respiratory diseases. The drug developed by InterMune against idiopathic lung fibrosis – a rare disease of which the main symptom is a gradual difficulty in breathing normally – called pirfenidone, was expected to be very costly because there is no alternative therapy. In the United States alone where 48,000 patients are suffering from this disease, the annual sales of the new drug would amount to *ca*. US$1 billion. In order to acquire InterMune, Roche did not hesitate to grant the shareholders of the company a 38% premium over the share price. Furthermore the drug, marketed under the name of Esbriet, will allow Roche to complete its portfolio of drugs against respiratory ailments, while its products Xolair and Pulmozyme, against asthma and cystic fibrosis, respectively, were already ten years old (Hecketsweiler, 2014d). Earlier on, during the summer of 2014, Roche tried to acquire the Japanese firm Chugai, specialized in the development of anticancer drugs and inflammatory diseases such as rheumatoid polyarthritis. While Roche already owned *ca*. two-thirds of Chugai's equity it was willing to offer US$10 billion in order to acquire 100%. But it finally backed off (Hecketsweiler, 2014d).

Foundation Medicine ownership

On 12 January 2015 Roche announced it would acquire the majority stake of the Cambridge, Massachusetts-based tumour-testing company Foundation Medicine for more than US$1 billion. Such a deal aimed at improving cancer treatment and thus

strengthening Roche's position on that market. Foundation Medicine, listed on the Nasdaq with a stock value of *ca*. US$700 million, is the leader in the growing area of sequencing the genes of tumour samples, looking for mutations that can help predict which drug will be the most efficient for a particular patient. Furthermore Roche which has also a huge diagnostic business will sell Foundation Medicine's tests outside the United States. "We believe this approach will become the norm in oncology in the not-too- distant future, and this takes us another step in that direction," Michael J. Pellini, the chief executive of Foundation Medicine, stated in an interview. Under the terms of the deal Roche was expected to invest US$250 million in Foundation Medicine by acquiring five million newly issued shares at US$50 a share, a 109% premium to Foundation Medicine's closing price on 9 January 2015. Roche was also expected to make a tender offer at the same price to acquire *ca*. 15.6 million shares. In total Roche would pay just over US$1 billion and end up with a stake of between 52.4% and 56.3% on a fully diluted basis, the companies stated. The transaction, subject to approval by Foundation Medicine's shareholders, was approved unanimously by the company's board. Three venture-capital companies holding a combined 31% of Foundation Medicine's shares – Third Rock Ventures, Kleiner Perkins Caufield & Byers and Google Venture – agreed to vote for the transaction and to tender at least a majority of their shares (Pollack, 2015a).

Foundation Medicine main product, called Foundation One, sequences more than 300 genes in a sample of solid tumour, such as lung or breast tumour. A newer product, Foundation One Heme, is for blood cancers. The company which went public in 2013 had revenues of US$61.1 million in 2014, up from US$29 billion the year before. It had performed more than 24,200 tests for patient treatment in 2014, up from only *ca.* 9,000 in 2013. The company was still losing money in 2014. It was working to provide data showing that its tests really helped patients, which was necessary for the testing to be reimbursed by the American social-security system or Medicare and by private insurers. The Foundation One test had a list price of US$5,800 and the hematology test costs US$7,200 (Pollack, 2015a).

As another part of the deal Roche was to provide as much as US$150 million in research-and- development funding over five years. The companies will work together to develop the so-called liquid biopsy test – analyzing tumours from a non-invasive blood sample rather than a tumour sample obtained from biopsy or surgery. They will also work on tests to help develop anticancer immunotherapies. In addition to running tests to help guide patient care Foundation Medicine has a significant business sequencing tumour DNA for pharmaceutical companies to help in clinical trials and drug development. Some drug companies might be less interested in working with Foundation Medicine once its majority is owned by Roche, a competitor. But M.J. Pellini stated that this would not happen and that Roche would not have access to data from any of the other drug companies. Daniel O'Day, chief of Roche pharmaceutical business, said that his company wanted a majority stake to foster a deep collaboration, but also wanted to allow Foundation Medicine to remain independent and agile. In fact Foundation Medicine, that is partly funded by Google, had signed cooperation agreements with some 25 pharmaceutical companies,

including Novartis and Johnson & Johnson (Hecketsweiler, 2015d; Pollack, 2015a). Roche will have three representatives on a nine-person board. Under the terms of the agreement it will not be able to buy all of Foundation Medicine for at least three years (Pollack, 2015a).

Following the same business strategy Roche announced on 2 July 2014 that it was acquiring the Californian biotechnology company Seragon for *ca*. US$1.7 billion (or €1.2 billion). Seragon is developing a new generation of treatments against breast cancer.

23 and Me deal

At the beginning of 2015 another very coveted deal was made by Roche with the Californian startup 23 and Me, also specialized in genetic tests. Until that date this firm, partly owned by Google, had disputes with the FDA which questioned the sales by the company of unreliable diagnostic kits to patients. By the end of 2013 the FDA requested 23 and Me to stop a significant part of its activity. However, in 2015, the biotechnology firm made a comeback through trying to sell the data concerning the genetic profile of its 800,000 clients. In this ocean of data – big data – Genentech/Roche is coveting hints on the genetic causes of Parkinson's disease. With the help of Michael J. Fox Foundation – M.J. Fox is an American artist affected by this neurodegenerative illness – 23 and Me was able to recruit a "community" of 12,000 patients very eager in participating in research programmes. Genentech's intention is to select 3,000 within this community and sequence their whole genome in order to identify new targets for medicines. It should be mentioned that 23 and Me focuses on very precise DNA sequences. The deal with 23 and Me included the transfer of US$10 million by Roche/Genentech, and eventually another US$50 million if the research work is successful (Hecketsweiler, 2015d).

Genentech was a newcomer in the area of neurosciences. In 2006 it created a research department devoted to what it considered another search for the Grail by biotechnology companies. For instance Alzheimer's disease that is the most frequent form of dementia among old persons affected 45 million people in the world in 2014, and this number would jump to 135 million by 2035. The development of an efficient therapy against the disease has been unsuccessful; this remains a big challenge and also a potential considerable source of revenue in case an efficient medicine is developed (see p. 377). Roche in the 1960s had commercialized the tranquilizing drugs Valium and Lexomil, that are still used in psychiatry. But since then the exploration of the human genome has led to the identification of genes involved in the development of Alzheimer's disease as well as the patients with a high risk of becoming affected by the disease (Hecketsweiler, 2015d).

Ryan Watts who is responsible for Genentech research in neurosciences is trying with his team to find and use a new class of drugs, derived from biotechnology and not chemistry, in order to stop the destruction of neurons. In other words they try to use a similar approach as that which led to developing targeted anticancer drugs. The major

difficulty, as stressed by this brilliant scientist recruited from Stanford University, is that the brain is surrounded by biological barriers which are not easy to penetrate. Proteins that are *ca*. 375 times bigger than a synthetic molecule cannot get through. However Genentech scientists have identified a transporter and found in experiments carried out with primates that it could help proteins overcome the brain barrier. They may initiate human trials by the end of 2017 (Hecketsweiler, 2015d).

A merciless war of talents

Genentech work is closely followed by rival companies such as AbbVie and Amgen, but also by Roche itself. The latter owns an independent basic-research unit, christened pRED, that is a competitor of its Californian alter ego, gRED. This features the competition spirit that prevails in the Silicon Valley area. And Genentech indeed finds itself in a merciless war of talents. All biotechnology and pharmaceutical companies are competing to recruit the best scientists. For instance Gilead Sciences, well-known for its research on AIDS/HIV and hepatitis C, was able to recruit Philippe Bishop who was responsible for the development of the main anticancer drugs in Genentech. Newcomers like Google are playing the same game. Arthur Levinson, the former chief executive officer of Genentech, and Hal Barron, also from Genentech and one of its most talented researchers, have been recruited by Google (see p. 47). Some analysts think that in the heart of Silicon Valley the war among the biotech giants is just starting (Hecketsweiler, 2015d).

Immunotherapy has been the first major bet of Genentech, before it was significantly involved in the struggle against neurodegenerative diseases. The goal is to "educate" the immune system when it cannot or is not any more able to defend itself against the disease. To that end small proteins (instructors) are used to act on specific markers located on the cell surface. These proteins can act either on tumour cells or on cells of the immune system, the expected result being the stimulation, or silencing, of certain functions. According to Andy Chan, the deputy leading scientist of Genentech gRED research unit, "some cancer cells escape the attacks of the immune system thanks to signals sent to T-lymphocytes – the body's killing cells. We develop molecules, called anti-PDL1, that block this communication mechanism." There were five molecules being tested by Genentech which intended to make the first request for commercialization by the end of 2015. "Five years ago, everybody thought that immunotherapy was a science-fiction approach," commented Andy Chan (Hecketsweiler, 2015d). The American pharmaceutical company Bristol-Myers Squibb outpaced Genentech in the area of immunotherapy but the gap between them is narrowing. Other companies are being involved because they are convinced that this new category of anticancer medicines can become a new "gold mine" for business. It was estimated by Citigroup that the value of the market could reach US$35 billion (or €30.9 billion) per year during the next decade. This is unprecedented in the history of pharmacy (Hecketsweiler, 2015d; see pp. 309-310).

## *Sanofi*

A French and an international big pharma with a new road map

Sanofi was in 2014 the world's third-biggest pharmaceutical group among the top ten big pharmas. Its annual turnover amounted to €33.7 billion, including €27.7 billion in drug sales, €3.9 billion in sales of vaccines and €2 billion in sales of veterinary products. The company's consolidated profit reached €4.4 billion. In 2014 the company invested €4.8 billion in research-and-development (R&D) activities. Sanofi strength on the global market of antidiabetic drugs is best illustrated by the sales of its blockbuster drug called Lantus (glargine): €6.3 billion in 2014. It is a novel insulin that is injected only once a day. The patent protecting Lantus expired in February 2015 in the United States and in May 2015 in Europe, which meant that the drug will be challenged by generics or biosimilars (Hecketsweiler, 2015i).

On 2 April 2015 Olivier Brandicourt was appointed to take the lead of Sanofi and succeeded Chris Viehbacher and Serge Weinberg (the president of the governing board). O. Brandicourt has worked for a long time for Pfizer and made a short stay at Bayer. By contrast to his predecessor, C. Viehbacher, in 2008, O. Brandicourt was to lead a group with its marching orders and with a stock value of €122 billion, ahead of the French oil company Total. Historically Sanofi has been a chemical-industry corporation, but after the acquisition of the American biotechnology Genzyme in 2011 it became one of the biggest biotechnology groups. Its biotechnology-derived medicines made up *ca*. 10% of the sales of its pharmaceutical division (€27.7 billion in 2014). [Hecketsweiler, 2014a; 2015i).

In order to improve and increase its portfolio of innovative drugs and also in order to soften the shock of the so-called patent cliff (i.e. the decrease in the annual sales figure due to the marketing of cheap biosimilars competing with Sanofi drugs that were formerly protected by patents), Sanofi adopted a two-pronged strategy. Firstly to make alliances with several biotechnology firms having promising drugs in their pipeline. Thanks to these partnerships Sanofi expected to commercialize up to 18 medicines by 2020 in a sector where generally a period of ten years elapse between the first clinical trials of a drug and its commercialization (if successful and approved by the health regulatory authorities). Secondly the development of novel insulins aimed at replacing Lantus (Hecketsweiler, 2014a; 2015i).

On Friday 6 November 2015 Olivier Brandicourt presented the company's road map. The new measures included the launching of 18 new drugs from 2015 to 2018, as well as the increase in R&D expenses – up to €6 billion, compared with €4.8 billion in 2014 for an annual turnover of €33.7 billion. Furthermore thanks to making its organization simpler and to focusing on a more limited number of activities, Sanofi expected to make savings amounting to €1.5 billion by 2018. O. Brandicourt announced that he was reviewing all "strategic options" for its veterinary division Merial as well as for its generic-drug activity Zentiva. Both companies could be sold or listed on the stock exchange, but Sanofi did not exclude that they may remain in the group's purview. At the same time Sanofi wanted to strengthen its mainstream activities – diabetes, rare diseases, vaccines and emerging countries – and to catch up in the area of anticancer drugs (Hecketsweiler, 2015i).

On 2 February 2016 Sanofi announced that "*ca*. 600 jobs will be eliminated over three years" in France, i.e. 2% of its total staff in this country. Sanofi, in 2016, employed 27,000 persons in France out of a total of 110,000. It had 23 factories there and 45% of the group's research-and-development investments were made in France, amounting to €2.2 billion in 2014. In November 2015 Sanofi already announced a plan for savings amounting to €1.5 billion until 2018. Sanofi Winthrop, Sanofi's subsidiary which includes all the group's French factories, is particularly hit: 400 jobs were to be eliminated there. In October 2015 Sanofi management indicated that "the competitivity of the French sites should be improved." Through curtailing jobs and staff, Sanofi aimed to reach a production cost of 20 to 25 cents of a Euro per box of prescription drugs, compared with 33 cents by the end of 2015; the cost of a box of non-prescribed drugs was expected to reach between 15 and 20 cents of a Euro, compared with 24 cents in 2015 (Hecketsweiler, 2016a).

The category of jobs that is expected to be drastically reduced is that of salespersons, who visit with general practitioners and physicians in order to promote the sales of their company's drugs. This has been criticized for several years and all big pharmas slashed this kind of personnel. The competition from generics also led the big pharmas to retreat from the physician's office and to promote their most sophisticated drugs or molecules at the hospital level; this means more qualified and less numerous people (Hecketsweiler, 2016a). In the United States Sanofi was expected to send a layoff notice to New Jersey's authorities (Sanofi has a large campus in New Jersey). This may concern a higher number of persons than in France – it seems easier to lay off people in the United States than in France. Trade-unions have denounced Sanofi's layoffs as having a stock-exchange purpose. They deplored that the money used by the group to acquire its own shares – €1.7 billion in 2015 and more than €1 billion since the beginning of 2016 – was not preferably reinvested in the group (Hecketsweiler, 2016a).

Antidiabetes drugs : the core business

Antidiabetes drugs and treatments still made up 21% of Sanofi's annual turnover in 2014-2015, but 35% of its profits. This activity will remain a pillar of the group. In order to successfully face the harsh competition from its rivals Sanofi relies on the purchase of other medical biotechnology companies as well as on licensing. Thus O. Brandicourt announced on 6 November 2015 an agreement with the biotechnology firm Lexicon, based in Texas, which is developing a new generation of antidiabetic drugs. One day earlier Sanofi CEO unveiled a deal made with the South Korean Hanmi with a view to developing and commercializing three other drugs. To that end Sanofi already disbursed €400 million and will pay up to €3.5 billion more and royalties in case of successful results (Hecketsweiler, 2015i).

Sanofi had disbursed US$150 million in 2014 in a deal with the Californian biotechnology firm MannKind aiming at acquiring the market rights of the inhaled insulin, called Afrezza, that is prescribed to patients who systematically refuse insulin injections. MannKind was started by Alfred E. Mann, who made a fortune funding aerospace and medical device companies, including the insulin pump manufacturer MiniMed which was sold to Medtronic for *ca*. US$3 billion. But A.E. Mann, who was 90-years old in 2015, spent much of his accumulated wealth backing MannKind Corporation as

it suffered many setbacks before finally winning the approval of Afrezza by the FDA. A.E. Mann and other supporters of Afrezza stated the product would provide millions of diabetics with a new option that might be more attractive to them than injecting themselves with insulin up to several times a day. This would improve overall public health, they said, because many people with diabetes who could benefit from insulin do not use it in part because they do not like injections (Pollack, 2016a).

Pfizer had used the same arguments several years earlier yet suffered a costly failure in trying to market an inhaled insulin called Exubera. MannKind argued that it would succeed because its inhaler was much more discreet and it had some desirable medical characteristics, though there was debate on that. But insurers have been reluctant to pay for Afrezza which is more expensive than injectable insulin. There are also concerns over the safety of putting insulin into the lungs. The prescribing information for Afrezza recommends that lung function should be tested before patients start on the drug, and periodically thereafter. Sanofi had hoped that Afrezza which is taken at mealtimes would be an adjunct to its blockbuster antidiabetes drug Lantus. In addition to the US$150 million paid in 2014 to MannKind for the Afrezza rights, Sanofi made an additional payment of US$50 million early in 2015 (Pollack, 2016a).

On 5 January 2016 MannKind announced that Sanofi was terminating the agreement between the two companies concerning the marketing of Afrezza. This was not a surprise since the inhaled insulin has had dismal sales since June 2014, when it was approved by the FDA. Sanofi reported sales of *ca*. €5 million in the first nine months of 2015. MannKind shares plummeted 48%, closing at UScents 75, while when Afrezza was approved the shares were worth *ca*. US$10. The company, based in Valencia, California, was expected to face money problems without Sanofi's support, though it vowed to fight on. Both Sanofi and MannKind stated they would work for a smooth transition so that delivery of Afrezza to patients was not interrupted. Sanofi announced it would continue to fill orders until 4 July 2016 (Pollack, 2016a).

Matthew J. Pfeffer, the chief financial officer of MannKind, said that the patients who tried Afrezza really liked it and that the drug could succeed if patients and doctors were educated and insurers were persuaded to pay for it. However, "with a busted launch by one of the leading diabetes players, it is hard to see any other company stepping in to take on commercial efforts," stated Joshua Schimmer, an analyst at Piper Jaffray, on 5 January 2016. He said that because MannKind did not take questions on its conference call "we did not have an opportunity to ask how the company can avoid bankruptcy." M.J. Pfeffer tried to dispel speculation that MannKind would run out of money. The company had *ca*. US$60 million in cash, enough to last into the second half of 2016 at the current rate of expenditure, he stated. MannKind had a net loss of US$91.4 million in the first nine months of 2015, even with Sanofi paying much of the bill to market Afrezza. As of 30 September 2015 the company had an accumulated deficit of US$2.6 billion since its founding. Sanofi entire diabetes business, one of its major assets, is suffering from price and product competition, in addition to the setback for the maker of inhaled insulin and Sanofi dropping its deal with MannKind. O. Brandicourt stated in the fall of 2015 that Sanofi diabetes business was expected to decline 4% to 8% annually from 2015 to 2018 (Pollack, 2016a).

Development of new kinds of insulin

Sanofi strategy aimed at protecting its core business of antidiabetes drugs and at mitigating competition from rivals, as well as the setback of MannKind's Afrezza, has been to develop new kinds of insulin. This was the case of Toujeo, approved by the FDA by the end of February 2015. It was also approved by the European Medicines Agency (EMEA) on 27 February 2015. "After the United States in April 2015 Toujeo was commercialized in May 2015 in Germany," stated Pierre Chancel, director Sanofi diabetes division. "Toujeo is three times more concentrated than Lantus so that the injection is faster and less painful. Once in the dermis the insulin forms a more compact crystal, henceforth a longer diffusion of the hormone and a more lasting action: *ca*. 30 hours, compared with 20 to 24 hours for Lantus. Its concentration in the bloodstream remains very stable, henceforth a lower risk of hypoglycemia," added P. Chancel. Indeed severe hypoglycemia is the major risk of insulinotherapy, particularly in type-1 diabetes: when there is an excessive amount of insulin in the bloodstream glycemia plummets, and the patient runs the risk of falling into a "diabetic coma" – a vital emergency (Rosier, 2015c). See also p. 349.

"Toujeo has minimal differences with Lantus," thought Etienne Larger, a diabetes specialist at Paris Cochin hospital. "This new dosage is not more efficient in lowering the average glycemia. Studies showed that the new drug – Toujeo – lowers the number of hypoglycemia events overnight, sometimes quite significantly, among type-2 diabetics. Its action seems to last longer which would be beneficial for type-1 diabetics – Lantus' action lasts less than 24 hours," he added. Other French specialists such as André Grimaldi and Vincent Renard, considered that Toujeo's development and commercialization was above all a "marketing operation" correlated with the expiration of Lantus' patent protection (Rosier, 2015c).

Cost-effectiveness

According to the French pharmacologist F. Chast the prescription of glargine insulin, developed by Sanofi, seems justified, even though "the improvements provided by Lantus had been somewhat overstated twelve years ago." In 2003 the French High Health Authority (HAS, French acronym) gave Lantus a "moderate level of improvement of delivered medical service" (ASMR, French acronym). But afterwards this level of improvement fell down and on 7 May 2014 the HAS evaluated that Lantus' ASMR was "inexistent", compared with that of a former slow-activity insulin, called NPH, that cost 60% less than Lantus. However A. Grimaldi, a renowned diabetologist at Paris La Pitié-Salpêtrière hospital, considered that Lantus' benefit was still important in the treatment of type-1 diabetes where glycemia is more unstable. He stated: "This drug has driven insulinotherapy towards a scheme that is close to normal physiology. We have moved from 10-12 different insulinotherapy protocols that are very complex to handle to a single one that is more comfortable for the patient." Conversely the same French specialist estimated that the benefit provided by Lantus was very small in the treatment of type-2 diabetics (Rosier, 2015c).

In 2013 Lantus was the seventh most-consumed drug in terms of expenses in France, with an annual total of €221.5 million (reimbursed by the French health insurance/social security). This amount was 8.6% higher than that of 2012. The use of the so-called "slow analogues" of insulin such as Sanofi Lantus or Novo Nordisk Levemir, by type-2 diabetics resulted in an overcost for French social security that has been estimated at €100 million per year. It was often hammered by French pharmacologists that the price of this kind of insulin must be decreased in order to keep the social security's accounts on the safe side (in other words not to deepen its current deficit). Dominique Giorgi, chairperson of the Economic Committee for Health Products, in charge of fixing the price of drugs, promised that "Lantus price will decrease when its competitors will be marketed." It should be emphasized that since 2006 the costs of treatment of diabetes have been covered by the French social security, while in the United States in 2014 a diabetic treated with insulin used to spend US$120 to US$400 per month which are not included in his/her health (drug) insurance (Rosier, 2015c). See also p. 109.

Biosimilars and the competition from generics

Jeremy Greene and Kevin Riggs of Johns Hopkins University, Baltimore, in the 19 March 2015 issue of the *New England Journal of Medicine* (*NEJM*), wondered why, almost 100 years after the discovery of insulin at the University of Toronto by Frederick Banting and John McLeod (Nobel Laureates in 1923), there were no biosimilars of the hormone on the market. Insulin had been purified in 1921 after being extracted from animal pancreas by F. Banting and Charles Best; thereafter in 1922 it had been administered to the first patient: a 14-years-old teenager who was in a diabetic coma and whose life was saved thanks to the injection of animal insulin. The University of Toronto researchers realized that their discovery could have a considerable economic impact but they decided to grant their intellectual property rights to their university for just one symbolic US dollar. In 1923 the University of Toronto filed a first patent on insulin; thereafter it imposed licenses to the industrialists and this allowed it to control the price of the drug, but also its quality (Greene and Riggs, 2015; Rosier, 2015c).

The paper published in the *NEJM* and authored by J. Greene and K. Riggs denounced the cycle of "patent perpetuation" where are involved the manufacturers of insulin. Such race forces them to continuously slightly change their molecules or their formulations in order to patent them and deliver them with the help of marketing campaigns; this process was named "evergreening." Thus in 1936 was marketed the first insulin with extended action, called iPZ, ten years before the first slow insulin. In 1982 the first "human" insulin was produced by genetically engineered bacteria. In 1997 "fast analogues" of insulin were marketed in France and, in 2003, Sanofi's Lantus – a "slow analogue" of the hormone – was commercialized. Nowadays insulinotherapy mimics the activity of a normal pancreas: it combines one or two daily injections of an extended-action insulin with injections of a very fast-acting insulin before each meal (Rosier, 2015c).

The Danish insulin manufacturer Novo Nordisk produced a "slow insulin", called NPH, and patented it in 1946. The company filed another patent in 1996 for an NPH obtained with an analogue of human insulin produced via genetic engineering. In 2003 Novo Nordisk filed again a patent for a new formulation of NPH. So dozens of patents have been filed for any new form or kind of insulin. "Some bring real therapeutic benefits, and others less," stated Maurice Cassier, a patent specialist at the French National Scientific Research Centre (CNRS, French acronym). In his opinion this evergreening should stop so as to facilitate a much wider access to insulinotherapy at the lower cost possible and with molecules that provide real therapeutic benefits (Rosier, 2015c). See also p. 109.

According to François Chast, head of the department of clinical pharmacy at the Paris Cochin hospital, "the number of diabetics in the world would reach 400 million and more than 40 million among them would need insulin; this will represent a colossal market." The annual value of the latter was estimated at €26.4-€27.3 billion, the average growth rate being 16.7% per year since 2004, according to IMS Health. Over the period 2015-2020 the growth of that market was estimated at 15% (Rosier, 2015c). It is therefore understandable that there is a harsh competition among the main insulin manufacturers in order to conquer a large share of that market. In the case of Sanofi it faces the competition of Eli Lilly and Novo Nordisk, which markets an insulin similar to Lantus. Eli Lilly expected to market a *biosimilar* to Sanofi's Lantus, called Abasaglar in 2015, but this was postponed to July 2016, further to the fact that Sanofi was suing Eli Lilly for counterfeit. Beyond this competition among manufacturers of antidiabetes drugs and their relentless efforts to pursue the evergreening of their products with regard to their patent protection, there is the urgent need to provide insulin for all, as the unique response to the diabetes "pandemia"; this is particularly urgent in developing or least developed countries. "In most African countries mortality after ten years of type-1 diabetes is 50%," recalled A. Grimaldi (Rosier, 2015c).

Responding to new challenges in health-care

Besides facing the competition from the company's rivals with respect to antidiabetes drugs Sanofi new chief executive officer had, according to analysts, to hold the promises made through the launching of new products and focusing on those that can respond successfully to new challenges of health-care. Indeed Sanofi is present in a record number of therapeutic areas: diabetes, cancer, rare diseases, vaccines, generics, auto-medication, veterinary drugs. Such dispersion may become a handicap with respect to competitors which are increasingly specialized and focused on a narrower range of activities. That is why the new CEO was expected to clarify even more the group's strategy. For instance in the research and development of anticancer drugs Sanofi may not be as competitive as Roche or Celgene, Since the expiration of its patent on Taxotere the sales had plummeted to €1.5 billion in 2014 from €2 billion in 2010 (Hecketsweiler, 2015v). However on 3 November 2015 Sanofi announced a licensing agreement, with an initial amount of US$60 million (or €55.2 million), with the German firm BioNTech, specialized in anticancer immunotherapy. Earlier on, in August 2015, Sanofi invested US$2.2 billion in the development of four experimental immunotherapies in collaboration with the New York-based biotechnology company Regeneron (Hecketsweiler, 2015v,x).

Sanofi and Regeneron have also worked in partnership for the development of the new generation anticholesterol drug Praluent, as well as for the development of the anti-asthma drug Dupilumab, to be launched in 2017, and of Sarilumab, an antirheumatoid arthritis medicine to be commercialized in 2016. Sanofi owned 22% of Regeneron's equity in 2015 and it could increase its share up to 30% but Sanofi often stated it did not want to buy the company. The last important acquisition of Sanofi had been that of the American biotechnology "star" Genzyme (for *ca*. US$20 billion). Genzyme sales increased 33% during the third quarter of 2015 due to the sales of its treatments against multiple sclerosis (Hecketsweiler, 2015v,x). See also p. 380.

Sanofi must also adapt its business model to the new challenges of the health-care sector. Like other companies Sanofi wanted to act beyond drug discovery and marketing competition, through offering patients and physicians a wide range of services. For instance Sanofi has launched applications and connected objects in order to help diabetics to better manage their illness. In 2013 Sanofi recruited a Chief Patient Officer, Anne Beal, an American physician in charge of involving patients as "partners" and of proving to the health authorities the added "value" brought in by Sanofi treatments. This approach is particularly relevant in the current situation when health authorities are trying to markedly decrease the price of drugs (Hecketsweiler, 2015x). Regarding the research policy within the group analysts, while admitting that new drugs will be commercialized during the period 2016-2018, underlined that the upstream pipeline of new molecules must be replenished. This is particularly true for antidiabetes medicines because of the harsh competition in this area with Eli Lilly and Novo Nordisk. In order to make the appropriate decisions in R&D and to remain competitive Sanofi chief executive officer did not seem to be in favour of making new acquisitions, but rather to sign other cooperation agreements with biotechnology firms or startups (Hecketsweiler, 2015i,v).

More generally Sanofi CEO did mention that the group had to increase its productivity across all its industrial sites: +20% to +25% in three years. Trade-unions were concerned because this might lead to laying off people. The group had lost more than 4,500 jobs since 2008. While Sanofi sales were expected to rise 3%-4% over the period 2016-2017, on 7 November 2015 the group's shares lost 5% at the Paris stock exchange where Sanofi was listed as the largest company with a value estimated at more than €120 billion (Hecketsweiler, 2015i). That is why the new road map of the company emphasizes the need to restore trust among the stakeholders and to respond to the current and future challenges of the health-care sector.

### *Teva*

Teva is the world's leading generic drug manufacturer. It is based in Petah Tikva, near Tel Aviv, Israel. When in February 2014 Teva new CEO, Erez Vigodman, took the lead of a group that was in a rather difficult condition, a managerial crisis was affecting the Israeli corporation. Moreover Teva was facing a strong pressure exerted by its generic-maker rivals. In particular emerging countries' companies are becoming formidable competitors. Teva profits decreased and the company had to adopt a vast

restructuration plan in order to reduce its costs. The group also focused its acquisitions on firms owning patent-protected drugs, e.g. Auspex, acquired in March 2015, for US$3.2 billion. This American startup was developing treatments for motricity ailments. Teva lost its exclusivity on the blockbuster drug Copaxone. This antimultiple sclerosis medicine had been developed by the Weizmann Institute of Science immunologists (Michael Sela and others). Its sales made up one-fifth of Teva annual sales and half of its profits. On 16 April 2015 a first generic version of Copaxone, developed by Sandoz – Novartis subsidiary – and Momenta Pharmaceuticals, was approved by the FDA. Mylan, the American rival of Teva, was also developing a generic version of Copaxone (De Vergès, 2015).

Teva / Mylan merger

In order to maintain its global leadership and outpace its rivals, Teva made an acquisition offer of more than US$40 billion (or €37.4 billion) to Mylan, based in the Netherlands and the world's third-biggest generic maker. Before known publicly on 21 April 2015 this offer had been mentioned by analysts in the pharmaceutical industry as one of the largest acquisition offers in 2014-2015 (De Vergès, 2015). With Mylan Teva expected to create a behemoth of generic-drug production and commercialization, with annual revenues of *ca*. US$30 billion and Teva, said, US$2 billion cost savings. In 2015 Teva share of the global generic market was *ca*. 12%, while that of Mylan reached 8%. According to analysts the purchase of Mylan would be at least four times bigger than any other acquisition made by Teva before 2014-2015. Teva offered US$82 for Mylan stock, which was *ca*. US$74 on 21 April 2015 (*The Economist*, 2015e).

Such merger would allow Teva to emphasize the production of biosimilars. In addition to many economies of scale the merger would also increase the firm's global reach: although Teva already distributes its products in 100 countries, a merger with Mylan would strengthen its operations in some regions, particularly in Asia and the Pacific. Sam Fazeli, an analyst at Bloomberg Intelligence, considered that "a merger between Teva and Mylan makes sense from the financial and strategic viewpoint: this would enable Teva to become resilient to attacks against Copaxone and to reduce the negative impact on its profits" (De Vergès, 2015; *The Economist*, 2015e). But the company would have to make some divestments to meet the requirements of the competition (antitrust) authorities.

At the beginning of May 2015 Mylan rejected the US$40 billion bid from Teva, arguing it lacked "industrial logic or cultural sense," stated Robert Coury, the president of Mylan governing board. He also mentioned the risk for the merger to be opposed by antitrust authorities. As part of its plan to escape Teva takeover Mylan made three successive acquisition offers (US$28.9 billion) to Perrigo, a smaller Irish rival, only to be spurned each time. Perrigo seemed likely to attract interest from other companies (De Vergès, 2015; *The Economist*, 2015e). On Friday 5 June 2015 Teva Pharmaceutical Industries increased its stake in Mylan to nearly 2.2%, as it pressed on with an unsolicited bid for its generic drug-making rival. Teva had accumulated *ca*. 10.5 million shares in Mylan

as of 4 June 2015. Teva was looking to increase its stake in Mylan to *ca*. 4.6% which would allow it to have standing to potentially challenge Mylan refusal to enter tie-up talks (*Haaretz*, Tel Aviv, 8 June 2015, p. 7).

On the other hand Teva announced its plan to move its global headquarters from Petah Tikva to Ra'anana. Teva has been trying to focus all its operations in one location in Israel rather than at several sites as in 2015. To that end Teva was in talks to buy land from a number of companies which own assets in Petah Tikva. But it preferred to purchase land in Ra'anana owned by Toyota Israel importer, George Horesh. Purchase of the land and construction of the new headquarters will cost Teva an estimated €100 million, at least. The move is part of Teva streamlining process and is designed to save the cost of leasing properties. Furthermore some of the land that will be vacated and is owned by Teva can be sold. The new campus which will be completed in five years will unify operations in the global headquarters (*The Jerusalem Post*, 8 June 2015, p. 16).

Impact on the prices of generic drugs

The Teva/Mylan merger proposal has triggered speculation that this kind of consolidation could cause the price of generic drugs to rise. All around the world health-care providers are keen on buying cheaper generic copies of branded drugs whose patents have expired. In large emerging markets such as India spending on drugs in general is growing fast, because of a combination of population growth, programmes to increase public access to health-care and new medicines, and governments' economic-stimulus measures. Generics represent there an even bigger share of drugs spending than in richer countries. But prices are a topic of great concern in the wealthy world, too, especially in the United States, thanks to some surprising spikes in the cost of some generics. According to Express Scripts, a drug wholesome dealer, the price of digoxin, a treatment of congestive heart failure, went up by 1,127% in 2014. This was because, for a period, only two companies were making it. Pricing concerns in the United States have triggered a congressional investigation as well as a review by the Department of Health and Human Services (*The Economist*, 2015e).

Such debates matter. Generics have succeeded in bringing down the overall cost of medicines by providing competition to branded drugs. So far, at least, the overall trend for generics prices has been downwards. Express Scripts' prescription price index showed that whereas the average price for branded medications in the United States had risen 127% over the seven-year period 2008-2014, the average for generics was down by 63% over that period. However, Michael Farr, the CEO of Farr, Miller & Washington, an investment-management firm (which holds a position in Perrigo), stated that consolidations in the generic industry tend to create both scale and negotiating power with purchasers of drugs. That is why, he indicated, the markets have rewarded consolidations through increases in their share prices. Kathleen Davenport of Decision Resources Group, a health-care consulting firm, meanwhile, thought that a Teva/Mylan merger had the potential to lead to higher generic prices in the short-to-mid term (*The Economist*, 2015e).

## Spain

In Spain, since 2010, drug expenses have been plummeting due to the lasting economic crisis and, according to the consulting firm IMS Health and the association of national pharmaceutical companies, Farmaindustria, such a trend will not change before 2018. Confronted with such a situation Spanish pharmaceutical corporations focused their development strategy on overseas markets so as to mitigate the decrease in their profits. In fact they could achieve an improvement in their sales and profits during the first half of 2014. In addition to drastic restructuration measures and to the search for new pharmaceuticals the exchange of marketing licenses and rights between Spanish laboratories and foreign ones (such as Pfizer, Merck, Johnson & Johnson, Astra-Zeneca and Novartis) has been a recurrent approach. To the point that one-fifth of the overall turnover of the Spanish companies Almirall, Faes, Rovi, Zeltia and Esteve, consisted of the recovery of royalties and other rights (Gómez, 2014).

Almirall started in 2014 to reap the fruits of its strategy: with sales of €433.4 million during the six-month period, January-June 2014, it made a profit of €19.1 million. By the end of June 2014 Almirall had €85.5 million in its cash and a financial debt of €319.5 million. The company, controlled by the Gallardo family, stated that 2013 was an inflexion point with the launching of 28 products and setting the bases for growth during the following years. By early 2014 Almirall finalized the acquisition of the American company, Aqua Pharmaceuticals, for €238 million, and by the end of July 2014 it sold to AstraZenecca its rights for the franchise of respiratory-ailment drugs, amounting to €1.562 billion. Analysts of Ahorro Corporación, a financial firm, considered that it was a positive transaction. Another important event for Almirall was the approval by the European Medicines Agency (EMEA) of the combination of two pharmaceuticals of the company used in the treatment of chronic obstructive lung disease (Gómez, 2014).

Grifols, the biggest Spanish pharmaceutical group, with sales of €1.610 billion during the period January-June 2014 and profits of €224.8 million, widened its international expansion as the world's third-biggest producer of of hemoderivatives, and expected to commercialize diagnostic products in China and India in the short term. Grifols had accumulated a total debt of €3.163 billion by the end of June 2014, largely due to the acquisition in 2010 of its rival Talecris (United States) for €2.8 billion, of Novartis unit of transfusion diagnostics for €1.24 billion and of 50% of equity of the Basque Kiro Robotics for €21 million (Gómez, 2014).

Zeltia, the Galician company, increased by 10% its sales during the period January-June 2014, reaching €78.2 million, and by 16% its net profit (€16.7 million). Half of the sales figure was recovered from its antitumour product, Yondelis. Zeltia had sold its marketing license of Aplidia (treatment of multiple myeloma) to the Japanese pharmaceutical company Chugai Pharma. In other words Zeltia is much more than Yondelis, stated the analysts of Intermoney (Gómez, 2014). Faes Farma, with sales of €99 million and a profit of €14.0 million over the period January-June 2014, i.e. a 3% and 10.6% increase, respectively, had created subsidiaries in Chile, Ecuador

and Colombia where it entended to triplicate its sales. It also expected to acquire a pharmaceutical firm. Natraceutical (with €17.7 million of sales and €1.2 million of profits over the period January-June 2014) and Reig Jofre were carrying out negotiations on a possible merger that would create the fifth-largest Spanish pharmaceutical group listed on the stock exchange (Gómez, 2014).

With respect to the generic-drug market, although Spain is still positioned among the trailing European countries, there are a few very successful national companies. Cinfa, from Navarra, is considered the star among them: it sells the highest number of generic "units" – 700 million – to the pharmacies, even more than multinational corporations. Cinfa's equity is 100% Spanish; the company owns two factories in Huarte and Olloki, it has almost 1,000 employees and its sales in 2013 amounted to €343.6 million (€320.6 million in Spain). Cinfa reinvests 90% of its profits into the company's development (Gómez, 2014). Esteve, a pharmaceutical and chemical company, had a turnover of €810 million in 2013, a 2.5% increase compared with 2012. Esteve bought a portfolio including 25 generic drugs from the American company Cypress and expected to increase by 20% the sales of these poducts in 2014. Regarding Esteve subsidiary Pensa, that sells drugs without license, its sales in 2013 amounted to €308 million – 35% of the group's total income. The prospects for the generics market in Spain are good, in so far as the patents of eight drugs with sales of €500 million were to expire between 2014 and 2015 (Gómez, 2014).

## China

### *Pharmaceutical research and development: initial steps*

China's potential to become a force in pharmaceutical research and development (R&D) had been evident since the 1960s when Mao Zedong ordered the Chinese army to find a treatment for malaria, which was a scourge among North Vietnamese soldiers in their jungle battles with United States-backed South Vietnam. The programme that was carried out discovered artemisinin which remains one of the most important weapons against malaria. It is derived from the sweet worm-wood plant (*Artemisia annua*) – a herb used in Chinese medicine for centuries – highlighting the potential to combine the country's traditional medical practices with modern science (Ward and Waldmeir, 2014). See p. 267.

However it was not until the 1990s, when an artemisinin-based drug was commercialized by Novartis of Switzerland, that this Chinese innovation was made available to the wider world. China wants to make sure future discoveries reach the global market more quickly – and with domestic companies taking them all the way. China has made faster development of research-derived pharmaceuticals a national priority – both to serve the growing health-care demands of Chinese society and to challenge the dominance of Western drug makers globally. In the government's five-year plan, launched in 2011, the sector was identified as one of the seven "pillar" industries to be promoted. Several big foreign drug makers have opened R&D centres in China or are planning to do so – including Novartis, Pfizer, Johnson & Johnson. But

most home-grown Chinese companies are yet to move beyond low-value generic medicines or ingredients for innovative drugs, made in Europe and the United States (Ward and Waldmeir, 2014).

This puts China in a rather similar position to India where companies such as Lupin and Ranbaxy have become big generic manufacturers but who little sign of developing their own high-value medicines. Where the two countries differ, however, is in their approach to the patent system underpinning the global pharmaceutical industry. Whereas India is battling with American and European drug-makers over the intellectual property rights that allow them to charge premium prices, China has shown less dissent. This, according to analysts and industry executives, reflects China's hope that it will eventually become a beneficiary of the patent system when its companies start developing drugs of their own (Ward and Waldmeir, 2014).

### *China pharmaceutical market and its stakes*

China is the world's second-biggest drug market behind the United States, and smoking, pollution and unhealthy food habits fuelled a rise in cancers and chronic heart and lung diseases. The country also has more diabetics than any other country in the world, with numbers expected to hit 151 million by 2040, up from 110 million in 2014, according to the International Diabetes Federation. That has made China an attractive spot for Novo Nordisk of Denmark, the world's biggest insulin producer, which has opened production facilities in the country since 1995. By 2010 it dominated China's insulin market. But it has been losing ground to local competitors. Made Krogsgaard Thomsen, Novo Nordisk chief science officer, confirmed: "Right now, the country is very focused on building domestic production." Local rivals are selling both cut-price basic insulin and sophisticated modern versions, including an almost exact copy of Sanofi's Lantus made by the Chinese biotechnology specialist Gan & Lee Pharmaceuticals (Hirschler and Jourdan, 2015).

Greater local competition is also evident in other areas, helping the top 10 Chinese drug makers increase sales 12% on average in 2015, according to IMS Consulting. That is twice the rate of multinationals which experienced a setback from a bribery scandal at GlaxoSmithKline (GSK) in 2013. Drug sales for GSK itself have slumped. Increasing local competition is part of a structural upheaval in China's hospital-dominated prescription drug market. Selling drugs to patients at a hefty markup – especially off-patent Western "branded generics" – often accounts for 40% to 50% of Chinese hospitals' revenue. But the authorities are now pushing a policy of zero markups, initially in smaller county hospitals. "Branded generics are something that exists today, but the need for them in ten years' time is not going to be there," stated Luke Miels, AstraZeneca's global portfolio head. That means foreign companies will have to rely more on new, patented drugs, although the scale of demand for such expensive products is uncertain in a country with only basic health insurance. At the other hand of the spectrum multinationals are trying to build up volume, often in partnership with local players, in the big markets outside of China's top cities. Distribution costs there are high and prices low. Pivotal to the transformation of the pharmaceutical market is the China Food and Drug Administration. The agency has promised to speed up

approval of innovative new drugs, which can take five to seven years, while cracking down on substandard local generics (Hirschler and Jourdan, 2015).

### *Partnerships with multinational corporations*

For several Chinese companies the first step in responding to the high stakes of the pharmaceutical market has been to strike partnerships with multinational corporations. Chi-Med, for instance, is developing its colorectal cancer drug, fruquintinib, with Eli Lilly of the United States, and is working with AstraZeneca of the United Kingdom on another drug, called volitimib, for renal cell cancer. With a market capitalization of just £550 million, London-listed China MediTech – known as Chi-Med – and backed by Li Ka-shing, Asia's richest man, is part of a growing Chinese life-sciences sector that authorities hope will become a driving force in new drug development (Ward and Waldmeir, 2014).

Much of Chi-Med revenues in 2014 still came from traditional Chinese medicines and herbal remedies sold through a marketing network that spanned 13,000 hospitals in 600 cities and towns. Christian Hogg, chief executive, believes the company can produce China's first truly home-grown blockbuster drugs. Both anticancer drugs being tested with Eli Lilly and AstraZeneca are in phase-2 trials, after promising early results. Normally *ca*. 20% to 30% of drugs that reach this stage go on to be launched on the wider market. Even if they fail Chi-Med has more candidate molecules in its pipeline, including one drug being developed in collaboration with Johnson & Johnson, and others it is developing alone. Whether through its traditional or innovative medicines analysts stated Chi-Med is well placed to benefit from growing health-care spending in China. Revenues rose 73% in the first half of 2014 to US$30million, producing profits of US$6.4 million. Shares in the company, listed on London's Alternative Investment Market, were up by two-thirds in 2014 with rising optimism over its prospects (Ward and Waldmeir, 2014).

Beijing-based BeiGene is also developing anticancer drugs with Merck of Germany. George Baeder, an adviser to local and multinational pharmaceutical corporations, predicted that more than a dozen Chinese-originated products will undergo clinical trials over the period 2015-2017. One of those domestic companies trying to make the leap from generics to innovative drugs is Simcare Pharmaceuticals which opened an R&D hub in Nanjing. Ren Jimsheng, the company's founder and chairman, stated funding remains a challenge. Compared with the US$30 billion invested by the United States government each year in fundamental drug research, the Chinese government invests less than US$1.6 billion and, at the company level, the gap is even larger (Ward and Waldmeir, 2014).

### *Biomedical research and the leap towards innovative pharmaceutical biotechnology*

Between 2007 and 2012 Chinese investment in biomedical R&D grew at a compound annual rate of 33%, compared with an average 7% in the rest of Asia-Pacific, according to the consultancy McKinsey. This increased spending is beginning to produce results:

the number of Chinese publications in respected life-science journals rose more than sixfold between 2001 and 2013, according Fangning Zhang of McKinsey in Shanghai. Skills shortages are being gradually overcome as China's universities graduate young scientists and those trained overseas return home. The regulatory environment is being improved thanks to the action of China Food and Drug Administration. To sum up favourable demand trends, coupled with the supportive environment for clinical research, mean the prospects for innovative pharmaceutical biotechnology are compelling (Ward and Waldmeir, 2014).

Thus, in addition to Fosun Pharma, which sees itself among the winners, a cluster of drug-research laboratories in eastern Shanghai highlights the promise of China's life-sciences and pharmaceutical biotechnology sector. The area brings together multinational and local companies, alongside contract-research businesses and small biotechnology operations. Among the latter is Hua Medicine – led by a Chinese-born, western-educated chief executive Li Chen, who used to run Roche China research-and-development centre. Now he is developing a novel diabetes treatment, licensed from Roche, while working on Hua's own promising leads (Hirschler and Jourdan, 2015).

Another standard-bearer for Chinese pharmaceutical biotechnology is the Beijing-based cancer specialist BeiGene, mentioned above, which in October 2015 announced plans for a US$100-million initial public offering (IPO) on the Nasdaq market. "There is a real chance for China to leap ahead in life sciences," stated Min Li, a returnee from the United States and Glaxo China research-and-development head. Dennis B. Gillings, executive chairman of the leading contract research organization Quintiles, said the number of drugs developed in China that were in the pipeline was rising fast. "As we hit the next decade in the 2020s, I'd be very surprised if there was not at least a top 20, if not top 10, global pharma player with headquarters in China," he added (Hirschler and Jourdan, 2015).

# KEY STRATEGIC DEVELOPMENT ISSUES FOR THE PHARMACEUTICAL AND MEDICAL BIOTECHNOLOGY COMPANIES

## Publication of the results of clinical trials

### *Theory and practice*

Though relevant clinical trials carried out by pharmaceutical companies should be reported to those responsible for licensing drugs and medical devices, there is no obligation on firms to make them public. That means such trials cannot be scrutinized by outsiders. The licensing authorities look at them, of course, so anything approved should, in theory, be safe, and have at least some beneficial effect. But the practitioners who go on to use them do not know all the details. Some estimates suggest the results of half of clinical trials are never published. These missing data have over several decades systematically distorted perceptions of the efficacy of drugs, devices and even surgical procedures. In the United States where most of the world's drugs first receive approval, the law was changed in 2007 to try to deal with this issue. Trials, with the exception of early safety evaluations, are supposed to be registered on a website, clinicaltrials.gov. Then within a year of the completion of data collection their results are supposed to follow suit. That, at least, is the theory. Practice seems different (*The Economist*, 2015g).

In 2015 a report by the *New England Journal of Medicine* (*NEJM*) combed through clinicaltrials.gov, looking to see how quickly after completion trials were reported. It found that, after the legal maximum of a year was up, just 17% of those paid for by industry had had their results published. Drug firms were not, though, the worst offenders. Only 8.1% of trials paid for by the National Institutes of Health (NIH), the United States government's main conduit for medical-research money, were reported within a year. And just 5.7% of the ones paid for by other government agencies and academic institutions were. Furthermore even though the FDA, which monitors the website, has the power to fine companies that do not comply, it has never actually done so (*The Economist*, 2015g).

### *AllTrials international campaign*

The assumption is that the missing trials tend to be those that show drugs in a less flattering light. Drug companies have an obvious incentive to play such trials down. Academic researchers may often prefer not to waste time on writing up results which show little effect. As an overall result many medicines look better than they are. Ben Goldacre, a British physician and author, is one of the instigators of an international

campaign called AllTrials which is designed to force researchers to publish all of their clinical trials. One drug he mentions is an antidepressant called reboxetine (branded as Edronax by its maker, Pfizer) that he used to prescribe to his patients. He said a couple of trials were published showing that this is as good for depression as any other drug. However unpublished data collected in trials involving three times as many people as those who participated in the published trials showed it was not (*The Economist*, 2015g). Something similar is true in the case of another class of antidepressant – selective serotonine-reuptake inhibitors (SSRIS), such as Prozac. These were introduced in the late 1980s but it was not until 2006 that an analysis of all clinical-trial data on SSRIS, submitted to the FDA over the years, showed that their use by adolescents was associated with an increase in the risk of suicide. That fact might have been discovered earlier if all those data had been publicly available. An independent review in 2008 showed that 94% of published trials of SSRIS suggested they produced positive results, whereas this was the conclusion of only 51% of the (more numerous, but often unpublished) trials submitted to the FDA (*The Economist*, 2015g).

Even if no medical harm is done financial harm can be. Since 2006 the British government had spent £424 million (US$660 million) stockpiling Tamiflu, an antiviral drug, in order to anticipate an influenza pandemic. At the time the decision was made 60% of the trial data about this drug remained unpublished. Those data have now been analyzed and that analysis raised questions about Tamiflu's efficacy in reducing hospital admissions, and thus about whether creating the stockpile was money well spent (*The Economist*, 2015g). A similar situation occurred in France where the antiviral drug had been stockpiled and the antiflu vaccine as well.

Nor is it just drug trials that are plagued by non-publication. In 1994 a study on surgery for bowel cancer found that, among those whose tumours returned, a second visit to the operation theatre to remove the resurgent carcinoma made no difference to life expectancy of the patients. Had this information been made public then, rather than as it was in 2014, countless very ill patients could have been spared surgical procedures. Also and just as importantly, the wider question of how useful repeat surgery of this sort is on other parts of the body would have been raised sooner (*The Economist*, 2015g).

## *Expected changes*

AllTrials and the groups behind it, such as SenseAboutScience, a British charity, have helped shape legislation that will regulate clinical trials in Europe when it comes into force in 2016. That law, like the existing American one, will require drugs trials to be registered and their results to be published. In the United States, meanwhile, regulators are proposing to write rules that will clarify and extend the scope of the existing legislation on order to remove the wriggle-room that has made them hard to enforce (*The Economist*, 2015g).

On the other hand companies' shareholders are increasingly concerned about the missing data. For instance Helena Viñes Fiestas, who studies companies' financial sustainability at BNP Paribas, a big French bank, stated many owners of drug-company

shares supported the push for publication and are requesting the firms in which they invested to publish plans that will make sure trials past, present and future are registered and their results reported. Long-term investors would prefer the truth straight away to reduce the level of risk in their portfolio. According to H. Viñes Fiestas, *ca*. 30% of a drug company's value is tied to the results of trials. There is also a more direct risk to firms that do not publish all their trial results. Between 2007 and 2014 the 21 big pharmas that H. Viñes Fiestas follows incurred collective fines of US$40 billion. Her studies showed that around half of this sum was a consequence of a lack of clear reporting of side-effects, which are often missing because trials containing these sorts of data go unpublished (*The Economist*, 2015g).

SenseAboutScience is working on an index, to be published by the end of 2015, that will rate pharmaceutical firms according to the extent of their commitment to publish all trials. This will, no doubt, be of interest to shareholders. The future, then, may be more transparent. A few firms have started to open their archives. GlaxoSmithKline published the results of all trials completed since its formation (via a merger) in 2000. Pfizer, founded in 1849, goes back to 2007. In 2015 the Institute of Medicine, the national academy of United States physicians, stated in a report that sharing data from old trials offered both risks and benefits, and should be considered case by case. One of the things the institute worried about was whether participants in old trials, which often happened before technological change made the question relevant, had given appropriate consent for the sort of sharing of their data which modern standards of transparency demand (*The Economist*, 2015g).

As that observation shows digging up the past has its dangers. But the advantages of knowing the truth about past trials mean it is worth trying to overcome these. In any case there is no excuse for not dealing with the trials of the future. Only if that is done will those such as Tim Crater, a research physician at the Hutchinson Clinic in Kansas, who perform the actual testing (T. Crater runs drug tests for big pharmas), be able to look at patients in the eye and tell them that their contribution really is making a difference (*The Economist*, 2015g).

## Quality control of drugs

### *Difficulties to monitor fraud and enforce quality requisites*

On 16 July 2015 the European Union demanded to its member states to stop as of 21 August 2015 the commercialization of 700 generic drugs, tested and made in India. This decision is a follow-up to "irregularities" reported in 2014 during a routine inspection by the French Agency for the Safety of Medicines and Health Products (ANSM, French acronym), on one of the sites of GVKBio, an Indian company based in Hyderabad, Andhra Pradesh State, south of India. The European Medicines Agency (EMEA) that has been alerted reviewed *ca*. 1,000 of drugs tested by GVKBio during the last five years and came to the conclusion that 700 of these drugs did not meet the safety requirements; and in January 2015 it recommended that these drugs be withdrawn from the market. Several countries, including France, Germany, Belgium and Luxembourg followed the EMEA recommendations (Bouissou and Hecketsweiler, 2015).

The "irregularities" pointed out by the French ANSM concerned clinical trials conducted by GVKBio between 2008 and 2014, with a view to establishing the bioequivalence of locally produced drugs with that of 25 generic drugs withdrawn from the French market (e.g. painkillers, antidepressant, antihypertensive and antiinflammatory drugs). Before being withdrawn from the market the 25 drugs targeted by the ANSM had been utilized by European patients. What had been their implications? According to Gaëtan Rudant, inspection director at the ANSM, "we should weigh the risks and benefits (of forbidding the sale of a drug). The 25 targeted generics are not widely consumed in France and there are alternative drugs. In Germany where they were also forbidden the situation is more complicated, because the risk of a lack of medicines is a real one" (Hecketsweiler, 2014j).

Regarding the 700 drugs their incommercialization has been suspended by the EMEA and the European Union; they have been listed on the EMEA website. They are copies of current drugs, such as Advil (painkiller), Seroplex (antidepressant), Aerius (antihistaminic) or Inexium (stomach protection). Several big generic manufacturers have to do with the withdrawal of these drugs: the Israeli Teva, the world's leader, the American Mylan and Abbott, the Indian Ranbaxy and Arrow, the French Sanofi. Both the French ANSM and EMEA commented that the drugs whose commercialization was suspended, did not present any risk for human health and their therapeutic efficacy was proved. They may be utilized if there is no alternative. Their withdrawal from the market was based above all on the lack of "fulfilment of good clinical practices." However the manufacturers of these drugs were given almost half a year (until January 2016) in order to provide the lacking clinical data. In France the health ministry stated: "This withdrawal should not put in question the trust in generics. It shows, conversely, that the methods of quality control are efficient and that we have the capacity to control what is going on in the sites of trials and production, including overseas" (Bouissou and Hecketsweiler, 2015).

In a press release GVKBio, a subsidiary of an Indian giant construction company, questioned EMEA's conclusions and declared that "it was disappointed by the fact that, despite a lot of intents on its side, a scientific dialogue could not be initiated." It also indicated that it proposed to carry out new trials at its own cost. Thereafter upon the request of GVKBio, an expert committee appointed by the Indian government, has conducted a counterenquiry and concluded that there was no anomaly in GVKBio clinical trials and quality-control practices. In April 2015 the Indian foreign-trade secretary, Rajeev Kher, threatened the European Commission that India would even bring this affair to the World Trade Organization (WTO) if the withdrawal of GVKBio drugs from the European Union market were confirmed (this was the case in July 2015). It was not however the first time when the quality of clinical trials carried out in India had been criticized. At the end of June 2015 the WHO issued a Notice of Concern regarding the Indian company Quest Life Sciences, after having found irregularities during clinical trials similar to those noted in the case of GVKBio. During their visit to Chennai (ex-Madras) the inspectors had caught laboratory technicians hastily filling missing forms, by postdating them. The same electrocardiograms had been found with several names of patients at different dates. In its mail to Quest Life Sciences the

WHO went farther and explained that these problems in India "seem to be systemic in nature and occur many times" in the case of other Indian companies (Bouissou and Hecketsweiler, 2015).

### *Role of India*

The reputation of India's pharmaceutical industry may be tarnished even more by the withdrawal from the European Union market of 700 generics, tested and made in this country. In 2013-2015 the United States, which import 40% of their generics from India, have made numerous warnings and decisions aimed at forbidding the import of products from several factories (e.g. leaders of the generics sector like Wockhardt which were requested not to get the supplies from some Indian companies). The FDA trebled its inspections of factories between 2009 and 2013, and strengthened its staff in India. Since 2013 four factories of the large Indian generic producer, Ranbaxy, had been banned by the FDA. Located in Dewas, Paonta Sahib, Mohali and Toansa, the factories have been producing drugs similar to several medicines prescribed in the United States. Before this ban Ranbaxy had to bring back to India significant volumes of Atorvastatin pills – a generic of Pfizer's blockbuster anticholesterol drug, Lipitor – because they had been contaminated with glass particles. Ranbaxy also acknowledged it had marketed adulterated antibiotics and antiepileptic drugs. In 2013 Ranbaxy was sentenced in the United States to pay a fine of US$500 million (or €406 million), because it had systematically falsified quality-control tests. "Ranbaxy has used fraud as a competitive advantage in order to expand in the United States," stated a few months later Vince Fabiano, the former vice-president of the group (Bouissou, 2015).

India is the world's first supplier of generics and it seemed to have emphasized lower production costs in its global expansion, but to the detriment of product quality. It is the future of the Indian pharmaceutical industry that is at stake, particularly when India has such strong competitors as China, Indonesia or Thailand. Improving the quality of its pharmaceutical products is expected to be a long and uneasy task. According to a study published in June 2015 by the consulting firm Deloitte 64% of Indian laboratories lacked qualified staff and 52% of trained personnel had difficulties in complying with the norms of the European and United States health agencies. The United States send inspectors only to factories that supply them. Other countries which do not have the same means are more harmed by the bad quality of Indian drugs. A study by the American National Bureau of Investigation in Economy, carried out in 2014 in 22 cities across Africa, concluded that 10% of antibiotics or treatments against tuberculosis, imported from India, were not efficient because they had a lower amount of the bioactive principle (Bouissou, 2015).

According to the WHO one out of five drugs commercialized in India is counterfeit, and in a country where the pharmaceutical industry is very fragmented, inspections are not easy to conduct; also the national agency for the control of drugs is understaffed. In a document published by the end of 2013 the ministry of petrochemistry and pharmacy clearly stated that quality standards of the pharmaceutical industry will be improved thanks to the construction of quality-control and certification laboratories, as well as to the training of technical staff (Bouissou, 2015).

Also by the end of 2013 the Indian Supreme Court stated that "clinical trials are a paradise for pharmaceutical laboratories, but they finally become a hell for India," and, in the light of former reports on the deleterious impact of clinical trials conducted between 2005 and 2012, decided to suspend these trials until new regulations are issued. In June 2015 the law was amended and new ethical committees were to be set up in order to check that the patients give their consent to participate in clinical trials and receive a financial compensation if an accident occurs. Also the medical follow-up of the patient must be fully documented and classified. India has not yet become the world's hub of clinical trials but intends to do so. With *ca*. 16% of global population in 2015 a great ethnic diversity and almost one out of five diseases reported across the world, India is an ideal place to test a large number of medical compounds. In 2015 only 2.7% of clinical trials carried out across the world were conducted in India, but this figure has risen 3.7% since 2007, whereas it went down in Europe and the United States. According to the consulting firm Frost Sullivan the revenues of the clinical-trial sector amounted to US$485 million in India in 2010-2011 and they were expected to double by 2016. Furthermore the Indian government wanted to propel the country as "the world's biggest supplier of quality drugs at reasonable prices" (Bouissou, 2015).

### *Controlling the quality of drugs: the road ahead*

"The laboratory which commercializes a drug under its brand is not necessarily that which manufactures it. It often relies on a cascade of subcontracted producers. And at the end of the chain one finds a limited number of factories, often Asian, which supply the whole world," stated Alain Astier, head of the pharmaceutical department at Henri Mondor hospital in Créteil, south of Paris. According to him traceability has become an illusion within this complex network, where health agencies cannot monitor everything. In Europe, however, they pooled their efforts, under the lead of the European Directorate for Drug Quality (EDDQ), a Council of Europe body that delivers to each drug commercialized in Europe a certificate based on the compliance with "good practices." Since 1999 this small body has been staffed with four inspectors who control *ca*. 50 production sites per year, in addition to those inspected by national agencies (e.g. the ANSM has eight inspectors who visit sites located outside Europe), following a planning agreed mutually (Hecketsweiler, 2014j).

"Around 20% of the sites which the EDDQ considers risky do not abide by the rules of quality control," commented Susanne Keitel, director of the EDDQ in 2014. Since 2011 the EDDQ had withdrawn its certification to two Chinese manufacturers of diclofenac (the most consumed antiinflammatory drug in France), to a French laboratory specialized in the production of paracetamol, to an Indian producer of metformin (a widely consumed and not-expensive antidiabetic drug) and to a producer of amoxicillin (a largely prescribed antibiotic) located in Bombay. Such decisions were made after having observed several kinds of problems: badly maintained factories, deficient quality-control processes, falsified data, hazardous dosage. In the latter case "one can find a 10% to 20% range of variation with regard to the standard dosage, when the authorized maximum variation is 5% (Hecketsweiler, 2014j).

Several inspectors come to the conclusion that locating production overseas has affected the quality of drugs. This concern is shared by the pharmaceutical companies and some of them are thinking to bring back their production to Europe, asserted S. Keitel. On his side, Sébastien Aguettan, the head of Delpharm, a French subcontracted drug producer, is struggling to create a "made in Europe" label in order to better inform patients and to value the know-how of European manufacturers. Furthermore he thinks that it is not just quality that matters, but it is also an issue of sovereignty. Nowadays 80% of chemical powders used to manufacture the most widely consumed drugs are imported from China and India. And this dependence could raise problems. "When the A(H1N1) influenza virus was detected in France the government asked us whether we could produce more paracetamol. We had not enough stocks and there are only two factories that produce paracetamol in the world, one in China and another one in the United States," mentioned S. Aguettan. "The Americans have struggled to keep one factory in the United States," so as to be less dependent on suppliers from overseas. The last European site for paracetamol production, located in France, was closed down in 2008 by the chemical company Rhodia (Hecketsweiler, 2014j).

## Negotiating and setting the price of drugs

Negotiating the price of their drugs with the government's health-care authorities is a key strategic issue for the development of pharmaceutical companies (and their biotechnology-firm partners). The price that is finally agreed upon should enable these companies to remain competitive and to invest in research and development (R&D), so as to have and broaden a portfolio of promising drugs. But not all pharmaceutical innovation is valuable. Though some drugs are breakthroughs, some offer marginal benefits at exorbitant cost. On the other side national health-care authorities are eager to lower the price of drugs in order to alleviate the enormous budgets (and debts) devoted to cover the drug expenses of all citizens who have a health-care insurance or are affiliated to a social-security system, e.g. in many European countries.

### *Cost of developing innovative drugs: an important factor in setting their price*

A study by the Tufts Center for the Study of Drug Development, a university institute partly subsidized by the pharmaceutical industry, has estimated at US$2.6 billion the cost for developing one new drug. This amount takes into account the cost of the failures in the development process (e.g. 80% of anticancer-drug candidates never reach the market) and it also includes the interests (profits) that would have cashed the investors if they had put their money elsewhere, the total being estimated at US$1.1 billion. These estimates have been questioned, particularly by the French NGO Doctors Without Borders (MSF, French acronym), in November 2014, before the publication of the Tufts Center's study. A few months earlier Andrew Witty, head of GlaxoSmithKline (GSK) in the United Kingdom, admitted himself that the one-billion-dollar production cost of a single new drug is "one of the greatest myths of the pharmaceutical industry." He added: "It is perfectly possible to improve the efficiency of our research and make sure that the resulting savings have an impact on the price of drugs." In this regard a survey made by Deloitte of 12 pharmas showed that the average development cost of a drug ranged between US$315 million and US$2.8 billion (Hecketsweiler, 2015p).

## *R&D costs versus marketing and advertisement costs*

Although R&D costs are considered a justification for a high drug price, sometimes R&D expenses are lower than those devoted to marketing and advertising drugs. For instance Novartis, the world's biggest pharma at the beginning of 2015, spent US$15 billion in 2015 for the commercialization and promotion of its drugs, compared with less than US$10 billion for the development of new compounds. In the case of Bristol-Myers Squibb (BMS) both kinds of expenses reached the same amount of US$4 billion approximately. However Roche seems to be one of the very few big pharmas that invests more in research and development (*ca*. a little less than US$9 billion) than in marketing (*ca*. US$6 billion).

Denouncing the advertisement of drugs on television screens in the United States

The United States are the only country in the world that authorizes the advertisement on television (TV) screens of prescribed drugs. The big pharmas are touted by the great television channels; they spent US$4.5 billion (or €4.1 billion) in 2014 in order to promote their molecules, 30% more than two years earlier. Pfizer is the leading group with US$1.1 billion spent in advertising its drugs, including US$246 million for Lyrica, an antalgic and antiepileptic drug, US$232 million for Viagra and US$221 million for Eliquis, an anticoagulant drug prescribed for preventing strokes. In 2015, according to the data gathered by Kantar Media, the advertisement investments made by the pharmaceutical groups rose 10% during the first quarter and 13% during the second, although the market was on the slump. Among the drugs mostly advertised the antihepatitis-C drug Harvoni, launched in 2014 by Gilead Sciences, was expected to require US$150 million. In October 2015 Johnson & Johnson spent more than US$13 million for advertising on TV screens its drug Invokana, according to the data published by the study firm iSpot.tv. This antidiabetes drug could be chosen by the 29 million Americans who suffer from diabetes; even though most of them cannot afford a therapy whose annual cost can reach US$ 4,600, i.e. 15 times more than the currently utilized drug, metformin (Hecketsweiler, 2015z).

A poll published in October 2015 by the Kaiser Family Foundation (KFF) has shown that advertisement is a significant leverage for pharmaceutical groups. One adult person out of eight claimed that he had obtained a prescription for a drug after having discussed with a physician an advertisement (on TV screens) which highlighted its merits. According to half of the interviewed persons these TV spots provide a clear information on the disease targeted by the drug, the benefits of the latter as well as its side-effects. In the opinion of Sharon Levine, one of KFF's spokespersons, the impact of this communication campaign is a source of real concern. "The amounts of money invested in marketing by the pharmas have an impact on the price of drugs and the communication campaigns lead the consumers/patients to choose branded drugs instead of generics." That is the case of Nexium, a drug produced by AstraZeneca whose patent has expired, but which is still highly appreciated by Americans further to advertisement clips which are systematically shown on TV screens (Hecketsweiler, 2015z).

On 27 November 2015 the American Medical Association (AMA) – an influential association which defends the physicians' interests and the ethics of the profession – expressed its serious worry regarding the impact of advertisement of drugs on health expenses – a major issue of the campaign for the election of the president of the United States in November 2016. The AMA's president, Patrice Harris, stated that "the multiplication of advertisement TV spots triggers the demand for the most expensive molecules, while there are less costly and still efficient alternatives; it also encourages the patients to demand the most recent and expensive drugs even though they are not always the most appropriate" (Hecketsweiler, 2015z).

Sharon Levine of KFF added that 'the advertisements whose allegations are often at the limits of legal regulation entice the patients to exert pressure on physicians in order to obtain prescriptions for these drugs which are not always efficient." She quoted the example of Opdivo, an anticancer drug of Bristol-Myers Squibb (BMS), that has been approved for the treatment of some lung cancers. "The advertisement spot shows patients looking at a skyscraper, with a slogan 'A chance to live longer'. In fact this molecule that is very expensive increases life expectancy by only three months compared with conventional chemotherapy." In her opinion this is a scandal. It should be underlined that the poll conducted by KFF showed that the cost of prescribed drugs is a matter of concern for 77% of Americans. No wonder then that the candidates for the November 2016 presidential election have dealt with the inflation in drug prices (Hecketsweiler, 2015z).

### *Linking the price of drugs to their efficacy*

On 2 April 2015 the Swiss pharmaceutical group Roche presented a programme aimed at monitoring patients treated with its anticancer drugs, with a view to linking the cost of the treatment to the efficacy of the drug used. Also the Swiss big pharma Novartis stated that in the future the pharmaceutical companies will be paid according to the results obtained. This approach – satisfied or reimbursed – was applied in 2014 to Gilead Sciences' Sovaldi – an antihepatitis-C drug – whose price (*ca*. €41,000 per patient in France) was considered a threat to the French social security's budget. Gilead accepted the new deal, i.e. it will to a large extent reimburse the cost of the treatment if the latter failed (Hecketsweiler, 2015h).

#### Performance contracts

These so-called performance contracts were considered a very useful tool for the Economic Committee for Health Products (CEPS, French acronym) – the French body which negotiates the price of drugs with the pharmaceutical companies. This approach must be based on an evaluation "in real life" of the drug's efficacy that can be different from the efficacy recorded during clinical trials (Hecketsweiler, 2015h).

Celgene – a large American biotechnology company – commercializes the drug Imnovid against rare blood cancers. The CEPS agreed with Celgene on a high price for its drug: €8,900 per cycle of treatment (patients go through five to six cycles). As a counterpart, Celgene, in August 2014, committed itself to reimburse the French

social security if the treatment failed. To that end Celgene set up a register aimed at monitoring the patients in real time. At the beginning and at the end of the treatment, and at every consultation, the physician should fill a form. All the collected data are made anonymous and thereafter transmitted to a service firm that is in charge of their statistical analysis. *Ca*. 1,000 patients out of the 2,000 that are potentially concerned were listed on the register by early 2015. By the end of the year the number of those patients that do not react positively to the treatment, will determine the amount of funds Celgene should reimburse. "The comprehensiveness of this register is unique," underlined Franck Auvray, Celgene's director. "The very fact that we work on real-life data facilitates the dialogue with the health authorities and this also enables us to consolidate the data recorded during clinical trials." Celgene intends to extend this approach to other molecules of its portfolio, such as the anticancer drug Revlimid, sold at a price of between €155 and €190 per capsule (depending on the dosage of the drug) [Hecketsweiler, 2015h).

Roche has also decided to follow in real life all the women treated with its renowned anticancer drug Herceptin – recommended in the treatment of some breast cancers, at a cost of *ca*. €270 million in 2012 for the French social security. In a first stage Roche hopes to collect the data concerning the present use (at the pilot stage) of Herceptin (e.g. indication, dosage, duration), and, in a second stage, the data relating to its efficacy. In the medium term the Swiss pharmaceutical group hopes to find a formula aimed at linking the price of the drug to its efficacy. "The price of the drug must reflect the differences in performance," stated Corinne Le Goff, chairperson of Roche French subsidiary. For instance Avastin (bevacizumab), the most widely sold anticancer drug in France, is efficient in the treatment of colorectal cancer, less efficient in the treatment of lung cancer and is not interesting in breast cancer. Its price is nevertheless the same for these three kinds of treatments: *ca*. €1000 per dose (Hecketsweiler, 2015h).

Creation of reliable databases

Such approach – satisfied or reimbursed? – implies the "creation of reliable databases, comprehensive and prospective, so as to improve the treatment of patients," stated Luis Teixeira of the Centre of Breast Diseases at the Saint-Louis hospital in Paris, where for several years physicians have been using softwares to better monitor the patients. But there is not yet a national register where all patients are included, as it is the case in Italy (Hecketsweiler, 2015h). The Belgian group UCB has accepted to bear the cost of its drug Cimzia for the patients suffering from rheumatoid polyarthritis and whose health did not improve after three months. The pharmaceutical company trusts the databases of the social security system which record interruptions of the treatment. In 2013 and 2014 UCB reimbursed to the social security the costs of treating the patients who did not react positively to Cimzia. In exchange UCB was able to negotiate a price for its drug that was slightly less expensive than its rivals – €9,900 per year –, while it was not more efficient and arrived late on the market (Hecketsweiler, 2015h).

Other mechanisms

France is not the only country that applies the approach linking the price of the drug to its efficacy or performance. In Germany Novartis signed a similar agreement with the

health and social-security authorities regarding its drug Aclasta, a treatment against osteoporosis; if there is a bone fracture the pharmaceutical group reimburses the cost of the medicine. In the United Kindgom Johnson & Johnson accepted to make a similar deal for its anticancer drug Velcade (Hecketsweiler, 2015h).

When it is too complicated to obtain a result on a patient basis, the drug's performance can be evaluated through the "marks" awarded by the French High Health Authority (HAS, French acronym) and, in particular the mark which shows the contribution of the drug compared with current therapies, i.e. the improvement of the medical benefit (ASMR, French acronym) for the patient. This mark has a scale from I (major improvement) to V (no therapeutic advantage) and it determines, to some extent, the price of the drug. In 2013 90% of the drugs reviewed by the HAS obtained a V mark and only 5% obtained an ASMR of I, II or III. The pharmaceutical companies or laboratories that question the mark awarded to their drug can rely on real-life studies in order to renegotiate the deal and subsequently the price of their medicine(s). Among the first contracts of this kind was the one signed by the French CEPS in 2005 with Johnson & Johnson. The HAS gave a IV mark to Risperdal, a drug used in the treatment of schizophrenia. Despite complementary trials Johnson & Johnson could not convince the HAS to change its evaluation and the American big pharma had to reimburse *ca*. one-third of its revenue to the French social security (Hecketsweiler, 2015h).

### *Reducing the price of drugs in the United States*

All the above-mentioned mechanisms, including the linkage of drug prices with their efficacy and without inhibiting the capacity of pharmaceutical groups to innovate, should lead to a significant decrease in prescription drug prices. In the United States American politicians, such as Hillary Rodham Clinton and Bernie Sanders, both Democrat candidates to the United States 2016 presidential election, have promised to act in this respect. For instance on 22 September 2015 H.R. Clinton presented a plan aimed at alleviating taxes for those pharmaceutical companies which reinvest part of their profits into research and innovation. In the same wane six American States adopted in 2014 laws that requested the pharmaceutical companies to justify the price of their drugs, particularly through indicating the amounts of funds really invested into their research-and-development activities (Hecketsweiler, 2015h,p). A report of the American Senate, published on 1 December 2015, revealed that Medicaid – the health-insurance system managed by the government in order to assist poor families – had spent in 2014 US$1.3 billion to buy two very expensive antihepatitis drugs, produced by Gilead Sciences (Sovaldi and Harvoni). But these funds could help treat only 2.4% of the patients. This led Ron Wyden, a Democrat senator of Oregon, to make the following statement: "The primary objective of Gilead has always been to maximize its profit, without taking account of the human implications ..." "If this price policy is followed by other pharmaceutical laboratories, treating just a portion of the patients will cost billions and billions," he added (Hecketsweiler, 2015h,p; see also pp. 108-109).

Indeed the controversy on the costs of specialty drugs and their affordability to the largest number of patients has given rise, not only to a political and economic, but also to a moral debate in the United States, where business is generally separated from

moral issues. Steve Miller, medical director of Express Scripts – the most powerful pharmacy benefit manager of the United States – which banned many drugs from its lists or forms, stated in an interview published in July 2015 in *The American Journal of Managed Care*: "In the past, pharmaceutical laboratories or companies used to abide by a non-written social contract and commercialized their drugs at a reasonable price in order to be affordable to the patients. This contract has now been broken and drug prices have soared and reached levels that are completely disconnected with respect to the investments made by the companies to develop their molecules." This kind of discourse was rather unusual in the United States. But it expresses a real concern among patients and the American pharmacy benefit managers such as Express Scripts and its rival CVS Health, which has also banned from its lists a large number of specialty and expensive drugs, including for instance Bristol-Myers Squibb's Abilify, prescribed for the treatment of schizophrenia and whose costs for the American health-insurance system amounted to US$3.8 billion in 2014 (Smith, 2015).

It is true that drug business is more profitable than ever. According to the consulting firm McKinsey the pharmaceutical sector represented in 2013 10% of the profits recorded through the stock index S&P 500 (Standard & Poor 500) which corresponds to 500 big American companies listed on the stock exchange, compared with 6% of the S&P 500 index in 1990. Globally the average net profit margin of the big pharmas is *ca*. 16%. Only real-estate business, finance, tobacco and software development are more profitable (Hecketsweiler, 2015h,p).

Reference pricing

According to Austin Frakt, a health economist with several governmental and academic affiliations, there is a way to keep prices low, while encouraging drug companies to innovate: reference pricing. This has led to drug-price decreases and significant savings in the Canadian province of British Columbia, as well as in Germany, Italy, Norway, Spain and Sweden. A study published in *The American Journal of Managed Care* found that price reductions ranged from 7% to 24% (Fendrick, 2015; Frakt, 2015). Reference pricing works in the following way: drugs are grouped into classes in which all drugs have identical or similar therapeutic effects. For instance all brands of ibuprofen would be in the same class because they contain the same bioactive compound or molecule. The class could include other non-steroidal antiinflammatory agents like aspirin and naproxen because they are therapeutically similar. The insurer pays only one amount, called the reference price, for any drug in a class. A drug company can set the price higher, and if consumers want that one they pay the difference. Setting the reference price low enough therefore puts considerable pressure on drug manufacturers to reduce prices for drugs for which there are good substitutes. If they don't consumers will switch to lower-cost products. In British Columbia and in Italy the reference price is set at the lowest-price drug in the class; Germany uses an average price across drugs; Spain also uses an average, but only of the lowest-priced products that account for at least 20% of the class' market (Frakt, 2015).

Impact of reference pricing

In pushing prices down reference pricing does not squelch innovation; it encourages a different form of it. The market still rewards the invention of a cutting-edge drug with novel therapeutic effects. Such a drug might be placed in a new class and therefore

could be priced high. But, within classes, the market also rewards innovations that lead to lower-priced drugs, because consumers switch to them to avoid out-of-pocket costs. In this way reference pricing promotes cost-effectiveness. For instance the price of new anticholesterol (LDL) drugs known as PC-SK9 inhibitors (see p. 326) is *ca*. US$14,000 a year (in 2015 in the United States), and a report from the Institute for Clinical and Economic Review (ICER) received considerable attention when it argued that the drugs were priced too high for the value they offered to patients. Reducing the prices to close to US$2,000 would make them both cost-effective and would help keep American health spending below a widely accepted growth target, according to ICER analysis. Reference pricing could keep drive down the prices of the PC-SK9 inhibitors if they were put in the same therapeutic class as other, cheaper generic anticholesterol drugs like statins. If that happened PC-SK9 inhibitors manufacturers – Amgen and Regeneron Pharmaceuticals – would face powerful incentives to reduce their prices. However some people might reasonably argue that PC-SK9 inhibitors are superior to statins and therefore they should not be grouped in the same class. Because ICER price is based on cost-effectiveness, it incorporates such performance differences by recommending a high price for more efficient drugs, although in the case of PC-SK9 a lower price than the manufacturers may want (Frakt, 2015).

And such a situation raises the issue of a reasonable profit margin for the pharmaceutical groups. "What is a reasonable profit?" asks Andrew Hill, a pharmacologist and researcher at Liverpool University, United Kingdom. "Nowadays the price of drugs is less associated with the profits they generate than with the relation of power between the big pharmas and the health (or disease) insurance systems," explained A. Hill. He thinks that this explains the difference between the drug prices in the United States and in Europe where they are three to four times less costly (Hecketsweiler, 2015h,p).

The promise of reference pricing goes beyond prescription drugs. In a paper presented at the Brookings Institution – an independent organization of social and economic studies – in October 2015 the Harvard University economist Amitabh Chaudra, the University of Michigan law professor Nicholas Bagley and Austin Kraft proposed extending the approach to a wider range of medical technologies. They suggested that Medicare should pay more for a new therapy for a given condition only if that new therapy is better than existing ones. In no case the authors of the paper proposed, should Medicare pay more for a therapy than a generally accepted cost-effectiveness standard. If patients wanted cost-ineffective therapies they could pay the difference out of pocket, a departure from current Medicare policy (Frakt, 2015).

For instance Peter B. Bach, a physician at Memorial Sloan Kettering Cancer Center, New York, proposed a variation on reference pricing that considers how the cost-effectiveness of a cancer drug varies by what disease it is used to treat. He noted that the drug Tarceva costs the same whether it is used to treat patients with a kind of lung cancer or patients with pancreatic cancer. But the results are quite different: on average Tarceva extends a lung-cancer patient's life by just over three months; it extends a typical pancreatic-cancer patient's life by a mere week and half. P.B. Bach therefore proposed that the price of Tarceva be sharply reduced for pancreatic-cancer patients to bring the cost per duration of life gained in line with that of lung cancer

patients (Frakt, 2015). A spokesperson of the big Swiss pharma Novartis stated that "the price of drugs is determined by factors that are beyond their production costs and involve the benefits provided to patients and health-care systems." This is in line with P.B. Bach's opinion and proposal, as well as with the shared view that drug pricing should be linked to drug efficacy.

## *Situation in France*

### Pharmaceutical market

In France, as in the United States, pharmaceutical companies are equally concerned about the impact of lowering drug prices on the competitivity of the French pharmaceutical industry. By mid-October 2014 when the draft law on the financing of social security was to be reviewed by a committee of the French National Assembly (parliament), Patrick Desbiens, president of GlaxoSmithKline (GSK)-France, called for a "new economy of the medicine." GSK is the leading international pharmaceutical group present in France, with three industrial sites and two research centres GSK annual investment in research and development (R&D) amounted to *ca*. €50 million for an annual turnover of €880 million in 2013 (GSK global annual turnover amounted to €33 billion). GSK employed 3,300 persons in France and commercialized *ca*. 100 medicines including its antiasthma blockbuster drug Seretide (€6.7 billion sales in 2013 in the world), children's vaccines (Infanrix, Rotarix, Engerix B), antibiotics (Augmentin, Clamoxyl), antidepressant Deroxat and antimalaria drug Malarone (Hecketsweiler, 2014g).

In an interview with the French daily newspaper, Le Monde, P. Desbiens indicated that the French pharmaceutical market was less favourable than formerly. Except for antibiotics and anxiolytic drugs the consumption of medicines in France is close to the European average. But drug prices are often lower than those prevailing in the five leading European countries. France is the world's fifth-largest pharmaceutical market, behind the United States, China, Japan, and Germany. The value of drug exports reached in 2013 *ca*. €26 billion, half of these exports being sent to the European Union. In 2013 the value of the global pharmaceutical market had been estimated at €639 billion. P. Desbiens underlined that drugs made up 15% of health-care expenses in France (in 2012 €525 per inhabitant per year, or 382 "units" per person per year, compared with 456 in the United Kingdom, 329 in Germany and 298 in Italy). But 50% of the savings earmarked in the law for the financing of social security were to be made in drug consumption. Conversely 45% of the health-care expenses corresponded to hospital expenses, but only 15% of the savings were to be made there (Hecketsweiler, 2014g).

In addition to what was considered unfair with respect to the savings to be made in drug expenses compared with other items of the country's social security department in charge of health insurance, the pharmaceutical groups pay high taxes in France: up to 40% compared with a 33% average rate for companies. In the United Kingdom the tax rate is 20% and in Ireland even lower. But this situation does not impede the pharmaceutical groups located in France to have an annual turnover of *ca*. €50 billion,

almost half of it in exports. P. Desbiens indicated that there were still 220 factories in France, but most of them produce old drugs which are mostly exported to developing or emerging countries that may become able to produce these drugs. Out of the 72 drugs approved by the European Medicines Agency (EMEA) in 2013, only six were manufactured in France (Hecketsweiler, 2014g).

Another matter of concern is, according to P. Desbiens, the lack of flexibility on the French labour market which is not an incentive for investors. Thus GSK invested €1 billion in the United Kingdom during the two-year period 2013-2014 and did not select France for building a new factory there. Other countries, like Singapore, are becoming competitive and attractive for pharmaceutical companies in terms of research and development and local production of their drugs (Hecketsweiler, 2014g).

Pricing of drugs

The time lapse for a new drug to reach the marketing stage is another issue. Once a medicine has been approved for commercialization at the European level, it is reviewed by the French High Health Authority (HAS) with respect to its therapeutic value and thereafter the Economic Committee for Health Products (CEPS, French acronym) determines the price of the drug. This takes about a year (360 days). It would be more efficient to authorize at once the commercialization of the drug, as it is done in many countries. This would enable the patients to get access to an innovative therapy more rapidly. And the harvesting of medical data "in real life" would permit negotiations on the price of the drug that can be adjusted according to the results of treatment and drug efficacy (Hecketsweiler, 2014g).

For France to remain attractive and to keep its drug factories, P. Desbiens was of the opinion that the price of a medicine should be linked to its production site. In this respect the French government should not be afraid of saying: "We agree on such a price and guarantee it for so many years, and in exchange you commit yourself to invest and produce in France." The price of a drug is mutually agreed between drug manufacturers and the CEPS, on the basis of "marks" (I to V, see above, p. 97) which the HAS gives to each drug to evaluate its therapeutic value (Hecketsweiler, 2014g).

According to Jean-Michel Joubert, director of governmental issues at the Belgian chemical group UCB, "the principle of satisfied or reimbursed" seems fair, because "we are paid for the value we bring in," he explained; and he hopes that physicians will be receptive to this approach when they will have to choose among several medicines. But all pharmaceutical groups do not support this approach, stated Dominique Giorgi of the CEPS. He quoted the case of a biotechnology company that was ready to commercialize a drug for treating a rare respiratory disease. "As a counterpart of the high drug price it requested, we wished that it could guarantee that the patients taking its drug will keep a certain respiratory capacity," told M. Giorgi. "It refused to do so, because it thought that this requisite was too random. We therefore went back to a more conventional negotiation" (Hecketsweiler, 2015h).

To reduce the burden of the overall bill of medicines for the French social security the CEPS has been trying for many years to obtain lower prices from drug manufacturers, mainly correlated with the volumes of prescriptions. The CEPS can also decide to put a ceiling for the sales of a pharmaceutical company or laboratory and force it to reimburse all the revenue gained beyond that threshold. These conditions had been applied since 2014 to Gilead Sciences antihepatitis-C drugs, Sovaldi and Harvoni, as well as to other treatments of hepatitis C, in addition to the enforcement of the policy "satisfied or reimbursed." As a result the bill for the state has plummeted to €650 million from €1.2 billion. By early April 2015 Gilead Sciences was given only a few days before sending its voucher to the French social security (Hecketsweiler, 2015h; see also pp. 108-109).

On 25 March 2016 a decree was published in the *Journal Officiel* (Official Gazette) of the French Republic, where the conditions of reimbursement of the price for a drug were indicated. From now on this reimbursement will be based only on scientific and medical criteria, i.e. the "mark" delivered by the HAS and concerning the therapeutic benefit provided by the drug compared with that of current treatments. The improvement of the medical benefit for the patient or ASMR ranges from mark I (major benefit) to mark V (lack of therapeutic benefit). Only those drugs which obtain a "good" mark (ASMR I to III) will be put on the HAS list. There will be no exception to the new rule as this was the case earlier on with drugs having an ASMR IV to V (Santi and Hecketsweiler, 2016).

Some complained that there was not enough dialogue before the publication of the decree. Physicians also expressed their perplexity. "We are fully conscious of the fact that the prescription of costly molecules should be regulated and that the clarification of the rules regarding the listing of drugs on a table with their ASMR marks is a good thing," indicated Jean-Yves Pierga, an oncologist at the Institut Curie in Paris. "The debate is more about the evaluation of drugs: on which scientific and medical criteria is it based? How to take into account the data on the efficacy of drugs in real life?", he added. The French oncologist also raised the issue of the freedom that will be given to the health centres regarding the prescription of drugs that are taken out of the list (Santi and Hecketsweiler, 2016).

The stake for the French health ministry is to reduce the overall bill of drugs: the list of these costly molecules, called "on the top list" and created in 2004, included in 2016 *ca*. 100 drugs (e.g. many anticancer drugs and medicines for rare diseases). Their cost has been increasing: more than €3 billion in 2014 compared with €2.6 billion in 2012. On 14 March 2016 in the French daily newspaper *Le Figaro* two oncologists, Dominique Maraninchi and Jean-Paul Vernant, supported by 110 colleagues, signed an article warning the public opinion: "Confronted with the inflation of drug prices fixed by pharmaceutical companies that tend to optimize their profits, real threats exist with respect to the fair access of patients to innovative treatments of cancers, as well as to the sustainability of our health-care system based on solidarity." Catherine Simonin who administers the French League Against Cancer explained: "The prices of innovative drugs are excessive, they take into account only the 'medical benefit

for the patient' instead of the real costs. Nowadays the treatments are increasingly targeted, one drug can be efficient for a very small group of patients." The League Against Cancer is relentlessly requesting more transparency in fixing drug prices and in the reimbursement of innovative medicines. "The price must be fair, relevant and equitable. Formerly it was based on research and development, now it depends on the ASMR, without any ethical or socially-responsible thoughts; the economic stake prevails, while pharmaceutical companies are taking patients as hostages," denounced the lawyer Giovanna Marsico, director of the association Cancer Contribution (Santi and Hecketsweiler, 2016).

### *Pricing of anticancer drugs: a major issue for health-insurers or social-security systems*

The market of anticancer drugs is by far the most important at global level. Estimated at *ca*. US$80 billion in 2014 it should jump to *ca*. US$155 billion in 2020, according to the predictions made by Evaluate Pharma, another consulting firm. For instance the profits drawn from the commercialization of Glivec reached US$4.7 billion in 2014; the amounts had been US$1.3 billion for Tarceva and US$1.5 billion for Sprycel. In the United States the prices of anticancer drugs had been multiplied by 10 since 2000, from US$5,000 to US$10,000 per year (average), and up to more than US$120,000 in 2014. During that period the average annual income of an American family (of four persons) fell down by 8% to US$52,000. During the summer of 2015 a hundred of renowned American oncologists alerted the health-care authorities about this high inflation in anticancer drug prices (Hecketsweiler, 2015t).

Are really these high prices justified? For instance Glivec is a very efficient drug for treating rare blood and bone-marrow cancers. Commercialized in 2001 he has changed the condition of many patients whose life expectancy was until then very short. But Glivec is an expensive drug: more than US$100,000 or €90,000 per patient per year in the United States. How Novartis, the big Swiss pharma that produces Glivec, has calculated the price of its drug? This is one of the best kept secrets of the pharmaceutical industry. However, in the opinion of Andrew Hill, these drug prices are completely disconnected from the expenses really spent by the company. According to his calculations which he presented at the European Cancer Congress, held in Vienna, Austria, on 27 September 2015, the production cost of imatinib, the active substance of Glivec, is between US$350 and US$700 per kilo. Adding the cost of the other stages of drug production, transport and a profit margin of 50%, the drug should be sold at less than US$200 per patient per year. "This estimate is based on the already published data on the production costs of several bioactive compounds and on the proposals we have received from Indian laboratories/ companies," explained A. Hill (Hecketsweiler, 2015t).

A. Hill has also reviewed the case of four other molecules of the Glivec family – tyrosine kinase inhibitors – that are recommended for the treatment of different kinds of cancers. He reached similar conclusions: sold in the United States at US$78,000, US$135,000 and US$137,000 (per patient per year), respectively, Tarceva (produced

by the Swiss big pharma Roche), Sprycel (Bristol-Myers Squibb, BMS, United States) and Nexavar (produced by the German chemical and pharmaceutical company Bayer) could be copied and sold at US$230, US$330 and US$1,300 (per patient per year), respectively (Hecketsweiler, 2015s,t).

Other anticancer drugs, called anti-PD1, such as Keytruda (pembrolizumab), a monoclonal antibody produced by the American big pharma Merck (known in Europe under the name of MSD or Merck, Sharpe and Dohme), are blockbuster drugs. Keytruda was authorized for commercialization in Europe in July 2015 and the results of clinical trials presented in France on 17 September 2015 were considered impressive, to the point that some oncologists are beginning to use the word "cure". Keytruda was expected to be commercialized in France by the end of 2015. Another compound, belonging also to the anti-PD1 anticancer drugs, produced by BMS and named Opdivo (nivolumab), contributes to opening a new era for cancer treatment (Hecketsweiler, 2015s; see also pp. 310-311).

Global sales of anti-PD1 and anti PDL-1 (an analogous group of compounds) drugs were expected to reach the record figure of US$33 billion in 2022 due to the high price of these molecules: up to US$150,000 per patient per year in the United States. In France this is a matter of serious concern; the cost of Keytruda and Opdivo as well could reach €100,000 per patient per year, but the total bill for the French social-security system is not easy to assess. Negotiating the price of these drugs is the purview of the Economic Committee for Health products (CEPS). In determining the price of drugs the CEPS often plays on the rivalry between pharmas so as to strike the best deal. Moreover the French social-security department in charge of medical insurance will carefully watch and oversee the correct use of the prescribed drugs, so as to optimize anticancer treatments and thereby justify the price of the newly commercialized drugs (Hecketsweiler, 2015s).

### *Pricing of antihepatitis-C drugs; a puzzling variation in prices at national and regional level*

Hepatitis C is a viral disease that affects between 130 million and 150 million people across the world (e.g. 200,000 in France) and the death toll is *ca*. 500,000 per year (e.g. 3,000 in France). A major therapeutic progress has been the development of direct action antiviral (DAA) drugs. The latter eradicate the virus in 90% of the cases and they are better tolerated than the conventional treatment based on the use of interferon and ribavirine which cures *ca*. 50% of the patients. These drugs are very expensive: e.g. €74,000 in the United States for a three-month treatment and €41,000 in France (totally covered by the social-security department in charge of medical insurance). The big pharmas justify these costs by stressing their high research-and-development investments. This is the case of Gilead Sciences which manufactures two of these drugs called Sovaldi and Harvoni (Benkimoun, 2015b).

Gilead Sciences success story

Gilead Sciences was a biotechnology startup, founded in 1987 at Foster City, south of San Francisco. By the end of 2011 it acquired Pharmasset, a pharmaceutical firm which had developed several compounds, one of them being at the origin of Sovaldi. This molecule has been tagged as GS-7977. Gilead had to pay US$11 billion (or €9.7 billion) in order to acquire Pharmasset. Both Sovaldi and Harvoni were commercialized in December 2013 and October 2014, respectively. The drugs were sold at a price of US$1,000 and US$1,125 (or €947 to 1,065) a pill, respectively, and Gilead's revenue from the sales of both drugs soared to US$12.4 billion in 2014 and more than US$15 billion for the first nine months of 2015 (Benkimoun, 2015b; Hecketsweiler, 2015y).

Gilead Sciences is also well known for its anti HIV/AIDS drugs. Truvada, a combination of two antiretroviral drugs with a fixed dose, is expected to become the most widely prescribed antiHIV drug. This treatment had been approved by the FDA at the end of 2012 and it had been authorized in France by the end of 2015. The price of Truvada can reach up to US$1,500 per month and in 2014 its sales worldwide brought in a revenue of US$3.3 billion to Gilead. Investors have been attracted by Gilead success story so that the stock value of the company rose to US$150 billion from US$30 billion over five years (2010-2014), i.e. more than the French big pharma Sanofi (US$110 billion). With global sales estimated at US$30 billion in 2015 Gilead Sciences was propelled among the world's top big pharmas (Hecketsweiler, 2015y).

Gilead research-and-development budget amounted to *ca*. US$3 billion in 2015. Norbert W. Bischofberger who leads the company R&D department explained that "now, after we have found a treatment against hepatitis C, we turn to hepatitis B for which there is not yet an efficient treatment." Market in this area is promising because, according to the WHO, 240 million people suffer from an infection caused by the hepatitis-B virus, compared with 130-150 million for hepatitis C. But the real primary target of Gilead is the treatment of AIDS. "We have developed a wide range of drugs with a single uptake, which enable the patients to have an almost normal life, but our objective is to cure them," stated N.W. Bischofberger who nevertheless admits that this is still "a very long-term objective" (Hecketsweiler, 2015y).

N.W. Bischofberger who gained almost US$7 million in 2014, is one of the discoverers of Tamiflu, the drug recommended worldwide for the treatment and prevention of influenza. Launched in 1999 this antiviral drug is commercialized by the Swiss big pharma Roche because at that time Gilead Sciences was too small to be able to conquer the global market. Tamiflu sales had already brought a revenue of *ca*. US$20 billion to Roche, although some experts expressed doubts about its efficacy. "We have relentlessly tried to find a more efficient drug, but up to now we have not been successful," indicated Joe Hesselgesser, a biologist at Gilead (Hecketsweiler, 2015y).

A competitor of Gilead Sciences, the Cambridge-Massachusetts-based Idenix Pharmaceuticals, has developed three antihepatitis-C treatments, two of them belonging to the same category as Sovaldi (DAA drugs). The kind of scenario that

led Gilead to acquire Pharmasset had most likely enticed the American big pharma Merck to buy on 9 June 2013 Idenix Pharmaceuticals for US$3.85 billion (or €2.8 billion); this amount was 3.3 times the value of Idenix before the announcement of the deal by Merck and that was considered a record figure by Bloomberg. As a result the share jumped to US$23.79, a 229% increase. As in the case of Pharmasset the value of Idenix Pharmaceuticals may seem too high for a company that had accumulated losses of more than US$800 million since its creation in 1998 and that had not commercialized any drug. But the development of antihepatitis-C drugs changed the situation. "Two big rivals of Merck, Johnson & Johnson and AbbVie, were willing to buy Idenix Pharmaceuticals and consequently Merck did not want to miss this opportunity, because it was above all interested in the combination of Idenix Pharmaceuticals products with its own treatment in order to create a new drug that can compete with Gilead Sciences Sovaldi," explained John Mondoloni, associate at Wombat Capital, a consulting firm in mergers-acquisitions, based in New York (Lauer, 2013).

On the legal front Idenix sued Gilead Sciences because of an infringement of patent rights, while Merck also initiated a procedure along the same way. The purchase of Idenix Pharmaceuticals by Merck had an indirect positive impact on Achillion Pharmaceuticals, one of the last independent biotechnology firms working on hepatitis-C research and development. The share of Achillion rose 57%, as investors were foreseeing a public offering for purchase of the same type as that negotiated by Merck (Lauer, 2013).

Also on the legal front, on 10 February 2015 the NGO *Médecins du Monde* (MDM, Doctors of the World) focused its attacks on the patent granted to Sovaldi by the European Patent Office (EPO) since 2013. While recognizing a major therapeutic progress MDM challenged that patent. The active principle of sofosbuvir is derived from a molecule having the code RO2433. This molecule cannot penetrate into liver cells infected with hepatitis-C virus (HCV); to do so a chemical group must be added to the molecule so that it can be transformed in the hepatocytes into an active form called RO2433-TP. Sofosbuvir is one of the forms of the original molecule with a chemical group added to it. In 2005 Pharmasset had filed a first series of patents on RO2433 and the forms produced through its transformation in the liver, as well as on several thousand related molecules. These patent requests were still under review in 2015 by the EPO according to MDM. A second series of patents had been filed later on, covering several chemical combinations between RO2433 and the chemical group that facilitates the penetration of the molecule into liver cells. In May 2014 the EPO granted a patent that protects the various forms of RO2433 with the chemical group, including sofosbuvir. This is the patent that MDM challenged, because firstly of "the lack of inventive activity" and secondly because "the object of the patent goes beyond the content of the request as the latter has been initially filed." On the first point MDM argued that the addition of the chemical group which activates the molecule was derived from the state of the technology. That indeed was the result of the research work carried out by the team of Chris McGuigan at the British public university of

Cardiff, Wales, and published in 2007. Regarding the second point MDM underlined that the patent request had been filed before the identification of sofosbuvir. At that time, stressed MDM, sofosbuvir "was just a molecule among thousands of other potentially active compounds, over which Pharmasset claims a priority." At least one year and a half or even two years would be required in order to enable Gilead Sciences to review MDM arguments and respond to them (Benkimoun, 2015b).

Price variations of DAA drugs

In November 2015 a study published in the *Lancet Global Health* showed that the sale price of DAA drugs varied considerably, not only in high-income, intermediary- and low-income countries, but also within each category of countries (Andrieux-Meyer et al., 2015). "Every country can be informed about the negotiations carried out by another country with a view to obtaining more advantageous prices," explained Isabelle Andrieux-Meyer, a medical adviser to the Campaign for Access to Drugs promoted by the French NGO Doctors Without Borders (MSF), who co-authored the article in the *Lancet Global Health*. She added: "Our questionnaire included five questions regarding the certification of the drugs, their price and preferential agreements. About 50 members of the network collaborating with WHO had replied." The survey dealt with half a dozen of DAA drugs or combinations of DAA and collected informations from 38 countries: 14 with high income, 20 with intermediary income and 4 with low income. It was noted that the pharmaceutical groups applied differentiated prices so as to improve the access to drugs in the poor countries. There was therefore no wonder to discover that the price of sofosbuvir in Switzerland (*ca*. €18,500) was much higher than those applied in India or Pakistan (€270), or that daclatasvir (Daklinza, sold by Bristol-Myers Squibb) cost a little less than €13,500 in Germany and 85 times less in Egypt. By contrast MSF noted strong disparities among countries with similar incomes. For instance the price of daclatasvir in South Korea is €1,000, while it is 13 times higher in Germany; that of simeprevir (Olysio, sold by Janssen) jumps from €8,300 in Spain to €13,400 in Australia (Benkimoun, 2015j).

The study also pointed out some "aberrations" in intermediary or low-income countries. For instance Côte d'Ivoire paid sofosbuvir at a price that was almost three times higher than India did, despite a significantly lower income. South Africa bought simeprevir at a price that was six times higher than Brazil did, while its income was lower. Gilead used to sell the combination ledipasvir-sofosbuvir at €19,800 in Turkey, which was more than the price paid by many high-income countries. Like Argentina, Brazil, China, Mexico, Peru and Ukraine, Turkey is not included in the countries where Gilead intends to sign licenses with local generic-drug producers (Benkimoun, 2015j). In France Sovaldi and Harvoni are sold at €41,000 and €46,000, respectively, for a three-month treatment. Launched in September 2015 a rival drug of Harvoni is sold at a 10% lower price and new drugs expected to be commercialized in 2016 will allow the French Economic Committee for Health Products (CEPS) to negotiate even lower prices (Hecketsweiler, 2015y).

Regarding the existing disparities in terms of prices of DAA drugs several hypotheses have been suggested in order to explain the current situation, bearing in mind that the negotiations occurring around the agreed prices are very secret. These assumptions include the role of renowned persons who support the pharmaceutical company's requests; the skills of negotiators; financial counterpart (e.g. in France Gilead is supposed to reverse part of the revenue derived from its sales after a threshold is reached); the wish of the pharmaceutical company or group to brighten its image through conceding lower prices or to be more present on a market. Even though a lower price is obtained "patients should be able to buy the drug," underlined I. Andrieux-Meyer who is concerned about the risk of monopoly, due to the late response by Gilead's competitors. "One has the feeling to be strongly tied up to the will of a company to speed up or not. Very efficient compounds are being marketed, but MSF cannot always use them," she stated. And the French medical adviser added: "The recommendations regarding the treatment of hepatitis C are changing rapidly. Should one therefore have no other option than recommend what is financially accessible" (Benkimoun, 2015j).

Access to Gilead drugs: economic and social issues

The Kaiser Permanente (KP) group, based in California, is one of the pillars of the United States health-care system. This health-maintenance organization founded in 1945, had more than 10 million affiliates in 2015. Sharon Levine, the physician who manages KP medical network in northern California, took the example of Sovaldi and indicated that it is out of question to refuse a treatment as efficient as Sovaldi to the patients suffering from hepatitis C, but the drug price is outrageous: it is threefold the price announced by the discoverer (Pharmasset) of the bioactive molecule, before its acquisition by Gilead Sciences (Hecketsweiler, 2015y). In France, according to a 2014 report by Daniel Dhumeaux – a professor of medicine and specialist of viral hepatitis – who recommends to treat with DAA drugs patients showing even a moderate degradation of liver function, *ca*. 120,000 patients suffering from hepatitis C should need this kind of treatment. The director of *Médecins du Monde* (MDM) in France, Jean-François Corty, stated that beyond the major issue concerning the access of hepatitis-C patients to Sovaldi there was a need "for triggering again the debate on the issue of drug-price fixing in France" (Benkimoun, 2015j). In September 2014, in addition to MDM, several associations, including AIDES and SOS Hépatites, expressed their concern about the price of Sovaldi (sofosbuvir), which they considered "exorbitant" (Benkimoun, 2015j).

Also the AIDS Healthcare Foundation which provides treatments to more than 500,000 patients infected with HIV worldwide and is one the most important clients of Gilead, is campaigning in California in order to impose a ceiling price for drugs by law. Dale R. Gluth who is behind this campaign by the foundation in the region of San Francisco, claimed that "they had already collected more than 500,000 signatures" by the end of November 2015. On the other hand 18% of American gross domestic product (GDP) is devoted to health-care and the commercialization of a new wave of very expensive drugs (such as anticancer and antihepatitis drugs) that target large numbers of people and must be taken during the whole life, is a serious challenge to the health-care system. Thanks to the health-care reform in the United States, named "Obamacare" (Affordable Health Care Act), another 10 million people are being

provided with health insurance. But in the majority of cases patients have to co-pay for part of the prescribed drugs (Hecketsweiler, 2015y).

Bearing in mind these challenges and the averse public opinion concerning the pricing of its drugs, Gilead Sciences set up in the United States programmes aimed at improving the access to drugs for patients having no health insurance. "We have adopted a policy of differentiated prices, that is adapted to the situation prevailing in each country," underlined Clifford Samuel who manages the programmes for the access to drugs at Gilead. "In the emerging countries the price of our antiviral drugs has fallen 80% since 2006," he added. According to Gilead data 8 million patients with HIV – out of the 14 million who follow a treatment – receive drugs developed by Gilead. The most prescribed drug, Viread, cost US$3.60 per month in 2015. "We have granted licenses to manufacturers, most of them in India, who are authorized to commercialize generics of our drugs in 112 countries," indicated Clifford Samuel. He is also convinced that global competition will decrease drug prices even more (Hecketsweiler, 2015y).

### *Pricing of antidiabetes drugs: an international harsh competition*

#### Competition and its impact on prices

As mentioned above (see pp. 76-77) Sanofi Lantus insulin, one of the biggest blockbusters in the history of the pharmaceutical system, brought in *ca.* €35 billion in ten years to the company; its sales in 2015 reached more than €6.3 billion. Lantus is the world's fifth-most widely sold medicine and is one of the pillars of Sanofi, whose annual turnover reached €33 billion in 2014. But this success story is over. During the spring of 2015 the patent that protected the bioactive product of Lantus – glargine insulin – expired. A biosimilar drug, named Basaglar, was commercialized during the summer of 2015 by the American company Eli Lilly and the German firm Boehringer Ingelheim. Marketed in the United Kingdom and Germany, the drug was 15%-20% less costly than Lantus, and it was expected that when it is marketed in France, the price would be at least 30% lower than that of Lantus (Hecketsweiler, 2015i).

In the United States where two-thirds of Lantus sales are made, Sanofi has tried to mitigate the competition: after suing Eli Lilly because of patent counterfeit, the French big pharma signed an agreement with its rival that postponed the launching of a biosimilar to 15 December 2016 in the United States. However Novo Nordisk, the Danish global leader in insulin and antidiabetes drugs, is a formidable competitor on the American market. Novo Nordisk insulin – considered less efficient than Lantus – is sold at a 15% lower price; the result was that Novo Nordisk Levemir encroached on 25% of the American market. The health-care insurers made the following calculation: if the 4.7 million patients used Levemir instead of Lantus the savings would amount to US$2.6 billion (or €2.53 billion). Consequently in order to remain among the drugs that insurance companies will reimburse, Lantus was sold in 2015 with a discount amounting to more than 40% the "catalogue" price. The latter had increased markedly, from US$2,035 per year and per patient in 2011 up to US$3.630 in 2014 (Hecketsweiler, 2015i). Nevertheless Sanofi suffered a setback when, on 2 August 2016, the American group CVS, which

provides health insurance to 80 million people and manages 9, 600 pharmacies, decided to eliminate Lantus from its list of reimbursed drugs from 1 January 2017. CVS replaced Lantus (US$260 the vial) by Basaglar.

Sanofi strategy to keep abreast of new drug development

Thanks to such a strategy Sanofi hopes to have enough time for replacing Lantus by another patented drug: Toujeo which received the approval for commercialization by the FDA in February 2015. But the FDA thought that this new drug did not bring a real benefit compared with Lantus. According to Sébastien Malafosse, an analyst at Oddo, "Sanofi wanted to stress the fact that Toujeo decreased the level of night hypoglycemia, but the FDA did not authorize Sanofi to indicate such effect on the drug's brochure. Its salespeople have not therefore the right to mention it." The overall result is that Toujeo sales are not taking off: they amounted to €13 million during the first quarter of 2015 and to €20 million during the second quarter. These are, according to S. Malafosse, "anedoctic" figures. And there was no much hope for improvement, with the launching by the end of September 2015 of Tresiba, a rather similar drug developed by Novo Nordisk and already commercialized in Europe (Hecketsweiler, 2015i,v). The American group CVS decided to eliminate Toujeo from its list of reimbursed drugs – another setback for Sanofi.

In order to mitigate this negative impact Sanofi developed a programme christened Toujeo Coach, aimed at coaching the patients. The objective behind such a programme is not just to sell a drug but a package that includes advice, the possibility to be coached individually and applications for ensuring a better management of one's diabetes on a daily basis. Pierre Chancel, head of Sanofi diabetes division, stated that "a significant proportion of patients have been enrolled and the feedback is very positive." "We are carrying out studies with a view to demonstrating the impact of this coaching programme on the patients' compliance with their treatment, in particular. The overall challenge is to correlate the price of the drug with its therapeutic efficacy," he added (Hecketsweiler, 2015i,v).

Another drug is supposed to help Sanofi recover from the implications of Lantus becoming an off-patent drug. LixiLan is a combination of glargine insulin – which regulates glycemia during the whole day – and lixisenatide – whose function is to regulate the amount of blood glucose after a meal -, and it has been developed by the Danish company Zealand Pharma. Sanofi did acquire the rights to commercialize the drug in 2003. It was expected to be marketed by the end of 2016. At the same time Novo Nordisk will launch a similar drug: Xultophy. Harsh competition will then continue while it was estimated that LixiLan's sales would amount to €1.5 billion in 2022 (Hecketsweiler, 2015i,v).

In order to speed up the commercialization of its future blockbuster in the United States Sanofi could use a priority voucher, a regulatory procedure some pharmaceutical companies may benefit when developing drugs to treat rare children's diseases. In May 2015 Sanofi was awarded such voucher for an amount of US$245 million. Sanofi did not unveil for which drug it will use the priority voucher, but many analysts

thought that LixiLan was the target; this would allow this antidiabetes drug to be commercialized before Novo Nordisk Xultophy. Sanofi CEO, Olivier Brandicourt, who presented the group strategy for a five-year period on 6 November 2015, announced the creation of a new division, called "Diabetes and cardiovascular diseases," with a view to repackaging in the same division Lantus and LixiLan, but also Praluent, a new anticholesterol drug. Many changes were also expected within the group's teams. For instance, in the United States, one-third of the commercial staff has been renewed, as well as an important proportion of the directorate (Hecketsweiler, 2015i,v).

## Shifting intellectual property overseas to shield profit

A strategy of the pharmaceutical companies aimed at increasing profit, consists of avoiding taxes by moving their headquarters overseas. The United States administration is trying to stop this move but the manufacturers of some of the world's most lucrative and expensive medicines are using another tactic to reduce their payments to the federal government. An illustrative example is that of Gilead Sciences, which has come under severe criticism for the high cost of its antihepatitis-C drug, Sovaldi, sold at a price of US$1,000 a pill, or US$84,000 for a typical course of treatment. Nearly all of the US$6-billion sales of Sovaldi since the drug approval in December 2013 through June 2014 were made in the United States (see pp. 108-109). Much of the cost of Sovaldi will be borne by taxpayers, since many people with hepatitis-C are on Medicaid or Medicare, in prisons or in the Veterans Affairs health system. Although Gilead Sciences is an American company based in Foster City, California, the patent rights relating to Sovaldi have been transferred to an Irish subsidiary. So Gilead profit from the booming sales of Sovaldi are taxed at Ireland's rate which is well below the American one (Pollack, 2014b).

"In addition to fleeing the government with thousand-dollar pills, that they do not even want to pay their share of taxes is no surprise," stated Michael Weinstein, president of the AIDS Healthcare Foundation who has often complained about the prices of Gilead drugs for HIV/AIDS and hepatitis. The company expected an effective tax rate in 2014 of 17.5% to 20.5%. A previous forecast that excluded Sovaldi sales was for a tax rate of 28% to 29%. Gilead overall tax bill is going up because of profits from Sovaldi. But the lower rate on the drug is saving the company hundreds of millions of dollars from what it would otherwise owe (Pollack, 2014b).

Big pharmas have adopted this strategy of shielding profit through shifting intellectual property overseas but now biotechnology companies are following suit. Transferring individual products is not as advantageous, tax-wise, as moving the company's official headquarters, which is known as an inversion. Pharmaceutical companies, including Pfizer, AbbVie, Actavis and Mylan, have been active in pursuing this inversion strategy, and President B. Obama's administration is trying to rein it in (see pp. 65-66). But for many biotechnology companies the transferring of product rights is providing a substantial tax break. That is because the value of a drug is in its patent which protects it from competition and allows it to be sold at a higher price. Experts say companies cannot just put a patent in a low-tax country, but generally should have some other activity there, like manufacturing or distribution (Pollack, 2014b).

Celgene had a tax rate of only *ca*. 13%. It does some research-development and manufacturing in Switzerland, where it is exempt from most income taxes under an agreement with the Swiss government. About 60% of Celgene sales and assets are in the United States. Yet it attributed only *ca*. 2% of its pretax profits to the United States in 2013, according to Gradient Analytics, a research firm that questioned Celgene practices. In addition to Celgene, *Investor's Business Daily* cited Alexion and Regeneron as having among the highest profit margins of any company in any industry. Alexion Pharmaceuticals stated that as of 2014 certain intellectual property for Soliris, its superexpensive drug for rare diseases, is being held in Ireland, where it has established some operations. Regeneron Pharmaceuticals has made Ireland the tax base for sales outside the United States of its big-selling eye drug Eylea and for some drugs still in development, like the powerful cholesterol-lowering medicine, alirocumab. Regeneron agreed to pay US$67.5 million to BioMarin Pharmaceutical for a voucher that would entitle the anticholesterol drug to a faster review by the FDA. Although Regeneron is based in Tarrytown, N.Y., and BioMarin in San Rafael, California, the transaction was made between the Irish subsidiaries of both companies. BioMarin earned the voucher as a reward for developing a drug for a rare childhood disease, Vimizim, which treats an enzyme deficiency called Morquio A syndrome; this drug will eventually be manufactured mainly in Ireland, the company stated (Pollack, 2014b).

Tax savings would allow the American biotechnology companies to invest more in drug development and to remain competitive with overseas rivals that do not pay the 35% federal tax rate. The average rate in Ireland is 12.5%. Still Karl Wündisch, president of Transfer Pricing Pharma-Biotech, a Berlin-based consulting firm on tax issues, stated such profit-shifting was considered an "international abuse of taxation". Critics of the practice commented that American companies benefit from the country's higher-education system and from basic research supported by the federal government. Companies that make drugs for rare diseases also earn so-called orphan-drug tax credits to subsidize research and development. "I do think there is something problematic about the companies utilizing all of that and not paying their fair share of taxes by putting their intellectual property in low-tax jurisdictions where they do not really do much," stated Reuven S. Avi-Yonah, a law professor at the University of Michigan, referring to intellectual property (Pollack, 2014b).

## e-health

### *Impact of digital technologies on the health-care system*

On 27 January 2015 Novartis announced a 2014 turnover amounting to US$57.9 billion (or €51.5 billion) and made the health digital market a strategic priority. Its ambition was "to go beyond the capsule" in order to be closer to the patient but also to hamper its new competitors such as Google. The Swiss group anticipates that "the mobile and digital technologies may potentially change the practices of medicine and pharmaceutical industry." In 2014 Novartis created a specialized team for that purpose. On

12 January 2015 it announced an alliance with Qualcomm, the Californian giant of mobile technologies. Both groups created an investment fund of US$100 million (or €89 million) in order to support startups. The fund is based in San Diego where Qualcomm headquarters are located (Hecketsweiler, 2015c).

Qualcomm subsidiary, Qualcomm Life, has already invested money in success stories like FitBit which sells high-tech bracelets that measure heart rhythm, monitor the sleep, count the number of steps made or the quantity of burnt kilocalories (see p. 51). Qualcomm Life has also in its portfolio Noom a startup that develops virtual coaches: its applications aim to assist people who wish to lose weight or simply to adopt a healthier lifestyle. On its side Novartis has developed a dozen applications for smartphones, and intends to grow in this field. According to David Epstein, in charge of Novartis pharmaceutical division, "the objective is to integrate digital tools to medical routine with a view to improving patients' life." Novartis goal is to develop technologies that complement drugs. "In 2015 we are launching a molecule that reduces by 20% the death of patients suffering from heart disease. Even though we know that their health will worsen, we could equip them with a device that would send to the physician's smartphone an alert as soon as the first symptoms of heart infarctus appear." In 2013 Novartis had thus organized in the Silicon Valley a contest where 160 startups and developers competed. Their task consisted of designing in 48 hours an application aimed at assisting patients with heart disease during their daily life. Connected objects and mobile applications could also be combined to deliver to the patient the right medicine at the right time. This is what a French startup Voluntis is developing: "companion" softwares (i.e. complementary to a treatment). Qualcomm had invested in Voluntis (Hecketsweiler, 2015c).

Hervé Ronin, director of the health section at the bank Bryan, Garnier & Co., commented that "an increasing number of pharmaceutical groups consider themselves as providers of solutions and not just only suppliers of capsules. They intend to widen their main job so as to include a number of services: applications which will remind the patient to follow his/her treatment, or will monitor certain biological parameters. In this regard chronic diseases such as diabetes, multiple sclerosis or high cholesterol are ideal targets." These applications can add some value during the negotiations between a pharmaceutical company and public-health authorities or insurance companies, as well as when a rival corporation presents an almost similar offer (Hecketsweiler, 2015c).

### *Clinical trials of the future*

Being a pioneer in this area Novartis also intends to use these novel technologies in order to boost its clinical trials. Its programme named Trials of the Future is based on connected objects used to collect in real time the data that would enable the evaluation of a drug efficacy. D. Epstein explained: "Currently these parameters are measured at regular intervals by physicians. It is a major constraint for the patient and this image does not reflect what is going on in real life. A connected device would permit a more precise evaluation of the effect of an experimental drug." If the clinical trials are made more simple, more reliable, they will be less costly (Hecketsweiler, 2015c).

In order to carry out its project Novartis signed an agreement on 5 January 2015 concerning the use of Qualcomm platform of data exchange. This anticipation and rapid action on the part of Novartis are justified by the increasing new competition between big pharmas and giants of the digital industry (GAFAs). The big pharmas may lose ground to these industrial groups which can collect with the help of startups valuable information about patients' behaviour, as well as key data derived from the sequencing of the human genome or biological research. For instance Google and others have developed very powerful algorithms for the storage of data – a competence that is not in the realm of big pharmas (see p. 46). Therefore Novartis decided to make strategic alliances with its potential rivals and, thanks to the availability of cash, it could even acquire the most threatening ones (Hecketsweiler, 2015c).

### *e-health and ethical issues*

In all western countries the ageing of populations results in a relentless prevalence of chronic diseases and these are representing the largest part of the expenses covered by the health-insurance systems. With regard to medical treatments their cost is amplified by the bad compliance of the patients: only 40% of chronic patients follow their prescribed treatment correctly. This average proportion has been given in a report of IMS HEALTH CRIP on the compliance with medical treatments (12 November 2014). In France, in addition to the public expenses, estimated at *ca*. €9 billion per year, 12,000 deaths a year are caused by the non- or insufficient compliance. The pharmaceutical industry is particularly concerned; Pascal Witz of Sanofi stated in this respect on 2 September 2015: "It is useless to develop new drugs if the patients do not take them as prescribed" (D'Ivernois, 2016).

But how to make sure that a patient suffering from a chronic disease is fully following the prescription he/she has been given? This is a complex issue which has been drawing the attention of researchers for a long time. Non-compliance or insufficient compliance can be explained to some extent by many objective and subjective factors that can be dovetailed or added altogether: secondary effects of the drugs, misunderstanding by the patients of the objectives and modalities of treatment, complexity of the posology, negative beliefs concerning the efficacy of the therapy applied to oneself, denial of the disease, fatigue caused by the daily discipline imposed by the treatment, need to take a break, difficult periods of life, financial problems, etc., but also the fact that the patient takes a risk so as to express – consciously or not – his/her autonomy vis-à-vis the physicians. The approach to responding to the extreme complexity of the non- or insufficient compliance with prescribed therapies may be an easy but simplistic one: detection of non-observance with the help of digital techniques and thereafter penalize the patient by a lower reimbursement of the health expense (D'Ivernois, 2016).

This is what happened exactly in 2014 when the French health ministry revised the reimbursement of the cost of a device installed in the residence of patients suffering from sleep apnea. As the device is connected it was foreseen that the cost of the treatment would vary according to the degree of compliance by the patient; the device could even be withdrawn if the patient is not using it for several months in accordance

with the prescription. Such a proposal caused an uproar and the State Council (the institutional body which checks whether any proposal made by a public institution is legally valid or relevant) recalled that the health ministry is not authorized to decide that the reimbursement of the cost of a health device is subordinated to its actual use by the patient. But further to this attempt that was blocked a new era started: that of remote surveillance which deviated from its primary goal – the quality of treatments – and was oriented towards checking the "good behaviour" of patients and penalizing them when they do not follow their prescriptions (D'Ivernois, 2016).

The means for a remote surveillance of patients are already quite numerous and they are being developed and diversified at a fast pace. Electronic devices, "objects connected to the Internet", such as tensiometers, electronic pill distributors, captors recording glycemia continuously, ingested nanoparticles, bracelets or watches, iPhones, etc., all transmit a huge volume of clinical and biological data. The latter also include, for instance, the walking distances of a patient during the day, the calories used, the heart rhythm, the duration of sleep. The giant companies that make the digital "revolution", the GAFAs (Google, Apple, Facebook, Amazon and others) and which the press already names the "3.0 doctors", are joining the pharmaceutical companies in order to conquer this huge e. health market, valued at *ca*. US$10,000 billion (or €9,300 billion) [D'Ivernois, 2016].

Regarding the data collected by these connected devices they are supposed to be delivered to the physicians who follow their patients. But this is not realistic because of the huge volume of data. A more realistic view is the creation of huge health "clouds" which nobody can guarantee that the patients will be their only beneficiaries. Thus good compliance with the treatments prescribed is becoming an imperative that makes the connected health devices monitor the behaviours of the patients and detect any lack of compliance, rather than being tools which help the patients in the daily management of their illness and in preventing incidents. In other words e. health tools can be used in such a way that they raise a real ethical risk. Jean-François d'Ivernois, an emeritus professor of University of Paris XIII and who worked at the laboratory for health education and practices of that university, wondered whether the patients may become unwillingly submitted to these connected objects (D'Ivernois, 2016).

The French specialist recalled that there is a different approach to the non-compliance with the prescribed treatments, when one looks at the health system in a more humanitarian way. Firstly research in human sciences has still a lot to say about the reasons for the non-compliance issue and this research must be fostered. It is indeed a wrong approach to think that non-compliance is due to a kind of "human, too human" weakness. One should not forget that drugs have undesirable secondary effects that worry the patients and which are not always mentioned in the small drug pamphlets; that physicians have still a lot of difficulties in explaining their prescriptions, especially when they deal with patients suffering from several pathologies or whose knowledge of health-care is low. Also one should not forget that the patient is already punished by his/her illness and that it is not necessary to add an economic sanction, explained Jean-François d'Ivernois (D'Ivernois, 2016).

According to a report by the French General Inspectorate for Social Affairs (IGAS, French acronym) the financial or economic sanction against the non-compliance is considered as blaming the victim and it is not advisable for the state to move in this direction. Instead of a remote surveillance or monitoring of the patients via electronic or digital tools it is much more reasonable to focus on therapeutic education which, by contrast, aims to help the patient to become autonomous through coaching him/her in adopting the most appropriate lifestyle and following the prescribed treatment. Since 2009 therapeutic education is being carried out and funded by the French social-security system. In 2015 more than 3,700 therapeutic education programmes have been authorized by the Regional Health Agencies; they target chronic patients and they are being carried out in health-care institutions, but also in the cities and across the whole French territory. Thus the non- or insufficient compliance with prescribed treatments is not a fatality. Jean-François d'Ivernois considers that there is room for improvement if the decision is made to focus on the people through research and education, instead of relying on sophisticated digital techniques to spy the individual (D'Ivernois, 2016).

# PART TWO

## *SEQUENCING THE HUMAN GENOME AND ITS CURRENT AND FORESEEABLE IMPACT ON HUMAN GENOMICS, GENOME EDITING AND BIOMEDICAL RESEARCH*

# CONTENTS

# SEQUENCING THE HUMAN GENOME AND ITS IMPACT

## The history of a harsh competition

On Monday 26 June 2000 Bill Clinton, president of the United States, entered the large lounge located in the White House east wing, with Francis Collins and J. Craig Venter, who sequenced the human genome. Many distinguished guests were invited to attend this ceremony, including journalists (e.g. James Shreeve, chief editor for sciences at the *National Geographic*) and television teams. While a string quartet was playing Mozart, on the screens displayed in the room was written the sentence: "*Decoding the book of life. A milestone for humanity*." In his speech President Bill Clinton mentioned "the robust and healthy competition" between the public and private sectors in sequencing the human genome, and thereafter declared: "Today, we learn the language through which God has created life. We even more admire the complexity, the beauty and the marvel of the most divine and holy gift of God." These worlds should have soothed the ears of Francis Collins, a brilliant physician and scientist, who had been leading since 1994 the Human Genome Project (HGP) and is convinced that the complexity of the genome reflects the immense intelligence of God (Benkimoun, 2015h).

### *The actors*

The nice words of President Bill Clinton could not hide the very harsh competition between both American scientists, their teams and institutions. F. Collins' HGP was an international consortium including 20 public-research centres in the United States, United Kingdom, Japan, France, Germany and China, and having a total budget of US$3 billion. Born in 1950 in Staunton, Virginia, he has been raised by a Mormon family. During his career at the National Institute for Research on the Human Genome, of which he was the director, he isolated genes involved in severe diseases such as cystic fibrosis and he filed patents on them (Benkimoun, 2015h).

J. Craig Venter, born in 1946 in Salt Lake City, Utah, the world's headquarters of the Mormon Church, has been raised in the suburbs of San Francisco. He participated in the Vietnam war as a physician and started his university career on his return from the Far East. Excellent swimmer and surfer, he was not highly appreciated by the scientific community because of his arrogance. He created the private company Celera (speed in Latin) Genomics Corp. with the funding provided by the company Perkin Elmer, specialized in medical equipment, in order to sequence the human genome using the most-sophisticated equipment available. In May 1998 J. Craig Venter informed Francis

Collins about the creation of Celera Genomics Corp. and his objective to outpace the HGP, two days before informing Nick Wade, *The New York Times* journalist in charge of sciences; that was a scoop: Celera would have sequenced the human genome in 2001, four years earlier than it was scheduled for the HGP (Benkimoun, 2015h).

When he was informed F. Collins was to return to Los Angeles from Newark (New Jersey); he made a stopover in Washington, D.C., and met with J.C. Venter and colleagues in the VIP lounge of United Airlines at Dulles airport. James Shreeve who authored the best book on the harsh competition and race between the two teams (*The Genome War*, 2004, Ballantine Books), reported the words of J.C. Venter during that meeting: "We want to coordinate our efforts with yours in all the possible ways. There is enough work for all of us. For instance the mouse genome is as much important for research and biomedicine as that of humans, and to sequence both would be far more better than to have one or the other sequence." And J.C. Venter concluded: "Therefore, when we do the sequencing of the human genome, you can do that of mouse." "After the announcement of J.C. Venter in 1998, Nick Wade told the French daily newspaper *Le Monde* that "Collins and Harold Varmus – then director of the National Institutes of Health (NIH) – declared that they would be happy to let J.C. Venter go ahead. Why should the government waste its funds to duplicate what a private corporation could do? But When I published the information in *The New York Times* there was an outcry of the scientists involved in the governmental project. They required that the NIH remain in the race. F. Collins and H. Varmus hurried up to declare that they had not made such proposal" (Benkimoun, 2015h).

## *"Genome war"*

James Shreeve in the *Genome War* wrote after having attended the 26 June 2000 ceremony at the White House: "I have the feeling of having attended a mock ceremony (he remembered 15 years later). The president of the United States was aware of the conflicts between the two teams of researchers. He called Ari Patrinos who led the genome programme of the ministry of energy (MOE) and told him: "Make meet these two guys. End this rivalry. We need a better image!" Ari Patrinos negotiated a truce during a meeting held at home. But this again was a mock meeting. As soon as the latter ended each followed his course and started again to plot against each other. These guys did not like each other (Benkimoun, 2015h).

Francis Collins and the scientists in charge of the HGP were convinced that, contrary to J. Craig Venter, they were only those who could guarantee the free access to the sequenced genome. "F. Collins has tried to present the situation as a confrontation between science and business," analyzed J. Shreeve. "He wanted to clearly demonstrate that the public programme was on the side of the future of humankind, and that J. Craig Venter's motivation was mainly to make money, while restraining the access to information on the genome. Conversely, Craig conveyed an opposite message: of course, there was money at stake, but this was the kind of large-scale project a private company could carry out better. In his view the HGP researchers were inefficient, overwhelmed and focused on their own reputation" (Benkimoun, 2015h).

J.C. Venter thought that "he was to supply information more rapidly, at a lower cost and of better quality. Everybody could have access to it, but the fees should be high for analyzing this information and looking for targets for drugs. And you will have to pay, because you are Roche and Merck. He was selling speed," explained J. Shreeve. "The irony was that HGP researchers carried out their sequencing work more rapidly than they thought in order to prevent J.C. Venter from being the first to deliver the overall result. Craig was the foe they needed," added J. Shreeve. The harsh race therefore continued and each camp made announcements. On 5 April 2000 Celera claimed it had sequenced a human genome that will be finalized "in three to six weeks," said Craig Venter. The share of Celera jumped. Five days later Francis Collins warned a journalist who published his declaration: "One should not believe, during at least two years, any claim made by a group that it has completely sequenced the human genome." The share of Celera went down. And the seemingly harmonious behaviour of both leaders during the White House ceremony on 26 June 2000 went astray rapidly (Benkimoun, 2015h). *Science* was expected to publish the first drafts of the human genome by each team. But Celera did not wish to transfer all its information to the public site GenBank, fearing that it might fall in the hands of its competitors. *Science* proposed a compromise but the HGP considered it unacceptable and sent its article to *Nature*, which published it on 15 February 2001 (International Human Genome Sequencing Consortium, 2001). The draft genome by Celera was published one day later in *Science* (Venter et al., 2001).

### *Missing the Nobel Prize*

The complete versions of the human genome sequence were published in April 2003, two years before the initial target set up by the HGP. A two-day conference was organized in Washington, D.C., in order to celebrate the event. Despite the efforts made by Ari Patrinos in order to convince F. Collins to invite J.C. Venter, the latter was not invited and his name was hardly mentioned. "The HGP hated Craig and did not want him to be part of the History," commented J. Shreeve. "But this was his main motivation, and not money, even though he liked to buy yachts and racing cars. He wanted to be the man who changed the world." This rivalry and harsh race between the two camps and their leaders have probably played a role in the fact that F. Collins and J. Craig Venter missed the Nobel Prize for Medicine or Physiology which was within their reach. Since 2009 F. Collins has been the NIH director, while J.C. Venter, after leaving Celera in 2002, was facing a new challenge at the J. Craig Venter Institute: synthetic biology – creating new microorganisms with synthesized genomes (Benkimoun, 2015h). See pp. 169-170.

## Lowering the cost of human DNA sequencing

In 2005 the United States National Institute for Research on the Human Genome set the challenge to lower the cost of human genome sequencing to US$1,000. In 2007 were commercialized DNA-sequencing machines belonging to a new generation with high throughput, while Moore's law predicts the duplication, every 18 months, of the power of informatics calculation. In 2014 the San Diego (California)-based company

Illumina was charging US$1,000 (or €750) for the sequencing of the human genome. Therefore, in just a decade, this cost fell from US$100 million to US$1,000, and the prospects are that this cost could still be decreased. The world leading company in DNA sequencing, Ilumina, was created by the end of the 1990s in La Jolla, the scientific heart of San Diego, California. Many biotechnology start-ups are established there, as well as renowned research centres such as the Jonas Salk Institute, and communication technology giants like Qualcomm. Rather unknown from the large public Illumina has become the global leader in the manufacture of the highly sophisticated robots that equip most advanced laboratories worldwide and can sequence human DNA at very high speed and great precision. Illumina is also a global leader in the analysis of DNA and its genetic tests are widely requested by American physicians. In 2013 Illumina annual turnover amounted to US$1.4 billion, while its stock exchange value was estimated at US$25 billion. In 2016 Illumina annual turnover was expected to reach US$2.4 billion (Hecketsweiler, 2014c).

## Illumina venture and its impact on human genome-sequencing initiatives

Illumina performance was illustrated by the commercialization in January 2014 of a new robot, christened HiSeq X Ten, that can sequence a human genome in less than 24 hours at a unit cost of US$1,000. The objective is to identify the genetic components of an increasing number of diseases so as to better detect and treat them. This low-cost DNA sequencing also means that physicians and patients will have access to more sophisticated and affordable genetic tests. Jay Flatley who has been the executive officer of Illumina, almost since its creation, considers that the 21st century is the golden era of DNA. In an interview with the French daily newspaper, *Le Monde*, published on 19 August 2014, J. Flatley said that the huge amount of data derived from genome sequencing at a lower cost will be analyzed and compared with clinical data in order to establish correlations between genes and diseases. Between 20,000 and 25,000 genomes must be sequenced before drawing any meaningful conclusion, but in five years the advances will be important, he stated. For instance the British government launched the Genomics England project with a view to sequencing the genome of 100,000 persons over the period mid-2014-2017. *Ca*. 20,000 people suffering from a genetic disease and 20,000 patients having a cancer will be included in the targeted population. The British researchers are hoping that by comparing the genome of a healthy person with that of a patient, pathologies will be better understood and appropriate, efficient and targeted treatments will be developed. The initial budget of Genomics England, announced by the United Kingdom prime minister, David Cameron, was £300 million. This was added to a previous and first budget allocation of £100 million in 2012. The total is *ca*. £500 million. Thanks to this commitment "the United Kingdom will be in a few years at the forefront of genetic research in the world," stated David Cameron (Hecketsweiler, 2014c).

There are also private initiatives such as that of J. Craig Venter who aims to sequence 100,000 genomes in order to find the clues for health and longevity. Starting in 2015 J.C. Venter wanted to recruit tens of thousands of participants across the world, including several 100-years-old persons, in order to decipher their genomes and gather all their physical and biological features. With the help of powerful

algorithms these data may reveal the structure of the genes whose expression can explain the exceptional longevity and health of certain persons. This could be a "gold mine" for pharmaceutical groups which would buy the information supplied by J.C. Venter. His company named Human Longevity, Inc., has been able to collect US$70 million (or €53 million) in an initial public offering. J.C. Venter's objective is to enrol 40,000 volunteers a year in a first stage, and thereafter up to 100,000 in a second stage. In addition to their genomic features other data will be collected: on their microbiome (i.e. on the microorganisms living in the body) and metabolism. Cooperation with pharmaceutical companies will help obtaining the genetic profile of the patients involved in their clinical trials. Gathering such a wide range of data from a high number of people is an unprecedented achievement. J. Craig Venter declared that "medicine has entered into the big data era" (Hecketsweiler, 2014c,f).

J.C. Venter's company is recruiting bioinformatics specialists and statisticians in order to analyze the huge amount of data and extract interesting and meaningful correlations. This kind of work corresponds to 80% of the company's activity while 20% is devoted to the collection of the samples. With respect to seeking the clues for longevity and good health J.C. Venter's goals is to allow people to live longer and in good health understanding why during the process of ageing cells undergo deleterious changes and why certain persons seem naturally immunized against a kind of disease will help finding new treatments and ways to delay ageing; if they are better informed the patients can adopt a lifestyle that is more in tune with their genetic heritage (Hecketsweiler, 2014f).

In his interview with *Le Monde* Jay Flatley underlined the current availability of an increasing number of tests that contribute to the diagnosis of an individual's likely susceptibility to a disease (like cancers), or the characterization of a tumour or even to predicting the reaction of a patient to a drug. J. Flatley insisted on the fact that Illumina through its tests does not impose any prescription to the physician or any advice to a patient. The entire decision remains with the physician who analyzes the results of the test (eventually with Illumina help) and makes the appropriate prescription. He also indicated to *Le Monde* journalist that he had his genome sequenced in 2010, and the analysis, using the existing databases, showed that he suffered from a dozen of pathologies and he should have died several years ago. Since then the databases have been improved and his genome has been reviewed using the new information available; he thus discovered that he may die from a general anesthesia during a surgery (Hecketsweiler, 2014c).

By the end of August 2014 Illumina announced the conclusion of agreements with the French pharmaceutical company Sanofi, with the British AstraZeneca and the American Johnson & Johnson. The objective of these cooperation agreements was to develop a single test that allows the physician to know the genetic profile of a tumour and helps him to choose the best drug cocktail. Until September 2014 125 genes that could play a role in the initiation or development of cancers had been identified. "The number of targeted therapies is still limited but the majority of the 800 anticancer drugs that are being developed target specific mutations. There will be therefore an increasing demand for adapted diagnostic tests," explained Illumina (Hecketsweiler, 2014c).

## Personal Genome Project (PGP)

George Church, professor of genetics at Harvard Medical School, has designed the Personal Genome Project (PGP) for which he was the first subject. G. Church's 1984 Harvard PhD included the first method for direct genome sequencing determining the exact order of nucleotides within the DNA molecules. He also came up with the idea of "multiplexing", where many pieces of DNA are sequenced simultaneously rather than one by one. In the late 1980s G. Church helped organize the internationally funded Human Genome Project whose aim was to sequence all 3.3 billion nucleotide-pairs within a human genome. Even before it started he was not satisfied with the programme because the goal was one genome and a very expensive genome at that time. The project took 15 years, cost *ca*. US$3 billion and delivered a genome that was a blend of different individuals and riddled with gaps. Although it was an historic milestone "it had relatively little value in practical personal or medical terms," according to G. Church. "What I really wanted was for everybody to have their genome and ideally everybody to share their genome, and for that we needed to bring the price way down" (*The Economist*, 2014c).

In 1994 his automated technologies led to the first commercial genome sequence, that of a bacterium that causes stomach ulcers, and later on to dramatic improvements in the accuracy and cost of sequencing human genomes, reducing the cost to *ca.* US$1,000 in 2014. Since 2007 G. Church had co-founded 12 biotechnology companies and advised many more. One of these, Genia, is commercializing a process called nanopore sequencing that G. Church devised in 1988. Because it relies on electronics rather than optics nanopore sequencing promises faster, cheaper sequencing of DNA, down to US$100, according to G. Church. In June 2014 Genia was acquired by the Swiss pharmaceutical group Roche (*The Economist*, 2014c).

The Personal Genome Project (PGP) is all part of a grand experiment to help researchers explore the interactions between genetics, environment, behaviour and disease, with the ultimate goal of developing customized therapies for individuals. The idea is that linking genes to outcomes, whether deadly diseases or talents, requires a huge amount of raw data about people's lives, diets and their environment; data that are not always compatible with the safeguarding of privacy. G. Church is seeking volunteers willing to waive confidentiality and lay bare their genetic code, medical records and daily habits to the world. More than 3,500 people have done so. The PGP expected to eventually enrol 100,000 subjects (*The Economist*, 2014c).

## Soaring market of genetic tests

In 2014 the market value of DNA sequencing and genetic tests derived from that sequencing was estimated by Illumina at US$20 billion including : US$12 billion for cancer (development of targeted diagnostics and therapies); US$5 billion for the study of the genomes of several species and for plant selection; US$2 billion for diagnostic and detection tests concerning genetic diseases or associated with an isolated genetic mutation. In France, for instance, there are 1,439 detectable genetic diseases and

350,000 persons have been provided with a genetic test in 2013. The total number of diagnostic tests carried out in 2013 amounted to 403,500, compared with 350,400 in 2010, while the number of pharmacogenetic tests reached 25,700 compared with 10,800 in 2010 (Hecketsweiler, 2014c).

Pharmacogenomics is a promising area for the big pharmas. Nowadays many drugs are not approved by the health authorities because of their lack of efficacy and the side-effects observed among patients. The variability noticed in the patients' reactions to drugs can sometimes be explained by the genetic profile of the patients. If a pharmaceutical group discovers this correlation and devises a test that could "differentiate" the patients it may be able to obtain an approval of the drug that otherwise would have been refused. According to Illumina chief executive there are such kinds of tests for a dozen of current drugs, but they are not yet very much used. A related issue is what do we propose to those patients who do not respond to the existing treatments? The truth is that we have not enough drugs, stated Jay Flatley. The latter thinks that the market of tests designed for the large public will soar in the forthcoming years. Patients have the right to know what their genes contain but this market must be regulated. Illumina is working with many companies which commercialize tests directly for the consumers, but it is very careful with regard to the ethical behaviour of the companies. Illumina supports Google initiatives in the area of human genetics and it is working with it on cutting-edge projects (Hecketsweiler, 2014c).

### *Tests for mutations in genes associated with high risk of breast cancer*

The growth of genetic tests market is so impressive that some analysts consider that in the United States these tests will progressively "replace the physician's stethoscope". The most widely prescribed test targets mutations of BRCA1 and BRCA2 genes, which are associated with a higher risk of occurrence of a breast or ovary cancer. The test was popularized by the actress Angelina Jolie, who after making the test decided to undergo a double mammectomy. Following her example many women decide to proceed similarly and thereby almost eliminate the risk for developing a cancer. The test is an undeniable progress for the women at risk, e.g. those who have in their family several cases of breast cancer. But it is not really useful in most cases; because the mutations of BRCA1 and BRCA2 genes are extremely rare (*ca*. one woman out of 1,000) and are involved in only 5% to 10% of breast cancers (Hecketsweiler, 2014c).

The biggest company that commercializes these tests is the American firm Myriad, based in Salt Lake City, Utah, co-founded in 1991 by Mark Skolnick – a pioneer in bioinformatics and one of the first researchers who explored the role of BRCA1 and BRCA2 genes and sequenced them. Based on M. Skolnick's discoveries Myriad has launched in the mid-1990s the first test for the susceptibility to breast and ovary cancers. Since then the portfolio of Myriad has been broadened and sales soared: in 2013 they amounted to US$613 million, compared with half of this amount in 2009. Each test costs *ca*. US$2,000. In order to protect its monopoly on this susceptibility test Myriad did not hesitate to patent the DNA sequences identified by its scientists.

This so-called "privatization" of a person's DNA was invalidated in June 2013 by the United States Supreme Court which ruled that "the naturally produced DNA is a product of nature and cannot be patented simply because it has been isolated" (Hecketsweiler, 2014c).

Since the Supreme Court judgement many tests that compete with Myriad's are being commercialized. For instance, by early June 2014, Pathway Genomics launched its own test, christened BRCATrue; this could become the best-selling product of this biotechnology startup, founded in 2008 in San Diego. The company strategy director, Ardy Arianpour, was enthusiastic about the future market of this test: "a US$-1-billion market value," he stated. He already had a wide range of the so-called "susceptibility" or "predisposition" tests which allow the evaluation of the risk for a person to have a cancer or a cardiovascular disease. This approach includes several advices regarding food and nutrition, physical exercise and the identification of the most efficient drugs for the patient. However the results of these tests should be interpreted with great caution. For instance, by the end of 2013, the United States Food and Drug Administration (FDA) warned the California-based company 23andMe, Myriad's rival, about a misbehaviour indirectly targeting consumers. The company, created by Anne Wojcicki, the wife of Sergueï Brin, one of Google's founders, has since then oriented its genetic test activity towards genealogic research which is another fast-growing use of genetic tests (Hecketsweiler, 2014c).

### *Forensic medicine*

DNA sequencing and analysis has been used in forensic medicine and investigations since the mid-1990s. In the United Kingdom as well as in other countries the technique has been very useful in unravelling many criminal cases. For instance, in 1998, members of the French police were unable to trace the authors of a series of non-elucidated crimes in Paris. In each case DNA traces were found and they all were identical. But the "serial killer" or "SK" could not be identified. However the genetic fingerprints of a suspect Guy Georges, well known from the police services, was well recorded in his judiciary file, but nobody made the correlation between this suspect and the series of murders, due to the lack of a centralized filing system. The judge working on this affair, therefore, requested all the French laboratories working with the police to compare the DNA of "SK" with the genetic fingerprints existing in their databases. After a meticulous work Olivier Pascal, one of the French pioneers in forensic analysis, could make the correlation between the murders and Guy Georges (Hecketsweiler, 2014f).

Further to this affair the National Computerized Filing System of Genetic Imprints was created; in 2014 it contained *ca*. 2.5 million DNA profiles, 75% of which correspond to suspected or involved people but not sentenced. It also contains 150,000 non-identified DNA traces that may be one day used to arrest the author of a crime. Since this national system had been created *ca*. 75,000 criminal cases had been resolved thanks to the comparison and analysis of genetic fingerprints. "Still, it is just a tool," said O. Pascal, who now heads the forensic division of Eurofins – the French biggest

laboratory of DNA analysis. "Police enquiry remains a key component, because DNA analysis does not reveal anything about how this DNA occurred on the crime location; and barristers always draw the police's and the court's attention to this fact," added O. Pascal. Another constraining factor of this kind of DNA analysis is its cost: €80 for a simple analysis in 2014 and much more in complex cases. For instance "this occurs when minute DNA traces have to be sought on large areas; the complete analysis of a T-shirt can cost several thousand euros," indicated Olivier Pascal (Hecketsweiler, 2014f).

### *Detection of genetic diseases and genetic counselling*

An increasing number of genetic diseases can be detected among newborns, e.g. cystic fibrosis, congenital hypothyroidy, phenylketonuria and congenital hyperplasia of adrenal glands. In the case of **drepanocytosis**, considered as the most frequent genetic disease in France, it is affecting at least 12,000 persons, mainly from sub-Saharan Africa, some regions of the Arabian Peninsula, India and the Mediterranean basin. Like cystic fibrosis drepanocytosis is due to a hemoglobin anomaly that is transmitted in a recessive autosomal mode; that is to say it is expressed only when an individual inherits both mutated genes (SS), derived from each genitor, who are heterozygotes (AS) and are not expressing the illness. The risk for a newborn to be affected by the disease is one out of four when both genitors are healthy heterozygotes (AS). But if one genitor has the disease (SS) and the other has the gene (AS), the risk for the offspring becomes one out of two (Cabut, 2014a).

Due to the ethnic aspects related with drepanocytosis, a neonatal detection programme has been set up in 2000 throughout the French territory. The detection of the disease in obstetric clinics is systematic in French overseas departments and, in France, it targets newborns whose genitors are from regions where the incidence of drepanocytosis is high. But several associations as well as many physicians have made a strong plea for a universal detection programme. Their arguments are both scientific and ethical: if the disease is not detected early enough, the risks of complications increase, while targeting some populations could be judged as a kind of discrimination – drepanocytosis being labelled as a "disease of Black People." The High Health Authority (HAS, French acronym) was requested by the Health General Directorate (DGS, French acronym) to give an advice on this issue. On 11 March 2015 the HAS issued a 130-page report on the subject and concluded that a systematic neonatal programme in France was not justified (Cabut, 2014a).

In France, at the Henri-Mondor hospital in Créteil, south of Paris, Frédéric Galactéros is in charge of the Unit that is the reference centre for drepanocytosis over the French territory (excluding the overseas departments). Patients who attend this hospital unit suffer from painful crises resulting from the obstruction of small vessels or capillaries by blood red cells (erythrocytes) with abnormal shape (e.g. sickle-cells). Drepanocytosis is also called sickle-cell anemia. Other symptoms include joint and bone aches. Thousands of patients are looked after by the unit while 10 to 30 patients are treated in the hospital on a permanent basis; some of them even need a bone-marrow graft. The

unit is also carrying out genetic counselling for *ca*. 250 couples a year. This approach, also adopted in the early detection of genetic diseases, would decrease the risk of having newborns with the illness (Cabut, 2014a).

Another example of genetic tests and counselling among people having a specific ethnicity or whose disease is associated with specific regions, is the case of **Tay-Sachs disease**, a lethal neurodegenerative illness, that has been diagnosed systematically in Israel and the North-Eastern States of the United States among Ashkenazi Jews. The result has been that the disease almost disappeared in these communities. In Israel the health ministry has set up a list of diseases for which genetic tests are recommended, such as Tay-Sachs disease: the detection is carried out in this specific case by a committee called Dor Yeshorim, but the persons tested do not know if they have the pathological mutation, they just have a code number. When a marriage is foreseen the committee checks whether the code numbers of the future couple are genetically compatible. Thereafter follows the genetic counselling which has been very successful in the almost disappearance of this lethal disease (Cabut, 2014a).

In Cyprus, Greece, northern Italy and Cuba, systematic detection of **hemoglobinopathies** – drepanocytosis and thalassemia – among couples has resulted in a significant reduction in the number of patients, as indicated by Agnès Lainé, a French historian and researcher, who has been following these issues for 30 years. She is of the opinion that in France the number of healthy heterozygotes with one of the mutated gene (AS) justifies a campaign of information among young adults that can be genitors. An investigation is often suggested during pregnancy; if the pregnant woman and her partner are found heterozygotes, a prenatal diagnosis can be carried out through the sampling of fetal cells, but this is often too late, stated A. Lainé. Henceforth the need before marriage for disseminating the relevant information among future genitors (Cabut, 2014a).

### *Bioethical issues relating to genetic tests*

In France the 2011 law on bioethics prohibits the individual request for a genetic test without a physician's prescription. Marc Delpech, head of the department of genetics at the Cochin hospital in Paris, expressed his profound disagreement with the current trend of genetic testing for disease susceptibility, because "most diseases involve several genes, and the studies carried out on true twins show that the environment also plays a key role." That is why in France this kind of tests is exclusively prescribed to those patients whose family history is worrying, e.g. several generations of women who suffered from a breast or ovary cancer (Hecketsweiler, 2014c).

Diagnostic tests are more currently used, but they are also regulated by the law. The physician should prescribe them only on the basis of solid clinical information and if the patient can draw a benefit (e.g. because there is a treatment available or because the fact to know could bring some relief). The patient can object to the test or he can request not to be informed about the diagnosis. The 2011 law on bioethics emphasizes the need to accompany the patient. This is a key point, according to

Arnold Munnich, director of the department of pediatric genetics at the Necker-Enfants Malades university hospital. "We cannot reduce genetics to just its technical aspects and deliver complex results with explanation, without holding a personal dialogue." This is particularly relevant when our knowledge is not that broad. For instance, "of the 25,000 genes which make up the human genome, only 250 have been identified as correlated with mental retardation and 40 have been associated with epilepsy. But there may be hundreds of others," indicated A. Munnich. In 2014 *ca*. 1,500 genetic diseases could be diagnosed, using a genetic test, and *ca*. 350,000 patients could have access to them, according to the French Biomedicine Agency (Hecketsweiler, 2014c).

## DNA analysis and the history of human populations (genealogy)

### *A booming market*

The search for a person's or a population's origins, using genetic analysis, is a booming market. For a few hundred euros or dollars dozens of firms across the world propose to their clients to find out the members of their families or to know the geographical origin of their ancestors. In this regard the California-based company 23andMe has the largest reputation; in 2014 it was selling its kit for sampling a person's saliva as well as the results of DNA analysis for *ca*. US$100 (or *ca*. €90); it had almost 1 million clients worldwide. Based in Switzerland, the firm iGenea is making three offers: "Basic," "Premium" and "Expert", with a cost between €229 and €1,338; the client receives detailed results as well as a certificate. The GenoChip test, proposed by the Genographic consortium – launched in 2005 by the National Geographic Society – is based on mitochondrial DNA, 150,000 signatures on the Y chromosome and other regions of the genome; it cost *ca*. US$200 (or €175) in 2014 and it gives information on the origin of the person's ancestors, as well as on the proportion of DNA inherited from the Neanderthal and Denisova humans (Mary, 2015).

### *Understanding the history of human populations*

Regarding the use of DNA and genetic studies in order to better understand the history of human populations and their migrations, there has been a great change in the understanding of that history, but also some controversy about the results. One hundred years ago physical anthropology was predominant in the study of the diversity among human groups; such biological differences as the colour of the skin or the skull's shape, combined with archeological studies, were referred to in trying to understand the origins of human groups. For instance Icelandic people had their saga, the Jews their scriptures, the French people their middle-ages literature, stated Gisli Palsson, professor of anthropology at the University of Iceland in Reykjavik and associate professor at London King's College. He authored and co-authored several books, including *Anthropology and the New Genetics* (2007, Cambridge University Press) and *Biosocial Becomings: Integrating Social and Biological Anthropology* (2013, Cambridge University Press). The Icelandic anthropologist considers that the introduction of genetics and genomics is changing the way we write human history

and particularly the way we can trace back the origins of a human group – this is a crucial aspect of that group's identity. It is important for anthropologists to understand to what extent the genomics approach modifies the traditional concepts of "race" and social hierarchy (Mary, 2015).

A study funded by the Wellcome Trust Centre for Human Genetics – an important British foundation that is involved in medical research – and published on 18 March 2015 in *Nature* (Leslie et al., and Donnelly, 2015) showed that "the inhabitants of the United Kingdom share a common genetic heritage to which have been integrated variations that characterize their various origins, particularly the Anglo-Saxon ones," according to Peter Donnelly who conducted the study. "Our study unambiguously shows that the Anglo-Saxons mixed themselves with the existing populations," he added. The study also "suggests the existence of a migration wave – unknown until then to historians and archeologists – which came from continental Europe into southeastern England before the arrival of the Anglo-Saxons," claimed P. Donnelly. "What this very refined study shows are the different historical events that shaped the present United Kingdom's people," indicated Lluis Quintana-Murci of the Pasteur Institute, Paris, who has been studying population genetics since the mid-1990s (Mary, 2015).

The Wellcome Trust Centre for Human Genetics study was initially conceived with a view to establishing correlations between genetic variations and disease susceptibility. It has been carried out on more than 2,039 persons who know their region of origin. For each one of these persons 500,000 DNA sequences distributed over their whole genomes and known to contain genetic signatures of geographic origin, have been analyzed. Once deciphered these signatures were compared with those contained in a database, using an informatics model, and subsequently the genetic profile of each one of the 2,039 persons participating in the study could be established. The Britons were thus classified into 17 groups that resulted from the mixing of populations they were derived from. When these genetic profiles were put on the United Kingdom map a great similarity was found between this form of mapping of origins and the geographic distribution of the Kingdom populations present in the year 600, after the Anglo-Saxon invasions. The genetic study provided therefore a reply to a controversy among historians and lasting for centuries: Have the Anglo-Saxons replaced the populations already present in the British islands or have they mixed with them? In the 5$^{th}$ century after the collapse of the West Roman Empire, the Angles, Jules and Saxons, coming from the present territories of Germany, Norway and Denmark, invaded the British Islands and founded several independent kingdoms. The study shows that these people mixed with the local populations (Mary, 2015).

Like the Wellcome Trust Centre for Human Genetics in the United Kingdom the Icelandic company deCODEGenetics has been carrying out many studies with a view to mapping the genome of thousands of Icelandic people. In this country the genealogy of families has been recorded for centuries; the data have been digitalized by deCODEGenetics in the site *Islendingabok* (Book of Icelanders). Moreover deCODEGenetics has carried out a nation-wide study on the correlations between the genetic profile of Icelanders and disease susceptibility (e.g. breast cancer or Alzheimer's disease). The results have been published in *Nature Genetics* in March 2015 (Gudbjartsson et al., 2015). Iceland

is considered an excellent laboratory for this kind of studies because the population is small and the family genealogical data have been well recorded. Iceland therefore offers a good opportunity to correlate genomic and medical data with the history of families (Mary, 2015).

### *Genographic Consortium*

The Genographic Consortium intends to trace back the first migratory flows of humankind through the study of indigenous peoples, present for several centuries in different areas of the world. Dozens of studies funded by the consortium have been published in this respect in renowned reviews such as *Nature* or *Science*, and also *PLos One*. A study published in *The American Journal of Human Genetics* by the team of Lluis Quintana-Murci and supported by the Genographic Consortium (Behar et al., and Quintana-Murci, 2012) has confirmed that the linguistic isolate formed by the Basque people had an origin different from the so-called Indo-European origin of all the other Europeans. According to this study the contemporary Basques share a partial genetic continuity with the first Mesolithic or Paleolithic settlers of the Catalonian region. Another study was carried out by the same team and published in 2004 in the same journal, on the origin of the various ethnic groups found in Pakistan; the study focused on slave trafficking in the Indian Ocean. The analysis of mitochondrial DNA of the members of the Makranis ethnic group, living in southern Pakistan, revealed the African origins of their maternal lineage. It is known that only women transmit mitochondrial DNA, and the signatures of that origin found there were absent in the Y chromosome, transmitted via the paternal lineage. "This shows, asserts Lluis Quintana-Murci, that women had been deported as slaves beyond the Indian Ocean, suggesting that they were used as concubines or servants" (Quintana-Murci et al., 2004).

### *Controversies among geneticists and anthropologists*

Finally a study published in *Nature Communications* in 2014, in which L. Quintana-Murci team has participated, indicates that the hunter-gatherer peoples would have expanded before the introduction of agriculture in sub-Saharan Africa, *ca*. 5,000 years ago. This finding shatters the dogma according to which population outbursts (fast-growth episodes) follow the appearance of agriculture. The study involved geneticists, linguistics specialists and anthropologists, and dealt with the relationship between Bantu peoples and Pygmies in Gabon and Cameroon; it enabled to find out some kind of agreement between the hypotheses formulated by the geneticists, on the one hand, and the linguistics specialists, on the other, about the fragmentation of the languages of these peoples (Patin et al., and Quintana-Murci, 2014). L. Quintana-Murci drew the conclusion that "it is through this kind of collaboration that one can get close to what could have been the real history" (Mary, 2015).

Population geneticists have been nevertheless often perceived as arrogant by their colleagues belonging to other disciplines. Thus, Jean-Jacques Hublin who is the director of the Department of Human Evolution at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, and is a specialist of Neanderthal man, considers that "geneticists are on the top of the podium, they feel they do not make errors and

often neglect, either on purpose or because of ignorance, the data provided by other research fields." Talking about the various scenarios that have been suggested in order to explain the development of Indo-European languages, he stated: "There is an avalanche of new data that must be integrated and checked with those provided by archeology, paleontology and linguistics, while keeping a critical distance versus genetics" (Mary, 2015). A good illustrative example of this situation is the controversy about the EPAS1 gene that is involved in the transport of oxygen and whose variations are related to the adaptation to altitude. In fact a variant of this gene has been found among 87% of Tibetans, compared with only 9% among the Han – China's most important ethnic group (Simonson et al., 2010; Yi et al., 2010). Using a mathematical model the authors of this study found that the two ethnic groups diverged *ca*. 2,750 years BC. By contrast, according to the archeological studies, the first neolithic villages appeared 5,000 years BC on the Tibetan plateau. The geneticists had to revise their model and they finally found a period that was more in tune with the archeological data. The flaws in the genetic studies were the uncertainties of the mathematical models used by the geneticists, the small number of persons involved in the study and the lack of reliability of the datation methods. The molecular clock on which the geneticists base their research is not that precise and, as a result, an event can be dated with an approximation of several thousand years. This molecular clock is based on an estimated rate of mutations in the genome at each generation as well as an average duration of a human generation. A study published in 2012 and carried out on a father, a mother and their child showed that the accumulation of mutations had a pace which was much slower than the estimation made until then by the geneticists. All this work led to a re-evaluation of the date of divergence of the EPAS1 gene (Storz, 2010; Mary, 2015).

A surprising result came out from a series of genetic studies published in the journal *Molecular Biology and Evolution*, that dealt with the genomes of 6,109 individuals from 41 geographic populations across the Tibetan plateau. Qi et al. (2013) suggested that this ethnic group evolved from a nomadic people, 30,000 or 20,000 years ago. Archeologists also agree on the existence of a "Central Asia corridor", in the middle of which would be Tibet. This corridor that may be considered as the equivalent of a Silk Road, led to the intermingling of populations. Another controversy on the origin of ethnic groups, in relationship with genetic studies, occured after the publication of the book titled *Legacy. A Genetic History of Jewish People* (2012, Oxford University Press) and authored by the geneticist Harry Oyster of Yeshiva University Albert Einstein College, New York. H. Oyster claims that there are genetic signatures which are common to all Jews: Ashkenazis from Europe, as well as those from the Middle East or North Africa. These genetic data would indicate, according to him, that Jews are the descendants of the Hebrews mentioned in the Biblical tales, and they would explain their intellectual superiority. H. Oyster's book and conclusions have been severely criticized by the Jewish geneticist Richard Lewontin from Harvard University. The analysis of the same regions of the genome using other mathematical models and carried out by other geneticists led to very contrasting results: according to the Israeli geneticist Eran Elhaik of Sheffield University, United Kingdom, the Ashkenazi Jews, to a very large extent, had a Caucasian Asian origin, and not a Middle-Eastern one (Mary, 2015).

## *Conclusion*

As a conclusion of this controversy that is often raised about the results and interpretations of genealogical studies based on population and molecular genetics, we could quote the landmark speech delivered in November 2010 by Rod McInnes, president of the American Society for Human Genetics and professor at McGill University, Montreal, during its annual meeting (7,000 persons in attendance). He requested the geneticists to develop collaborative research with other scientists working on the Amerindians and their origins. Since the 1990s geneticists have been trying to draw conclusions from their studies on Amerindians' genomes on how the United States were populated. Amerindians, who wanted to be more involved in the studies and to have the right to discuss their results before they were published, complained about what they considered a "colonial" approach. The latter has been denounced by the American anthropologist, Kim TallBear, of Texas University, in her book titled *Native American DNA. Tribal Belonging and the False Promise of Genetic Science* (2013, University of Minnesota Press). The situation has been improving over recent years: collaboration between geneticists and anthropologists/social and human scientists has become more effective and the Amerindians themselves are not just providing their genomes, but they have their say on the conclusions drawn from these studies.

But beyond the ongoing discussions on the limitations of genetic tests and studies relating to genealogical search and peoples' history, it is an undeniable fact that nowadays an increasing number of people are requesting genetic tests. But there should be also an increase in the awareness of the risks associated with the interpretation of these tests, e.g. by insurance companies, governments or employees with discriminatory purposes. There is therefore a need for a real democratic dialogue on these issues, as stressed by Gisli Palsson of Reykjavik University, as well as by Barbara Prainsack, professor of political science at King's College, London. The latter has co-authored with Alena Buyn a report for the Nuffield Foundation of Bioethics, titled *Solidarity: Reflections on an Emerging Concept in Bioethics*, which calls for new forms of biogovernance, including the application of the principle of solidarity, in order to deal with all these issues appropriately (Mary, 2015).

## To know or not to know about one's DNA?

Nowadays, besides the case of the Downe syndrome (trisomy 21), prenatal tests aimed at detecting diseases are carried out in exceptional cases: either because there is a family precedent or because an echography of the fetus induces a doubt. But only such grave diseases as cystic fibrosis, sickle-cell anemia or hemophilia are being sought, which amounted to *ca*. 3,000 genetic tests per year in France in 2014. Besides these genetic tests which aim to detect in one or several genes mutations that are responsible for a well-identified disease, there are genotyping tests; they consist of identifying in an individual a large number of "genomic variations", which are short DNA sequences with frequent variations from one person to another; these variations can be correlated with certain traits, such as the susceptibility to diseases or morphological and ethnical features. For instance, in 2012, the company 23andMe was proposing to characterize 1 million "variants" for a cost of almost US$100.

*Wider availability and affordability of genetic tests: the risk of a "eugenic drift"*

Then comes the high-throughput sequencing of the whole genome of the future newborn, starting from the DNA fragments found in the mother's bloodstream. This would allow to identify, without risk and great reliability, a large number of diseases or mutations associated with several illnesses. The fact that these tests will become more affordable in terms of cost may lead to their availability to pregnant women and not only to those who present a risk. Consequently, in France for instance, the National Consultative Ethics Committee has examined the "eugenic drift" that may occur as a result of this wider availability of genetic tests. "The issue of the voluntary interruption of pregnancy is a key one, because the genetic tests are carried out before the end of the 12$^{th}$ week of pregnancy, which is the legal limit for a request for pregnancy interruption," recalled Dominique Stoppa-Lyonnet, professor of genetics at the Paris-Descartes University and rapporteur for the Committee's advice on prenatal diagnosis published in April 2013. In this regard two risks have been highlighted: that the systematic proposal of the diagnosis be interpreted as an "incitation for giving birth only to newborns without any genetic defect"; and that this would subsequently lead to a lesser investment into the research on genetic diseases and the need to accompany the patients (Hecketsweiler, 2014f).

The principle of "free choice" that is at the heart of bioethics could also be hurt by the difficulty of interpretation of certain informations. "We all bear anomalies that may never express themselves," underlines Arnold Munnich; "And even when we know that a genetic mutation is associated with a disease it is very difficult to predict how it will appear in an individual. Some 20,000 ill children are examined in my department each year, and there are not two of them who have exactly the same disease," he added. Bearing in mind this difficulty of interpretation, what should we exactly say to the parents? And "if there is a right not to know, can we accept a prohibition of knowing?" asked the French National Consultative Ethics Committee. The latter suggests the intermediation of a physician for genetic counselling. "It would be a very heavy responsibility for the physician who will have to select the elements to be revealed to the patient, because there is always the risk of an oversight of something important," questions A. Munnich (Hecketsweiler, 2014f).

There is an alternative: instead of analyzing the genome of the future newborn in order to find a possible defect, one could try to search in the parents themselves the defective genes. This is the purpose of preconception tests that are authorized in some countries, such as Israel and the United States. Geneticists are therefore analyzing the genomes of the future newborn's mother and father in order to discover recessive mutations and to evaluate the likelihood for the newborn to inherit two defective copies of the gene (thus two healthy parents could have a child with cystic fibrosis). But even this alternative is not that simple, according to the French National Consultative Ethics Committee: the design of this "identity card of genetic risk" may interfere with the marriages and discriminate those persons

with an imperfect genetic heritage (Hecketsweiler, 2014f). Therefore, beyond the technical aspects concerning the increasing affordability of genomic analyses, the free access to genetic tests (even on line in the United States), the reliability of these tests, their medical predictive or preventive value, philosophical and ethical issues are raised: it is our human nature that may be questioned; could genetics deny the human beings' psychological, emotional and social dimensions? The scare about eugenics and the "perfect child" may surge again.

## *Stakes of the bioethical debate*

In order to better understand the stakes of this ethical debate the French daily newspaper *Le Monde* (6 May 2015) interviewed two French geneticists, Jean-Louis Mandel, professor at the Collège de France, Paris, who in 1991 discovered with his team a new mutation mechanism – an unstable repetition of short sequences in the DNA responsible for the mental backwardness linked to X chromosome; this kind of mutation is involved in more than 15 neurological diseases, such as Friedreich ataxia or Huntington disease. The other geneticist is Patrick Gaudray, a member of the National Consultative Ethics Committee; he has participated in the Human Genome Project during the 1990s and he is research director of the French National Scientific Research Centre (CNRS, French acronym) at the University of Tours (centre-west of France) [Rosier, 2015d].

### Individual's freedom to know his/her genome

Jean-Louis Mandel defends a very unorthodox view: he is one of the few French biologists who make a strong advocacy in favour of the individual's freedom to know the data concerning one's genome, particularly with a view to avoiding the transmission of severe genetic diseases. He challenged the French law by testing the advantages and limitations of genomic analyses through having his own genome sequenced. He explained that when he had his genome sequenced in 2010, he did not understand why such approach was *a priori* considered abominable by most of his colleagues. He discovered that he had the most frequent mutation of the gene for cystic fibrosis, but he is healthy and nobody in his family had been suffering from that disease. However, having only one mutated copy of the gene, the likelihood of transmission of the mutation to his children was 50%; if his wife also had a mutated copy their children would have a probability of 25% for being affected. J.L Mandel also recommended to his daughter who could still have children to be tested in order to see if she had the mutation. Cystic fibrosis is still a very serious genetic disease, despite the progress made in treating it. J.L. Mandel also discovered that he had several variants that indicate a susceptibility to developing an ageing-linked macular degenerescence. It is known that smoking increases the risk of this disease. J.L. Mandel does not smoke, but in case he was a smoker, knowing the susceptibility thanks to the genome analysis would have convinced him to stop smoking. And he also pays attention not to expose himself to intense sunlight (Rosier, 2015d).

However the French geneticist admits that for common diseases the predictive value of the estimated risks was not of great interest, because the etiology of these diseases is generally very complex. For each disease the impact of each genetic variant is often very weak. Each variant interacts with many other genetic factors as well as with environmental factors. In fact these predictions are relevant for only two or three diseases where a small number of genetic factors play a key role. This is the case of Alzheimer's disease and ageing-linked macular degenerescence. In 1993 a genetic variant, ApoE4, that plays an important role in the susceptibility to Alzheimer's disease was discovered. *Ca*. 25% of people have a copy of this variant and the risk to suffer from the illness is multiplied by 3 or 4, while for the 2% of people who have two copies of this gene, the risk is multiplied by 9 to 12. J.L. Mandel indicated that he had not this genetic variant, but this does not mean that he will not have the disease. What is therefore the usefulness of this kind of information, in so far as the prevention of Alzheimer's is limited – even though intellectual or physical activity seems beneficial? He thinks that a person, who is between 20 and 30 years old, will have no interest in having this kind of information, while for a 60-years-old person, why not? (Rosier, 2015d).

J.L Mandel was requested during the interview with *Le Monde* (6 May 2015) to give his opinion on the decision made by the FDA to veto the medical interpretation of the data relating to the genotyping of the new clients of the company 23andMe. He mentioned that the FDA, on the one hand, considered that these tests are medical devices, and that 23andMe should have requested an authorization before their commercialization. On the other hand the FDA estimated that 23andMe had not provided any clinical data which could ensure the safety, efficiency and reliability of these analyses. Regarding the predictive reliability it remains very limited for most common diseases. Besides that, these tests concern 1 million genetic variants and statistical errors could occur, with false positive or false negative on a few dozen variants. But 23andMe carried out its tests in an accredited laboratory. The FDA feared that some clients of 23andMe could make decisions without any medical advice because they might have been scared by the results. But several studies have shown that this was not the case, and, according to J.L. Mandel, the tests carried out by 23andMe do not seem dangerous (Rosier, 2015d).

Regarding the tests that indicate a greater susceptibility to some drugs, J.L. Mandel has learnt from his genome analysis that he had an increased susceptibility to an oral anticlotting drug (warfarine) belonging to the antivitamin-K drug family. This drug is used quite frequently in cardiology and we know that the response to warfarine varies among patients; this has a great impact on the efficacy and safety of the treatments – the risks of hemorragic events associated with antivitamin-K drugs are high. To know that his response to warfarine is a good one is not without interest, according to J.L. Mandel: if he had to take this medicine he would probably have to reduce the initial dosage (Rosier, 2015d).

With respect to the genealogical tests developed and commercialized by 23andMe and other companies, J.L. Mandel has learnt that 96% of his genome had a Jewish Ashkenazi origin, which is true. All his "potential cousins" have the same origin; some them, like himself, had ancestors in Lodz (Poland). J.L. Mandel mentioned that one of his post-doctoral collaborators had been able to find out, among 23andMe clients, three of his third-degree cousins, belonging to a branch of the family that migrated to the United States in the 19th century (Rosier, 2015d). Asked to comment on the decision made by the FDA on 19 February 2015 to approve the first genetic test aimed at detecting the risk of transmission of a rare disease, J.L. Mandel took the example of cystic fibrosis. In France, when a first child is affected by the disease, it is ethically justified to test his/her brothers, sisters and cousins. And when these have the mutation, it is ethically justified to test their spouses or husbands. It is also ethically justified to make a prenatal diagnosis during a second pregnancy in order to prevent another case of the disease. But it is not ethically appropriate to make a test in order to identify the healthy carriers of the defective gene in all the couples who want to have a child – i.e. to prevent a first case. J.L. Mandel has some difficulty to understand that … In the United States the detection of the mutation of the cystic fibrosis gene is proposed to all couples who want to have a child; in Israel it is a state policy and it has obviously a cost. Should the couples be free to know the risks of transmitting severe diseases to their children? J.L. Mandel is of the opinion that more studies are necessary. But because of the fear of the risk of a eugenic drift – undoubtedly real – the problem is not dealt with (Rosier, 2015d).

A more careful approach to genetic testing

By contrast Patrick Gaudray makes a harsh criticism of the genetic tests commercialized in the United States (and prohibited in France): it is a system based on business where the health of people is just a pretext. For instance he claimed that in January 2015 23andMe had sold to the biotechnology company Genentech (owned by the Swiss pharmaceutical group Roche), for US$60 million, the genetic profiles of 140,000 clients in order to support research being carried out on the genetic causes of Parkinson's disease. But P. Gaudray raises the issue of the consent eventually given by those clients to the company (Rosier, 2015d).

He considers that a major problem of these genetic tests is that their predictive and clinical value is overestimated. They only show the existence of mutations in genes, correlated with the appearance of common diseases, but there is not a clear-cut demonstration of a cause and effect relationship. In medicine probabilistic messages are difficult to understand and this uncertainty can lead to distress. Even in the case of a mutation of the gene BRCA1 which increases the risk of developing a breast cancer, we can only say to a woman: "You have a 60% risk of developing a breast cancer before the age of 70 years." The fact of not having this mutation does not protect against this cancer, he added. And there is also a risk of neglecting the usual ways of prevention, such as the detection of breast cancer via periodic mammographies: prediction may therefore counter prevention (Rosier, 2015d).

The interpretation of the data provided by genetic tests must be therefore a clever one and respectful of the person and of his family history. And P. Gaudray goes on and states that the greatest flaw of these tests is their tendency to put aside our psychological and social dimension: "We are not just the outcome of our genes." Should our life be entirely guided by our genome structure? In 2008 the United States Congress voted the Genetic information Non-Discrimination Act which forbids insurance companies and employees to use individuals' genetic information inappropriately. But this law is already being circumvented by insurance companies; and some people had their genome sequenced in order to show their insurance company that their fee should be decreased! (Rosier, 2015d).

P. Gaudray added the following remark: we all have mutations that may lead to more or less severe diseases. At what point should we consider these mutations as deleterious? It is a scientific nonsense to think that we may be able to define what is a "normal" DNA. Genetic tests may become a tool of the tyranny of standards which has been denounced by the French philosopher and physician Georges Canguilhem (1904-1995). Let us assume that the sequencing of the genome becomes widespread among couples who want to have children, the result may be a "marriage of genetic assets," and not of a marriage of persons; this would reactivate the myth of the "perfect child." It is true that when the Human Genome Project (HGP) was launched in 1990, we knew that this was feasible and at an increasing speed. But we did not foresee the magnitude of the acceleration of the sequencing process. While the HGP took 13 years of international collaborations, with a total cost of US$3 billion, in 2013 a complete human genome could be sequenced in a few hours, with a cost of *ca*. US$1,000. But sequencing a genome does not necessarily imply its medical interpretation. To that end we need a very advanced quality of sequencing so as to remove all ambiguities. That is why Elaine R. Mardis, a geneticist, wrote in 2010 in the journal *Genome Medicine*: "The sequence at a cost of US$1,000, its interpretation at a cost of US$100,000" (Mardis, 2010). On the other hand advanced research has led to questioning several concepts of basic genetics: for instance the same gene can be subdivided into several fragments that are distributed in different locations, and some genes do not encode proteins. In addition epigenetics may play an important role in gene regulation in relation with environmental factors (Rosier, 2015d).

## Conclusion

To conclude, all these debates about the ethical issues raised by the widespread utilization of genetic tests, particularly for medical purposes, underline that it is impossible to go back to the period before the sequencing of the human genome. The latter has definitely opened new vistas towards the medicine of the future. On the other hand people – not all of them of course – will want to know more about their genome; henceforth the increasing demand for genetic tests particularly in the countries where this is not forbidden by law, like in the United States. Then the key issue

is the interpretation of the results of the tests; the debates among specialists, including of course geneticists, physicians, but also anthropologists, lawyers and philosophers, highlight that these tests do not say everything, that a great caution must prevail in drawing conclusions, bearing in mind that in most cases we are dealing with a statistical probability of a risk and there are still many uncertainties. A purely genetic approach is not the right one because many other factors have to be considered: the history of the patient or person, the environmental and social factors.

No doubt that the post-Human Genome Project era which started in 2003-2004 has enabled humankind to know much more about its genetic heritage. We shall know more in the future about the functioning of our genes, as well as about the complex etiology of common and also genetic diseases. Genetic tests will therefore be improved and their interpretation will be refined; the treatment of the huge amount of genetic data provided by clinical trials will be improved, as well. Ethical issues will not disappear but will change or evolve according to the new informations provided by the life sciences, as well as by the analyses made by the human and social scientists.

# FROM GENOME READING TO GENOME EDITING: ENGINEERING GENES OR "GENE SURGERY"

In every cell of the human body there are two copies of the genome, one from the mother and one from the father. In each of those genomes there are *ca*. 25,000 genes, most of which generally encode the synthesis of proteins. The sequencing of a complete human genome (or reading) cost US$3 billion in 2000, whereas it can be done today for less than US$1,000 (or €872). This cost is plummeting: down to US$100 rather quickly and may be US$1, i.e. less than the cost of taking a blood sample, asserted George Church, professor of genetics at Harvard Medical School, member of the American Academies of Sciences and Engineering (Sciama, 2015); see p. 124.

## Genetic engineering: more accurate modifications of DNA

Recombinant DNA or genetic engineering was the first step in a series of ever-improving stages for manipulating genetic material. The main problem has always been one of accuracy, of modifying the DNA at precisely the intended site, since any off-target change could be lethal. In 1970 the French molecular biologist and biochemist, Jacques Monod, who shared in 1965 the Nobel Prize for Medicine or Physiology with his fellow countrymen François Jacob and André Lwoff, stated in his book *Le hasard et la nécessité* (Paris, Le Seuil ed., 1970): "Not only modern molecular genetics cannot provide us with any means aiming at modifying heredity with a view to introducing new traits (…), but it reveals the vanity of such a hope: the microscopic scale of the genome forbids for the time being and surely forever such kind of manipulations." Almost forty years after this statement new techniques of genome engineering demonstrate that it is possible to modify any gene in any living organism and in a "customized" way (Rosier, 2014c).

Two methods, known as zinc-finger nucleases and TALEN (TAL effectors – also enzymes –), in addition to meganucleases, come close to the goal of accurate genome modification. But the three of them are not easy to use. A fourth technique, known by the acronym CRISPR-Cas9, is easier to use and has become a widespread method of genome "editing". It should be underlined that the cost of the enzymes used in the four methods was divided by 10,000 in ten years which therefore facilitates the "editing" of genomes, including the human genome, e.g. for medical purposes (Alexandre, 2015).

## The CRISPR-Cas9 technique

The history of the CRISPR-Cas9 technique starts with the discovery in 1987 by a Japanese research team of an odd feature in the genomes of the bacterium *Escherichia coli*, that was described in 2002 as "clustered, regularly interspaced short palindromic

repeats" – CRISPR. Palindromic means that the DNA sequences can be read identically in both ways. Bacteria use these sequences to make small RNA fragments that recognize the DNA of viruses which prey on them (bacteriophages), marking that DNA for destruction by a protein (enzyme) called Cas9; the latter is a sort of a pair of molecular scissors that cuts through the DNA at the point where CRISPR RNA binds to viral DNA. Thus the bacteria protect themselves from infection by bacteriophages. When a second infection occurs the CRISP DNA is transcribed into two RNA molecules; one of these RNAs is complementary to viral DNA that can be cut off by Cas9. So bacteria can "remember" the DNA of viruses that attack them and they are therefore ready to react to further viral invasions.

Emmanuelle Charpentier, a French microbiologist who left France in 1996 and was professor at Hanover's School of Medicine, as well as professor at the Molecular Infection Medicine Sweden, University of Umea, and now head of the department of Regulation in Infection Biology at the Max-Planck Institute for Infection Biology, Berlin, explained that "the CRISPR sequences allowed bacteria and archaea to protect themselves against viral infections." E. Charpentier and her team disentangled the mechanism of this "primitive immune system": "It is when I was trying to understand the regulation mechanisms of virulence genes in a pathogenic bacterium that I discovered this system, the most simple for using it as a genetic tool," said E. Charpentier. In her opinion and as a microbiologist by training, this discovery confirms the interest for studying bacteria: "This is nowadays a very fashionable research area but it should be recalled that molecular biology tools are derived from research carried out on bacteria" (Rosier, 2014c, 2015a).

E. Charpentier and her team published in *Nature* (Deltcheva et al., and Charpentier, 31 March 2011) the details on the functioning of the CRISPR-Cas9 system and thereafter in *Science* (Jinek et al., and Doudna and Charpentier, 17 August 2012) they explained how it could be used in genome engineering or "surgery". This tool consists of expressing in a cell the Cas9 enzyme, using an artificial RNA; the enzyme is guided to the targeted DNA thanks to a CRISPR RNA sequence that is complementary to that DNA, and it cleaves it in a specific way. Scientists can make RNAs that target any DNA sequence they want. And because of the way that cells repair broken DNA, if they put a new gene into a cell along with the CRISPR-Cas9 system they can achieve that a new gene replace an old one. The effect is to give scientists something that works like the find-and-replace function on a word processor (Rosier, 2014c; *The Economist*, 2015h).

Because it is so simple and easy to use the CRISPR-Cas9 technique has generated huge excitement in the worlds of molecular biology, medical research, commercial biotechnology – and gene therapy. In this respect E. Charpentier stated: "We have predicted the performances of this tool but we have been surprised by the magnitude of its success." Monya Baker (in the 8 April 2014 issue of *Nature Biotechnology)* supported the researchers' view, when she wrote: "It is difficult to ignore the avalanche of publications on the CRISPR-Cas9 system during the last 18 months (129 in 2012 and *ca*. 1,000 in 2015). This is the type of technology which is developed once every ten years" (Rosier, 2014c; *The Economist*, 2015h). Alain Fischer, director of the Institute of Genetic Diseases (IMAGINE, French acronym), at the Paris Necker-Enfants Malades university hospital, was enthusiastic about the technique and its applications when he stated: "I should not be surprised if such a revolutionary innovation were rewarded very soon by a Nobel Prize" (Rosier, 2014c).

## Adoption and applications of the CRISPR-Cas9 technique

When E. Charpentier and Jennifer A. Doudna of the University of California, Berkeley, who are considered the co-discoverers of the CRISPR-Cas9 technique, and colleagues worked out how to turn the bacterial CRISPR-Cas9 system into a genome editor in 2013, there were already – as mentioned above – two other techniques for making specific and precise changes in genomes, but they were time-consuming and often complicated. The new technique was as good if not better, and far quicker and easier to use. Matthew Porteus, a pioneer in gene editing at Stanford University, stated that research which required a sophisticated molecular-biology laboratory three years ago can now be done by a high-school student (*The Economist*, 2015h). This viewpoint may be considered a little exaggerated, but "by the beginning of 2015 the regular analysis of "hot" research in biology put out by Thomson Reuters, which looks at which papers are being cited most by other scientists, had three CRISPR papers in its top ten" (*The Economist*, 2015h).

The technique has been applied to dozens of species, including zebra fish (much favoured by developmental biologists), yeast, fruit flies, rabbits, pigs, rats, mice and macaques – the first primates to be genetically engineered by this technique – but also to crop species. On 30 January 2014 the journal *Cell* published on line the very first application of this technique to a primate: Niu et al. (2014) of Nankin University described the efficiency of CRISPR-Cas9 in the *in vivo* simultaneous inactivation of two genes, Ppar-y and Rag-1. How? Simply through the injection of a molecule kit into one-cell embryos (zygotes). Two monkeys, Lingling and Mingming, were born after this experiment out of 10 pregnancies after making implants in surrogate mothers of 180 genetically modified embryos. Printed on the cover of the journal, they heralded the CRISPR-Cas9 technique worldwide.

It was often thought that the technique can be used in gene therapy where it may be possible to make changes with profound consequences. To date gene therapies have been designed to fix every day sorts of cells, such as those of blood or the retina, or the pancreas. CRISPR-Cas9 has been used to cure mouse versions of muscular dystrophy (Duchenne's disease), a genetic disease caused by one defective gene, and a rare liver disease. CRISPR-Cas9 makes it possible to think about aiming at the stem cells that make sperm and ovocytes, or the genome of an embryo awaiting implantation in the womb. In either case the changes made would pass from one generation to the next, and the one after that. But such an approach will raise serious ethical issues and many think it is a red line that must not be crossed (see below) [*The Economist*, 2015h].

Though highly efficient the technique occasionally results in cutting the genome at unintended sites. The issue of how much mistargeting could be tolerated in a clinical setting is one that J.A. Doudna's group wants to see thoroughly explored before any human genome is edited. Scientists also stated that replacing a defective gene with a normal one may seem entirely harmless but perhaps would not be (Wade, 2015a). However ways have been found to make the technique more reliable and less likely to make cuts where it is not supposed to do so; further improvements are on the way, not least at the startup companies built around the technique (*The Economist*, 2015h). American researchers have adopted the technique very quickly. Thus the genome-

engineering group founded by Keith Joung of the Massachusetts General Hospital (Boston) comprised 700 people by the end of 2012. At the beginning of March 2014 the staff number rose to 1,900 – mainly thanks to the use of the CRISPR-Cas9 technique. George Church of Harvard Medical School is involved in four startup companies associated with the use and applications of the technique. G. Church is also using CRISPR-Cas9 to edit the genomes of stem cells before turning them into nerve cells, so as to find the mechanisms behind a range of neurological disorders. Feng Zhang, a scientist at the nearby Broad Institute, has been using CRISPR-Cas9 to model Angelman syndrome, a neurological disorder. He also filed, by the end of 2012, a request for a patent on CRISPR-Cas9 in animals and plants. Earlier on, by mid-2012, J.A. Doudna et al. applied for patent on CRISPR gene editing (Rosier, 2014c; *The Economist*, 2015h).

## Introducing or removing several genes at a time

The CRISPR-Cas9 technique has the major advantage of being used to introduce, or remove, a number of different genes at a time. Most pathological disorders are not caused by just one gene going wrong; being able to manipulate many different genes in a cell line, plant or animal, opens new prospects for the study of diabetes, heart disease and autism where a number of genes are involved, along with environment. In the past a mouse with as few as three genes knocked out would have taken as many years to create; now it can be done in three weeks. That largely explains why the technique has been welcomed by a wide range of researchers. In this respect Alain Fischer, director of IMAGINE (Paris), recalled in an editorial published on line on 28 May 2014 by *Nature* that "in gene therapy, the conventional approach is to add a functional copy of the defective gene to the cell genome." But with this approach we cannot control the location or site where the gene is inserted and therefore we do not restore a physiological control of that gene. "A distinct strategy (such as that of CRISPR-Cas9) is based on the targeted repair of the defective gene, thus offering an attractive alternative," he added (Fischer, 2014). The defective gene is replaced by a functional one at its insertion site in the cell genome.

On 28 May 2014 *Nature* published on line an article that illustrates the potential of this genome-engineering approach. An Italian research team (Genovese et al., 2014) has been able to repair in human cells, *in vitro*, the genetic defect causing an X-linked severe combined immunodeficiency, called SCID-X1. That was probably done in a sufficient number of hematopoietic stem cells so as to eventually lead to a clinical application in humans. Also, on 30 March 2014, a research team at the Massachusetts Institute of Technology (MIT), Hao Yin et al., have published in *Nature Biotechnology* a study that showed for the first time the feasibility of CRISPR-Ca9 for correcting *in vivo* a genetic disease of the liver (correction of a Fah mutation in hepatocytes in a mouse model of the human hereditary disease tyrosinemia). Even more: not only the genetic defect had been repaired but the rodents could also recover their normal weight (Yin et al., 2014). Earlier on the *New England Journal of Medicine* (*NEJM*) published on 6 March 2014 a study carried out on 12 patients infected with AIDS/HIV (Pablo Tebas et al., University of Pennsylvania). Their CD4 T-lymphocytes were sampled and submitted to a treatment aimed at impairing *the functioning of gene CCR5 encoding a major receptor that allows the HIV to infect*

*human cells*. Thereafter these lymphocytes (11% to 28% of them had a modified *CCR*5 gene) were reinjected to the patients. This preliminary experiment using a zinc-finger nuclease (ZFN) to modify the *CCR*5-gene showed that the technique had a good safety level. However before applying this new genome-engineering approach to humans, its complete innocuity must be established. Alain Fischer stated that "undesirable modifications of DNA must be avoided and the toxicity of the transfer of genetic material into the cells should be minimized" (Rosier, 2014c).

Previous genetic-engineering technologies have tended to be species-specific; there have been many tools for manipulating *E.coli* and yeast, but quite often they have not been broadly applicable. This is another area where CRISPR-Cas9 excels; it can be used in organisms that have been resilient to previous attempts of genetic engineering. Another biotechnology application of CRISPR-Cas9 is to build a "kill switch" which allows many genetic modifications made to bacteria to be removed after they have been used, either for safety or to protect intellectual property (*The Economist*, 2015h).

## Gene drives

One particularly impressive – and potentially worrying – application of CRISPR-Cas9 is in the creation of genes that can spread themselves quickly through a population with disregard for the constraints of natural selection. Engineering the CRISPR-Cas9 system itself into an organism's genome makes it possible for such organism to edit its own genes, and there are ways that this ability can be used to "drive" a gene through a population (see below). Such a technology might be used, for instance, to make the mosquitoes that carry malaria or dengue fever pathogens unable to spread these pathogens (*The Economist*, 2015h).

Animals have two versions of any given gene on two different chromosomes and the two versions or alleles can have important differences. Offspring normally inherit only one of each pair of chromosomes from each parent, and thus each version of any gene typically is found in only half of them. Techniques like CRISPR-Cas9 make it possible to break this rule with what is called a gene drive – a gene that uses gene-editing techniques to copy itself from one chromosome to the other, so that whichever chromosome the offspring inherits they have the same version. The same will then apply to their offspring, too. In the case of normal inheritance (a gene on only one chromosome gets into only some offspring) the mating of a wild-type mosquito with a mosquito with a modified gene gives an offspring which have a 50% chance of inheriting the modified gene; in the case of gene drive inserted into one chromosome that copies itself into the other, the mating of a wild-type mosquito with a mosquito with gene drive gives an offspring where nearly 100% of the individuals inherit the modified gene (*The Economist*, 2015h).

Normally genes can only spread through a population if they confer an advantage. Gene drives can spread genes faster than the process of natural selection. A gene drive indeed should be able to spread through a population even if it is bad for its possessors. In 2003 Austin Burt – a professor of evolutionary genetics at Imperial College, London – suggested that this might be a way of altering wild animals so that they stop, for instance, spread disease. As mentioned above, if mosquitoes were given a gene drive that make them unable to transmit the malaria protozoan and then are released, the new trait's quick spread through the population at large would lower the burden of disease.

Gene drives remained theoretical until the beginning of 2015 when researchers tested CRISPR-Cas9-based implementation of the idea in yeast and fruit flies. This kind of work raises a number of concerns. One is that if an animal escaped from such experiments, its gene drive could spread far and wide even if no one wanted it to. Another is that well-intentioned use of such technology could have very bad environmental implications, if poorly thought through. This has led to calls for all workers in the field to develop "reverse drives" – systems which could undo the changes brought about by the gene drives they are working on. Another suggestion is that all drives be designed so as to require the presence of some exotic chemical that would not typically be available outside the laboratory (Oye et al., and Esvelt and Church, 2014; *The Economist*, 2015h).

## Intellectual property issues and the discovery of other endonuclease-based systems of gene editing

A dispute of over who invented what parts of the CRISPR-Cas9 technique first has threatened to curtail its potential. Feng Zhang of the Broad Institute in Cambridge, Massachusetts, is one of the parties to the patent dispute; the other parties are J.A. Doudna and E. Charpentier. This dispute of inventorship has caused some, though not all, potentially interested companies to give the technology a wide berth. Monsanto, for instance, has gone on record as saying that it is reluctant to employ CRISPR-Cas9 widely until it understands the intellectual property concerned. Drug companies have also circled at a distance (*The Economist*, 2015i).

But the discovery of another mechanism of gene editing by Feng Zhang and co-authors, announced in the 22 October 2015 issue of *Cell*, may not suffer from these intellectual property issues. The discovered mechanism is called CRISPR-Cpf1; it is a smaller and simpler enzyme (or endonuclease) than Cas9. That means it will be easier to deliver to the cells whose genes need modifying. CRISPR-Cpf1 discovery also raises the question of how many other endonuclease-based systems exist in bacteria. As mentioned above viral infection is serious threat to these microbes and the natural function of both CRISPR-Cas9 and CRISPR-Cpf1 is to recognize viral genes and cut them off before they can do harm. Conversely viruses are constantly evolving to escape such systems, meaning bacteria need to generate new ones. The chances are good, therefore, that CRISPR-Cas9 and CRISPR-Cpf1 are not alone. As F. Zhang himself puts it: "I cannot even begin to count how many there may be. There really is great diversity that we as a scientific community should go out and explore" (Zetsche et al., and Zhang, 2015; *The Economist*, 2015i).

The tools to carry out that exploration now exist. CRISPR-Cpf1, for instance, was found not by scrutinizing bacteria directly, but by searching a published database of bacterial genetic sequences for promising-looking bits of DNA. This yielded two species that contain the new mechanism. Further searches might be equally rewarding – and as more gene-editing systems are discovered, it will be harder to monopolize their use via the patent system (*The Economist*, 2015i). Despite the patent dispute over the discoverers of the CRISPR-Cas9 technique the latter has been rather widely adopted because of its numerous potential applications. And despite the reluctance of some companies it has spurred an important commercial activity and very significant investment.

## Biotechnology activity and investment

### *Medical biotechnology companies*

About a dozen biotechnology firms, mostly American, have been created in order to commercialize tool kits derived from the technique. Among these startups, Caribou Biosciences, which was founded by J.A. Doudna in 2011, raised initially US$11 million in funding and focuses on cell engineering for drug screening, agricultural and industrial biotechnology. Caribou has signed agreements with DuPont and Pioneer; it also engaged into a partnership with Genus, a specialist on animal genetics, with a view to developing pigs resistant to a virus causing a respiratory and reproductive syndrome. It has formed with the big pharma Novartis and a venture-capital firm a startup called Intellia Therapeutics (J.A. Doudna and L. Marraffini). With US$15 million raised in 2014 and an estimaded value on the stock exchange of *ca*. US$900 million in 2016, Intellia will focus its work on gene therapies in which cells are taken from patients, edited and put back (*The Economist*, 2015i).

Crispr Therapeutics, founded by E. Charpentier in Switzerland, has sold US$198 million of shares and made partnerships with pharmaceutical groups (Bayer) valued at US$440 million. Editas Medicine, founded by F. Zhang, raised by early August 2015 US$120 million from a group of investors that included Bill Gates. This came on top of US$43 million the company raised in 2013, and its stock value was estimated at *ca*. US$900 million in 2016. Although J.A. Doudna and E. Charpentier filed the first patent for CRISPR-Cas9 use in gene editing, F. Zhang was granted in April 2014 by the United States Patent and Trademark Office (USPTO), the first patent on CRISPR after his institution paid for an accelerated review. This might give him and the Broad Institute control over the key commercial uses of the technique in humans and research animals. The applicants (Charpentier and Doudna) for the same patents are challenging the ruling (*The Economist*, 2015i). Analysts thought that the result, expected in 2017 by the USPTO, would be a compromise with crossed licenses and shared royalties.

Sangamo Biosciences, based in Richmond, California, has been working for a decade On gene therapy experiments *ex vivo*, i.e. cells can be extracted, have their genes modified, and have their new genes tested before being put back to the body. It has been using a more cumbersome gene-editing technique than CRISPR-Cas9, known as zinc-finger nucleases. Sangamo Biosciences is trying to apply that technique to cure beta-thalasssemia, sickle-cell anemia or drepanocytosis, hemophilia and HIV/AIDS. In clinical trials of its HIV/AIDS treatment Sangamo Biosciences takes the immune cells that the virus infects out of the patient's bloodstream and edits in a mutation that makes them highly resistant to infection. It then grows up a large number of the edited cells and infuses them back into the patient, where it is hoped they will flourish. A similar approach can also be used to repair such blood disorders as beta-thalassemia and sickle-cell anemia which are caused by mutations in the globin gene: blood stem cells from bone marrow are extracted, edited so as to switch on the production of fetal hemoglobin (which the body stops producing shortly after birth, even if it cannot make the adult hemoglobin) and returned to the patient's body. It would be like a bone-marrow transplant – except that since the new genetically improved cells come from the patient's own body there is no risk of rejection (*The Economist*, 2015i).

Similar *ex-vivo* approaches could make gene editing a powerful tool for fighting cancer. A currently promising approach to that end is to retrofit the immune system T-cells with what is called a chimeric antigen receptor (CAR) – a protein that recognizes tumours (see p. 314). This CAR-T approach is likely to evolve as CRISPR-Cas9 makes it possible to add more, or subtler, genetic changes to the T-cells. Given the ease and speed with which RNA guides can be designed and tested, it seems only a matter of time until T-cells are tailored to mutations specific to a particular patient's cancer. But when it comes to a brain disease there is no way to take the cells out, repair them and put them back. Instead one has to deliver the molecular editing tools to the cells where they live – to do editing *in-vivo*. So far attempts at therapeutic *in-vivo* gene editing have been limited in scope. Sangamo Biosciences has done a little work in mouse brains where it has been able to repress the expression of the gene that causes Huntington's disease. Intellia has plans to look at *in-vivo* applications that include diseases of the eye and nerves, as well as hemophilia and some infectious diseases (*The Economist*, 2015i).

The easiest *in-vivo* applications of gene editing will be diseases where the damaged cells are easy to access – e.g. diseases of the eye. But gene-therapy companies also have strategies for harder-to-reach cells, with years of work that could now be applied to the delivery of gene-editing packages. For instance, Lysogene, the company Karen Aiach and her husband founded after their daughter's diagnosis with Sanfilippo syndrome – a rare disorder the prognosis of which is that from about the age of three the disease would gradually erode most of the cognitive abilities of the child, who is unlikely to live into her teens. Ornella, Karen Aiach's daughter, who has been diagnosed in the summer of 2005 that she was suffering from the Sanfilippo syndrome, could not produce a protein (because of a defective gene) which is involved in the breakdown of a complex sugar molecule, heparin sulphate. It is the build-up of that molecule in brain cells that lies behind the symptoms of the syndrome. Lysogene has a viral vector which, injected directly into the central nervous system, puts copies of the gene that children like Ornella lack directly into brain cells (*The Economist*, 2015i).

### *Applications to plants and crops*

Genome editing could be a big help to crop breeding, particularly in cereals and fruit crops. In fact Monsanto and many smaller crop-breeding or biotechnology companies are using and improving the CRISPR-Cas9 with a view to designing plants with useful traits. This would be another revolution of plant or crop genetic transformation, but this time without gene transfer (transgenesis). The new plants or crops derived from gene editing will not be called transgenic, but just genetically engineered, as this is the case with microbial, animal or human cells. See also Sasson (2013). Among the companies working on the application of genome editing to plants and crops, Israeli startups and more mature companies are good examples.

Danziger – "Dan" Flower Farm celebrated its 60$^{th}$ anniversary in 2013. Even though the company has over 220 employees in Israel, it acts like a startup at the forefront of innovations in the flower breeding, production and marketing. One of the company's greatest accomplishments in 2014 has been Danziger Guatemala: four hectares of new greenhouses, designed to provide high-quality cuttings to meet customers' needs. Danziger research-and-development (R&D) department is continuously

developing new products, e.g. two new and unique varieties of *Craspedia globosa*. The *Craspedia* flowers belong to the Asteraceae family and are native to Australia and New Zealand. The flowers are called billy buttons and woollyheads. Flowers are conventionally obtained from plants grown from seeds but Danziger has recently introduced a set of varieties, called the PaintBall™ series. The advantages of the new varieties, especially compared with those obtained from seedlings are: uniform flowering, significantly early flowering time, stable and upright stems, spherical inflorescences and large oval inflorescences, deployment of flowering over many months, extraordinary shelf-life.

In order to maintain and strengthen its position as a global leader in breeding, production and marketing of propagating material for ornamental and flower plants, Danziger – "Dan" Flower Farm is committed to constant innovation. To that end Danziger Innovations, a startup, was founded in 2008, with the mission to be a first-choice partner for breeding companies striving to maximize the potential of commercial plant varieties by using "precision breeding" to create varieties in the shortest time possible. Danziger Innovations has developed and owns the intellectual property rights of Memogene, a groundbreaking technology for delivering endonucleases to crop plants via uniquely designed viral vectors. These nucleases perform targeted and site-specific plant genome modifications. Due to regulatory constraints the technology has been optimized for gene deletions with the CRISPR-Cas9 system. The deliverables consist of plantlets or seeds with a designed, inheritable deletion in the genome and free of virus. The product is considered non-GMO by regulatory authorities in Israel and the United States.

Danziger Innovations, whose deputy chief executive officer and head of R&D is Hanne Volpin (2015), has been awarded a joint grant (BIRD Foundation) with Precision Biosciences to develop *Petunia* with extended shelf -life. H. Volpin recalls that plant breeding is the process of changing the traits of plants in order to produce desired characteristics, and that the most important factor in plant breeding is genetic variation. The source of the latter has been for millennia natural variation in breeding material (wild species → early domestication → agriculture and livestock husbandry). The problem is that 90% to 95% of natural variation, including beneficial alleles, has been lost during domestication. Enriching genetic variation can rely to some extent on wild relatives of crop species and varieties, but there can be a linkage with non-beneficial traits during that process. Genetic variation in crop breeding can be produced by induced mutations and genetic modification (associated with randomness and regulations). Danziger Memogene™ technology is used to make site-specific genomic modifications with the highest possible accuracy.

Another Israeli startup, TargetGene Biotechnologies Ltd., based in Rehovot (south of Tel Aviv) Technology Park, is working on creating efficient and specific gene-editing solutions in key crop species. It has been founded in 2012 by Yoel Shiboleth and Dan Weinthal, together with ADAMA Agricultural Solutions; it had nine employees in 2015. TargetGene was the first to invent RNA-guided gene targeting. It is currently implementing its novel proprietary "T-GEE" (Genome Editing Engine) platform which can be used to delete or insert genetic material in a living organism. TargetGene platforms include *in-vivo* assembling nucleases targeted to the cell's DNA. This startup is seeking partners in the sectors of plant breeding, gene discovery and seed industry.

## Editing the genome of newly created human embryos, or of germline cells

In the 18 April 2015 (published on line) issue of the journal *Protein and Cell* a team of Chinese researchers led by Junjiu Huang of Sun Yat-Sen University, Canton (Guangzhou), published the results of the first genetic modification in non-viable human embryos, using the CRISPR-Cas9 technique, as part of their research into beta-thalassemia or Cooley disease (Liang et al., and Huang, 2015). Their objective was to correct the gene causing the illness. Such kind of experiment is forbidden by the Oviedo Convention, ratified in 2011 by more than 28 European countries, because the mutations induced in the embryo could be found in the germinal cells and had therefore the potential to be transmitted to the offspring. Many of the 86 early embryos used in that experiment, which were supplied by *in-vitro* fertilization centres, had a so-called genetic mosaic structure; some cells had a cleaved endogenous β-globin gene while others in the same embryo had still the defective gene. Moreover the 86 embryos had lethal chromosomic abnormalities, even before the targeted mutation. The experiment did not therefore intend to reimplant the modified embryos in order to give rise to fetuses. It confirmed some of the results obtained in laboratory animals: the CRISPR-Cas9 technique is not totally reliable and must be improved. Indeed genetically modifying an embryo that is supposed to develop into a newborn entails a zero-error modification. That was not yet the case (in 2016), as shown by the fact that a small percentage of the modified embryos had the mutation corrected. It is estimated that these techniques will be operational in human embryos in 10 or 15 years. There is therefore time for a thorough discussion on the outstanding power we may acquire on human genetic identity (Alexandre, 2015; Morin, 2015a).

Yet some experiments are more than puzzling. Before the publication of the results of the experiment carried out by the Chinese researchers of Sun Yat-Sen University, the Massachusetts Institute of Technology journal revealed on 5 March 2015 an enquiry made by Antonio Regolado and published under the title *Engineering the perfect baby*. Luhan Yang, a young post-doc who was working in the laboratory of George Church (Harvard Medical School), reported that her research project aimed at collecting the ovaries of women who underwent surgery because of cancer; extracting immature ovocytes thereafter and cultivating them *in vitro*. Then the CRISPR-Cas9 technique would be used to correct the mutation in the BRCA1 gene which causes that cancer. This information provoked a shock in the scientific community and G. Church had to declare that this was a "non-project" (Regalado, 2015).

According to Florence Rosier (2015b), a French scientific journalist who writes for the daily newspaper *Le Monde*, G. Church's position seems ambiguous. During meetings of the so-called "transhumanist" groups he explained the potential of the CRISPR-Cas9 technique for mitigating heart disease and Alzheimer's. But he also explained in 2013 during an interview with the German newspaper *Der Spiegel*, how to recreate the man of Neanderthal whose genome is known. He later on revised his position by co-signing in *Science* a warning against the misuse of new genetic-engineering tools that could modify the human species (Baltimore, Berg et al., and Church and Doudna, 2015). In an interview with *Le Monde*, published on 2 September 2015 by Yves Sciama, G. Church did recall that he co-signed the warning statement in *Science* and he explained that in the case of genome modification (editing) of human embryos

there were very few situations where the CRISPR-Cas9 would be really useful. Patients suffering from cystic fibrosis or beta-thalassemia have other methods at their disposal if they want to have children without the respective defective genes – e.g. make an *in-vitro* fertilization, followed by a preimplantation diagnosis; in other words select the embryos without the defective genes and implant them. G. Church also mentioned in the same interview that molecular genetics can be useful for the recreation of animal species which had disappeared, like the mammoth (this is one of G. Church's team project). But he did not refer to the recreation of Neanderthal man. Finally he recalled that his laboratory was very much involved in gene therapy and personalized medicine (precision medicine). He stated that one very promising area is, in his view, the mitigation of the ageing process or deaging. G. Church thinks that ageing is an illness and should be treated as such. He is not sure that this will work but with his co-workers he brought the proof of concept. They have taken skin cells belonging to G. Church and they succeeded in making them evolve epigenetically into infant cells – but with G. Church's genes. Their research work also deals with the hippocampus that plays a crucial role in the memory process within the brain; it has therefore something to do with Alzheimer's disease. The great challenge is, according to G. Church, to make the brain younger, while at the same time conserving all the memories; in other words repair the engine while it is working (Sciama, 2015).

## Crossing a red line, again? The ethical debate

Scientists stated that replacing a defective gene with a normal one may seem entirely harmless but perhaps would not be. Alain Fischer of the Paris Necker-Enfants Malades university hospital and professor at the Collège de France – a renowned high-level academic and research institution in Paris – reckoned that the CRISPR-Cas9 technique "is an outstanding research tool that improves our knowledge of human diseases." This tool indeed could make a useful contribution to gene therapy in somatic cells, i.e. all the body's cells, except germinal cells. Henceforth there is a legitimate hope to cure some genetic blood diseases, as well as cancers or AIDS/HIV. Jean-Claude Ameisen, chairperson of the French National Consultative Ethics Committee, stated: "Germinal gene therapy does not only treat an individual, but it also affects his/her offspring" (Rosier, 2015b). Many ethicists have accepted gene therapy which implies that changes introduced into the genome die with the patient, but they drew a clear (red) line at altering the germline, since these changes will extend to future generations. In February 2015 the British Parliament approved the transfer of mitochondria to human ovocytes whose own mitochondria were defective. But that technique is less far-reaching because no genes are edited (Wade, 2015a; see p. 386).

### *A possible major ethical rupture*

Ethicists, for decades, have been concerned about the dangers of altering the human germline – meaning to make changes to human sperm, ovocytes or embryos that will last through the life of the individual and be passed on to future generations. The CRISPR-Cas9 technique makes these worries not any more theoretical because it has already been used to edit the genomes of mice, rats and monkeys, and even in human embryos (see above). The technique could be used to repair or enhance the expression

of any human gene. Alain Fischer shared the concerns about the misuse of that tool, for instance in modifying the genome of an embryo that will develop into a fetus and a newborn. This would be contrary to the patient's basic rights. "I think that it should not be done, neither today nor tomorrow. It would be a major ethical rupture," he stated. That is why, according to J.C. Ameisen, "a moratorium and an international debate are needed" (Rosier, 2015b).

France, like 28 other European countries, signed the Oviedo Convention by the end of 2011. This convention stipulates: "An intervention aimed at modifying the human genome can be carried out for preventive, diagnostic and therapeutic reasons, but only in the case when a modification of the offspring's genome is excluded." In these countries it is therefore strictly forbidden to manipulate (change) the genome of germline cells (e.g. in the case of medically assisted procreation). In France the Civil Code, in its article 16-4, modified by the law on bioethics (6August 2004), states that the modification of the genome of human germinal cells even for research objectives is not authorized. "The Civil Code adopts an evolutionary prospect: our species' genome must be protected. However the overall interpretation of different articles of the law leads to some ambiguity," reckoned Emmanuelle Rial-Sebbag, a bioethicist at the French National Institute for Health and Medical Research (INSERM, French acronym) [Rosier, 2015b].

## *Calls for a moratorium*

At the international level there have been several initiatives that called for a voluntary moratorium on all experiments involving germline modification and using the CRISPR-Cas9 technique. Thus a group of scientists which included the head of Sangamo Biosciences (that has been working for a decade on gene editing using zinc-finger nucleases instead of CRISPR-Cas9), published an article in *Nature* calling for such a moratorium (Lanphier et al., 2015); and this was before the publication in April 2015 of the results of the experiment carried out on the modification of embryos' genome by Chinese researchers. The Centre for Genetics and Society, a not-for-profit organization in Berkeley, California, that supports the responsible use of genetic technologies, opposed using CRISPR-Cas9 to conduct even basic research on embryos (like the French Civil Code does). It stated that the prospect of people modified in ways that would be transmitted to their children raises major safety, social and ethical concerns, running the risk not just of producing infants with unforeseen difficulties because of side-effects, but of opening the door to new forms of social inequality, discrimination and conflict (Center for Genetics and Society, 2015).

Jennifer A. Doudna and a number of eminent molecular biologists, such as David Baltimore, a Nobel Laureate and former president of the California Institute of Technology (CALTECH), have called on scientists to avoid any attempts at human germline modification, even if they live in countries where regulation might allow it, before there was a much deeper discussion of the implications. According to George Church 79% of the countries do not forbid such kind of modification, explicitly. In a paper published on line in *Science* on Thursday 19 March 2015 David Baltimore stated: "You could exert control over human heredity with this technique (CRISPR-Cas9), and

that is why we are raising the issue." Another member of the group who co-signed the paper in *Science*, calling for a moratorium, George Q. Daley, a stem-cell expert at Boston Children's Hospital, said about the technique: "It raises the most fundamental of issues about how we are going to view our humanity in the future and whether we are going to take the dramatic step modifying our own germline and in a sense take control of our genetic diversity, which raises enormous peril for humanity" (Baltimore, Berg et al., and Church and Doudna, 2015; Wade, 2015b).

David Baltimore reacted to the experiment carried out by the Chinese researchers by stating in the *New York Times*: "This shows how immature is this science. We have learnt a lot from that experiment, mainly on what could turn wrong" (Morin, 2015b). The biologists writing in *Science* (19 March 2015) supported continuing laboratory research with the technique and few if any scientists believed it was ready for clinical use. The call for a moratorium would not preclude using the technique for research purposes on embryos created as part of an *in-vitro* fertilization programme and not intended for implantation (in the United Kingdom and a number of other countries such research is allowed on embryos up to 14 days of life). The paper's authors, however, were concerned about countries that have less regulation in the field. Therefore they urged that "scientists should avoid even attempting, in lax jurisdictions, germline genome modification for clinical applications in humans" until full implications "are discussed among scientific and governmental organizations." J.A. Doudna, the lead author of the *Science* article, organized the meeting where the statement was prepared (*The Economist*, 2015h,m; Wade, 2015b).

Although such a moratorium would not be legally enforceable, there is a precedent. In 1975 scientists worldwide (including Paul Berg, a Nobel Laureate, David Baltimore) called for their colleagues to refrain from using some of the earliest tools of genetic engineering until rules had been established. A meeting of molecular biologists took place in Asilomar, California, and they issued their statement there. "We asked at that time that nobody do certain experiments, and in fact nobody did, to my knowledge," stated D. Baltimore. "So, there is a moral authority you can assert from the United States, and that is what we hope to do …" "We worry about people making changes without the knowledge of what those changes mean in terms of the overall genome. I personally think we are just not smart enough – and will not be for a very long time – to feel comfortable about the consequences of changing heredity, even in a single individual," D. Baltimore added (Berg, 2008; Wade, 2015b). The 1975 moratorium dealing with recombinant-DNA techniques and advocated at the Asilomar conference is often touted as a worthy example of scientists thinking a new technology's implications through before running into a lot of practical and philosophical issues (*The Economist*, 2015m).

With respect to regulations in this field George Church, whose group has been criticized because of some controversial research projects (e.g. recreating a Neanderthal man or ovocyte manipulation), said he supports regulation because it protects from the worst. It may be tedious to be regulated, but it is even worse to have to deal with such catastrophe like the effects of thalidomide – 50,000 children had been suffering from severe handicaps. Even the failure of the first attempt at gene therapy which caused three deaths has blocked the whole field for ten years, he stated. However it would be

very difficult to regulate some experiments. For instance the genetic modification of yeasts in order to synthesize opium-like compounds will raise very serious problems in the enforcement of law. George Church went on to say that an experiment which entails a morbidity or mortality risk is expected to be forbidden everywhere. But an experiment that is safer and more efficient than current practices will be most likely adopted everywhere (Sciama, 2015).

### *Will the human being dare change his heredity?*

Francis Collins who runs the National Institutes of Health (NIH), America's main government funder of biomedical research, stated in April 2015 that altering the human germline for clinical purposes is viewed "almost universally as a line that should not be crossed." Rudolf Jaenisch, a stem-cell biologist at the Whitehead Institute in Cambridge, Massachusetts, who was not a member of the Doudna group authoring the *Science* paper, stated: "It is very clear that people will try to do gene editing in humans …" "This paper calls for a moratorium on any clinical application, which I believe is the right thing to do." The International Society for Stem Cell Research supported the proposed moratorium. The Doudna group called for public discussion but it is also working to develop some more formal process, such as an international meeting convened by the United States National Academy of Sciences, to set up guidelines for the use of the genome-editing technique CRISPR-Cas9 in humans (*The Economist*, 2015m; Wade, 2015b).

This approach is justified because the red line that should not be crossed, as stated by Francis Collins, may not be strictly true. Mitochondrial DNA donation, an *in-vitro* fertilization technique that replaces a specific form of defective DNA from the mother with equivalent DNA from another woman, became legal in the United Kingdom. The issues surrounding mitochondrial DNA donation were widely discussed in the United Kingdom and the procedure voted on in parliament. The conclusion was that the risks were small and that helping people carrying certain diseases to have healthy children mattered more than rather formless worries about "playing God" (*The Economist*, 2015m; see pp. 386-387).

On the other hand the distinction between somatic and germline cells may become obsolete. "Cell-biology advances are blurring these frontiers," remarked J.C. Ameisen of the French National Consultative Ethics Committee. Researchers can now obtain stem cells called iPS from adult somatic cells, e.g. from the skin. And sexual cells could be "derived" from these iPS cells. "About 15 years of research work would be needed before human gametes could be obtained from iPS cells," stressed Gabriel Rivera of the French INSERM. But what would occur later on? It might be possible to modify the genome of iPS cells (this is authorized) and thereafter transform them into gametes that contain the introduced genome modifications. This likelihood raises the overall ethical issue: Will the human being dare change his heredity? (Rosier, 2015b).

In an interview with F. Rosier of the French daily newspaper *Le Monde* (25 March 2015) Philippe Kourilsky, former director-general of the Pasteur Institute in Paris, and

now honorary professor at the Collège de France, who participated in the Asilomar conference in 1975, underlined that a consensus had been reached after contradictory and heated debates among 140 lawmakers, physicians, scientists and journalists. The moratorium agreed upon in Asilomar could be lifted, except for the experiments that are considered more risky for human health. After 45 years, no particular risk has been detected, said the French scientist. Follow-up committees have been set up. Thereafter there has been a divergence between those applications relating to human health and those relating to agriculture (Rosier, 2015b).

The ethics debate has been triggered off again because of two scientific breakthroughs: the development of transgenic mice during the 1980s and the sequencing of the human genome (1998-2002/2003). Regarding the moratorium on the use of the CRISPR-Cas9 technique for modifying the genome of germline cells, P. Kourilsky stated it did not make sense in countries, like France, which already prohibited this kind of use. According to him modifying the genome of germline cells today would be premature: the techniques used are not yet safe enough; they may introduce modifications away from the targeted DNA sections. In experiments with laboratory animals, if applied to an ovocyte that has just been fertilized, "chimaeric" individuals can be obtained and only some of their cells contain the targeted modifications. A third risk is the following: something is corrected without knowing all the implications of that correction because living beings are very complex systems (Rosier, 2015b).

Therefore, on the technical front, CRISPR-Cas9, although being a good tool, is not perfect – it can make cuts in the DNA that are not desired as well as the ones that are. In research it is fine just to work with cells and animals; in the clinic we need a lower error rate. In germline editing, when any errors will end up in every cell in the body, the problem is particularly worrying. What is more, in most cases where there is a risk of genetic disease it will be safer, when using *in-vitro* fertilization, to choose an embryo that does not have the defect (preimplantation diagnosis) than edit one that does. Only when there are a number of genes to worry about would editing seem a plausible option (*The Economist*, 2015m). One can bet that gene-editing techniques will become more accurate and safer in the future. Would Man dare change his heredity? Having an *a priori* negative response to the issues surrounding this question is not easy to defend, according to P. Kourilsky. What would be the reason for prohibiting or tolerating the least modification of our biological heritage? However much the well worry about the nefarious applications of gene editing, the needs of the sick will continue to drive science and medicine forward (Rosier, 2015b; *The Economist*, 2015m).

## *The challenge of the future: a society becoming eugenic*

Laurent Alexandre (2015), a French geneticist, said that parents in future generations will demand embryo's genetic modifications in order to prevent the occurrence of diseases among their children and their whole offspring. It does not seem reasonable to impose on the families successive gene therapies at each generation, with a view to treating very serious diseases. For instance who would not wish to definitively

eliminate the risk of having children suffering from myopathy (Duchenne's disease) or Huntington's chorea? The debate on the genetic modification of embryos has to take into account the fact that society is becoming very eugenic. Thus the elimination of embryos carrying trisomy 21 (Downe's syndrome) is socially admitted, despite the opposition of some religious or ethicist groups. In 2015 97% of detected trisomic embryos have been aborted which is considered – rightly or not – morally acceptable. By contrast the correction in an embryo of a genetic mutation that is 100% lethal during infancy (e.g. myopathy or neurological disease) is perceived as a crime against humankind. L. Alexandre (2015) thinks that future generations will question this moral distortion and will do the reverse of what seems to us ethical, i.e. they will accept the editing of embryos' DNA instead of aborting fetuses with very serious malformations.

We should also bear in mind that there exists in the United States a rather strong transhumanist movement, almost non-existent in France, which considers that the combination of advances in genomics, robotics and digital applications would enable human beings to extend their life expectancy and evolve differently. Maybe some researchers belonging to this movement would dare target and modify certain genes associated with a longer life, stated Philippe Kourilsky (Rosier, 2015b). George Church of Harvard Medical School has chosen to work on deaging, using genetic modification to mitigate the effects of ageing.

## International Summit on Human Gene Editing

An International Summit on Human Gene Editing has been convened in Washington, D.C., from 1 to 3 December 2015. The organizing committee of the summit included The United States Academy of Sciences, the United Kingdom via the Royal Society (a team of British researchers requested in September 2015 the authorization for carrying out modifications of the embryo's genome; Wade, 2016). China was also associated with the organization of the summit, via the Chinese Academy of Sciences. *Ca*. 200 researchers participated in the summit; among them the Nobel Laureates and molecular-biology pioneers, David Baltimore and Paul Berg, who were the main promoters of the Asilomar conference and of the moratorium on DNA recombination; George Church and many outstanding researchers working on human genomics : Jennifer Doudna of Berkeley University and Emmanuelle Charpentier, of the Max-Planck Institute for Infection Biology, Berlin, as well as Feng Zhang, a prominent leader in biomedical engineering at Harvard University and the Massachusetts Institute of Technology (MIT). J. Doudna and E. Charpentier have been awarded the scientific prize of the Princess of Asturias, Spain, in October 2015, in the city of Oviedo, thus recognizing their role of co-discoverers of the CRISPR-Cas9 technique. Like Feng Zhang (who founded Editas Medicine), J. Doudna created earlier a private company (Caribou Biosciences in 2011) with a view to benefiting from the economic implications of her research work. In addition she set up a partnership with DuPont in order to share altogether their intellectual property rights (iPRs), just after the award of the 2015 Nobel Prize – which none of them received, as some analysts had expected (Lesnes, 2015c; see p. 144).

The Washington summit was not, like the Asilomar conference, a closed meeting. A limited number of journalists were invited, but all interventions wers retransmitted on line, so as to involve the public in the debate among researchers. Gene or genome editing has become a societal issue because of the prospects concerning its use in the treatment of diseases, as shown by the successful cure of Layla Richards, an 11-months-old British child who had leukemia, using genetically-edited T-cells. The British researchers altered T-cells from a healthy donor to help them recognize and kill the patient's cancer cells, to make them immune to her leukemia drug and to ensure they did not attack her healthy cells (see p. 36; Hecketsweiler, 2015w; Lesnes, 2015c). During the spring of 2015 J. Doudna made a strong plea in *Science* (19 March 2015) for a temporary moratorium on experiments to be carried out on the embryo's genome. Such proposal did not meet a unanimous support because it may lead to reducing funding for research. J. Doudna went on in *The New Yorker* and explained her mindset: "When I shall be 90 years old, should I be satisfied about having developed and applied this technology? Or should I wish that I had rather never developed it and how it works?" (Lesnes, 2015c).

## *Fears about the risks of gene editing*

In fact the scientific community is divided with respect to the evaluation of the risks of gene editing, compared with the benefits to be expected from its applications. Some underline that not a single one of the nightmare scenarios imagined in the 1980s has become a reality and that several techniques that may scare the public, such as *in-vitro* fertilization, are widely used. But Marcy Darnovsky, director of Berkeley Center for Genetics and Society, a non-governmental organization that was one of the representatives of the civic society at the Washington summit, did not accept such approach: "*In-vitro* fertilization did not modify the human being for generations," she stated. She expressed the concern regarding the possible drift of gene editing towards an "increase" in human capacities which is the dream of transhumanists. Francis Collins warned on the site of specialized information *Stat*: "Evolution has been working to optimize the human genome for about 3.8 billion years. Do we really think that a small group of genome "editors" or tinkerers with genes could do better without running the risk of making all kinds of unpredictable changes?" (Lesnes, 2015c).

"In the community there is a fear about a possible loss of control," explained Eleonore Pauwels, of the project on synthetic biology at the Woodrow Wilson Center, a public policy institute funded by the American government. Western scientists are worried by the interventions of Chinese researchers (especially since the announcement in April 2015 by Sun Yat-Sen University of experiments being carried out on human embryos, which were nevertheless not viable). "China carries out a lot of sequencing of plant, animal and human genomes. It is considered an actor that may not play by the rules," added E. Pauwels. According to the information drawn from the debates previous to the Washington summit, *ca*. one thousand of Chinese laboratories are using the CRISPR-Cas9 technique. Another worry concerns the potential spread of the technique that could be within the reach of any advanced student in biology. "The world may be ready or not, but the synthetic organisms already exist, such as more virulent influenza viruses or genetically modified embryos, and the instructions to make them will be found on the Internet," underlines the journal *Nature* (Lesnes, 2015c).

## *Promises of more accurate and targeted gene editing*

According to Paul Berg a major difference between the situation prevailing in the 1970s when he co-organized the Asilomar conference, and that of today is that many researchers are now working in the private sector and are using gene-editing techniques. On 18 September 2008 he published an article in *Nature* where he underlined that most of the researchers were then working in public institutions. And the precautionary principle or approach is not viewed or interpreted in the same way in both kinds of institutions (Berg, 2008). Conversely bio-entrepreneurs want to protect the right to innovation, as stated by Catherine Barton, an environmental engineer at DuPont or by Michael Flanagan who said: "We have not stopped the development of electricity because there were risks of fire" (Lesnes, 2015c).

Editas Medicine, a biotechnology startup founded by Feng Zhang and based in Cambridge, Massachusetts, announced it hoped in 2017 to start human clinical trials on CRISPR-Cas9 as a treatment for a rare genetic form of blindness known as Leber congenital amaurosis. Other companies are also testing gene-editing therapies. Moreover researchers at the Broad Institute, Cambridge, Mas., announced at the beginning of December 2015 that they had made changes to CRISPR-Cas9 which greatly reduce the rate of editing errors – one of the main obstacles to the technique's medical use. In November 2015 Anthony James, a molecular biologist at the University of California, Irvine, and co-workers announced they had used CRISPR-Cas9 to build what is known as a "gene drive" in mosquitoes (see p. 271). Gene drives can copy themselves and their associated gene directly into chromosomes that do not have them, meaning that all offspring of an organism will inherit a chromosome bearing the gene in question. This ensures the gene spreads through the population even if it does not confer advantages. In the case of mosquitoes the purpose is to spread genes that would make them resistant to the protozoan parasite causing malaria (*Plasmodium*). But some researchers worry that, once released into the wild, gene drives could induce unpredictable and irreversible effects (*The Economist*, 2015m). See p. 147.

Also before the Washington summit, at the conference organized at the beginning of November 2015 by SynBioBeta – the organization funded by the biotechnology industry – in San Francisco, Edward You of the biology unit of the Weapons for Mass Destruction Directorate at the Federal Bureau of Investigation (FBI) attended that conference and asserted that "the government is willing to help the researchers limit the potential risks of gene-editing techniques and work in total security" (Lesnes, 2015c). To sum up, the Washington summit was held against a backdrop of rapid scientific advance and also with the hope that, again like in Asilomar in 1975, a mixture of common sense and peer pressure will create a world in which scientists are trusted to regulate themselves, rather than having politicians and civil servants do it for them. Eleonore Pauwels stated that the challenge of the summit was "whether we will be able to control our hubris and resist the temptation to transform – and master – such a complex system as the human genome" (Lesnes, 2015c).

## *Conclusions and recommendations*

The academies that convened the International Summit on Human Gene Editing in Washington, D.C., from 1 to 3 December 2015, have no regulatory power, but their moral authority on the issues raised at the summit (as well as that of other renowned participants) seems very likely to be accepted by scientists in most or all countries. Similar restraints proposed in 1975 at the Asilomar conference on genetic engineering had been observed by the world's scientists (Wade, 2015b).

"The overriding question is when, if ever, we will want to use gene editing to change human heritage," David Baltimore said in opening the conference. Unlike gene therapy, an accepted medical technique that alters the body's somatic (ordinary) tissues, changes made to the human germline would be inherited by the patient's children and thus contribute permanent changes to the human gene pool. These, if sufficiently extensive, might in principle alter the nature of the human species. Some biologists think inheritable alterations to the human genome should be indefinitely prohibited. Others believe the science behind the technology should be pursued as vigorously as possible. Paul Knoepfler, a biologist of the biochemistry department at the University of California, Davis, who followed the debates of the Washington summit on his blog, noted that the replies to the issue of modifying the genome of germline cells varied from "Hell no" to "Yes now." Eric Lander, the Broad Institute's head, on the subject of germline editing, told the meeting it would be useful only in rare cases and said it might be a good idea to "exercise" caution before making permanent changes to the gene pool. The need for caution is advice that might also be heeded by those pursuing work in animals other than people, and in plants – subjects not covered by the Washington summit. And P. Knoepfler remarked: "There are already examples of dogs and pigs with genetically strengthened muscles. That might be an irresistible temptation for some parents. If we move in that direction it will be difficult to keep the public's trust" (Lesnes, 2015c; Morin, 2015b; *The Economist*, 2015m; Wade, 2015b).

### A wise middle way

The final declaration of the summit adopted a wise middle way. Basic and preclinical research is necessary and must be pursued on gene-editing techniques, as well as on the benefits and potential risks regarding their medical use. The summit has called for what would, in effect, be a moratorium on making inheritable changes to the human genome. It would be irresponsible to proceed "until the risks could be better assessed and until there was a broad societal consensus about the appropriateness" of any proposed change, stated the summit. The possibility for such work to proceed in the future was held open, and as knowledge advances, the issue of making permanent changes to the human genome "should be revisited on a regular basis." But "if, during research work, human embryos and germline cells are genetically edited, the modified cells must not be used for initiating a pregnancy," warned the summit (Morin, 2015b; Wade, 2015b).

The use of gene-editing techniques in somatic cells should follow the existing regulatory frameworks, that also evolve with the advances in knowledge, as suggested in the summit final declaration. On the clinical use of gene-editing techniques in germline cells the summit declaration highlights: the risks of mutations induced in other genes than those which are targeted; the difficulty to predict deleterious effects; the obligation to take account of the implications of these alterations for both the individual and future generations; the irreversibility of these alterations; the likelihood that privileged people or populations might benefit from these techniques, or, conversely, that some others be obliged to endure "improvements" of their genome (Morin, 2015b). In this regard Marcy Darnovsky had expressed before the Washington summit her worry about "the social stratification" that may result from a laxist approach to the human genome editing: only the wealthiest people might have access to the technique for "improving" their offspring (Lesnes, 2015c).

The overall consensus reached at the Washington summit, i.e. not to proceed until the risks could be better assessed and until there is a broad societal consensus about the appropriateness of any proposed change in the human genome, is very close to the position expressed by J. Doudna in *Science* (19 March 2015). If several speakers at the summit laid out plans for correcting various hereditary diseases in the egg or sperm by cutting and pasting the correct DNA sequence into errant genes, others noted there was no pressing medical demand now for making heritable changes to the human genome because diseases caused by a single errant gene were rare. Inheritable gene editing is inapplicable to common diseases like cancer or diabetes in which the hereditary component is associated with many different genes. Even in the case of single-gene defects, known as Mendelian diseases, germline editing is unnecessary in many cases because parents can have an unaffected child through *in-vitro* fertilization in which, after genetic screening, only healthy embryos are implanted in the womb. But in some cases, such as when a parent has two copies of the gene for a dominant hereditary disease like Huntington's chorea, editing the human germline may be the only way to ensure that a child will not also have the disease. George Q. Daley, of Boston Children's Hospital, described several such conditions in which germline alteration would be medically effective, while casting doubt on the feasibility of designer babies, a proposed outcome of germline editing (Morin, 2015b; Wade, 2015b).

The Washington declaration is not binding and George Church fears that the proposed moratorium might encourage "a black market and a medical tourism that would escape any control." On 3 October 2015 the Council of Europe announced "it is supporting the genome-editing techniques, but with a number of limitations." The Council recalled that, according to article 13 of the Oviedo Convention (1997), ratified by most European countries, including France, any intervention on the human genome "can be carried out only for preventive diagnostic and therapeutic reasons." This article also forbids "any gene modification in embryos that would be transmitted to future generations" (Morin, 2015b).

## Call for a continuing forum

The national academies that convened the Washington summit also called for a continuing forum in which potential uses of germline editing will be discussed. "The meeting in Washington is not an end, but a starting block, with future fora in perspective," underlined the French reproduction specialist, Pierre Jouanet, who was invited to participate in the summit in order to present the current research carried out in France. "We discuss a lot about the potential applications (of gene editing), but we cannot now achieve a 100% efficiency and security in the case of the human embryo," he recalled. "In the case of animals, when the mutation is found in three individuals out of ten, this is considered a success, but this cannot be satisfactory in the case of children," he added (Morin, 2015b). See also Wade (2016).

# OTHER APPLICATIONS OF HUMAN GENOMICS

## Small interfering ("si") RNA drugs

Gartner, an American consultancy, has described the life of a promising new technology, such as the CRISPR-Cas9 one, by reaching a peak of inflated expectations, then falling into a trough of disillusionment. After that, if it survives, it begins climbing the slope of enlightenment; finally it reaches the plateau of productivity (*The Economist*, 2015k). CRISPR-Cas9 was still ascending towards peak expectations by the end of of 2015. True to the Gartner hype-cycle, though, RNA interference, or RNAi, is well and truly in the trough. Google searches for the two technologies shows this. The question is, can RNAi climb the slope of enlightenment to become a productive and useful technology? Like CRISPR-Cas9 RNAi is based on a bacterial response to viral infection. Double-stranded RNA does exist naturally, but it is found only in viruses. For this reason, RNAi, a single-stranded version of RNA, recognizes double-stranded RNA and eliminates it (*The Economist*, 2015k).

When RNAi was discovered it looked tailor-made to be the basis of a new class of drugs. One of RNA main function is to carry information from genes in the nucleus to protein factories in the rest of the cell. If messenger RNAs could be inactivated, this would reduce or eliminate the proteins they generated. Since proteins, in the form of enzymes, signalling molecules, ion channels, etc., regulate all cellular processes, the range of diseases to which RNAi-based drugs might be applied seemed quite wide. Such drugs, known as small interfering ("si") RNAs, are short double strands of the molecule. The drug works because one strand of each siRNA is complementary to the messenger strand that is the object of interest. The RNAi system pulls the siRNA strands apart and uses the complementary strand to seek out and bind to the target messenger, thus disabling it. The result is, in principle, a precise means of knocking out proteins involved in a particular disease (*The Economist*, 2015k).

Drug companies showed great interest in these possible RNAi drugs. In 2006 Merck & Co. Inc. paid US $1.1 billion to Sirna Therapeutics, a biotechnology firm reckoned to be a leader in the field. Roche and Novartis, two Swiss top leading big pharmas, also made large investments about the same time. RNAi, it seemed, was going to bring a revolutionary approach to the drug industry. But siRNAs, so attractive in theory, proved impossible to end up in effective drugs. Roche ended its work in 2010, Novartis and Merck followed suit in 2014. Nevertheless a few biotechnology companies are still working on the idea, and some of them now think they have cracked it. Chief among them are Alnylam and Dicerna, both located in Cambridge, Massachusetts. These firms have, they believe, overcome one of the problems that cause RNAi to fall into the trough of disillusionment – transporting RNAi molecules across cell membranes to where they are needed (*The Economist*, 2015k).

The firms have done that in two ways. One is by encasing the RNA in fatty capsules having a diameter less than a micron. These capsules are easily absorbed by liver cells and the liver is a target for RNAi-based therapies. The other way (a method that is the subject of a legal dispute between them) is to attach the siRNA molecules to other molecules that are readily taken up by liver cells. According to Alnylam chief executive officer, John Maraganore, his company has seven siRNAs in clinical trials. The most advanced of these are two intended to combat TTR-mediated amyloidosis, an inherited disorder. In this case the siRNA involved knocks out the messenger RNA from the mutant gene which causes the disease. These two molecules were in 2015 in phase-3 of the clinical-trials process. But TTR-mediated amyloidosis is a rare disease. The treatment in the firm's pipeline that has the greatest market potential is directed against low-density lipoproteins (LDLs), the so-called "bad cholesterol", which increases someone's risk of heart disease. In this case the target is PC-SK9, a protein that regulates the production of certain receptor molecules found on the surface of liver cells. These receptors pluck LDLs from the bloodstream for disposal. Less PC-SK9 proteins means more receptors and henceforth lower LDLs concentration (*The Economist*, 2015k; see. p. 326).

For its part Dicerna hopes to be able to block the messenger RNAs from two genes called *KRAS* and *MYC* – or rather, by mutated versions of these genes. These genes are oncogenes, for when they go wrong they can lead to cancers. By late 2015 neither *KRAS* nor *MYC* genes were considered "druggable." In other words not only are no drugs available, but no good approach to developing them exists. Dicerna researchers intend to change that with their tailor-made siRNA. In Australia, meanwhile, Benitec Biopharma of Sydney is developing another way of introducing siRNAs into cells. This is to encourage those cells to make the molecules themselves. To do so means integrating genes encoding the siRNA in question into a cell's nucleus. For this Benitec uses viruses engineered for the purpose of gene therapy. The result which the firm calls DNA-directed RNAi, is being tested on hepatitis-C – a disease that kills *ca*. 500,000 people a year. If that works treatments for other illnesses should follow. It has been therefore concluded that RNAi, less hyped than it was in the early 2000s, may still have a bright future in the design of molecular biology-based drugs (*The Economist*, 2015k).

## "Good" versus "bad" mutations: a promising approach to treating or curing diseases

After years of looking for mutations that cause disease, researchers are now searching for those that prevent them. "The new approach is turning genetics research on its head," stated Eric E. Schadt of the Icahn School of Medicine at Mount Sinai Hospital in New York. "Instead of trying to fix things that are broken, let us look at people where things are broken but nature finds a way around it," he explained. In fact, in recent years, a few protective mutations have been discovered, pretty much by accident. One of them prevents the Human Immunodeficiency Virus (HIV) from entering blood cells and another considerably reduces the amount of LDL (low-density lipoproteins) cholesterol. Both led to drugs: the AIDS drug is a mainstay of treatment of the disease and the anticholesterol drug was in the final stages of testing in 2014. Using systematic searches of genetic databases researchers also found alterations in some genes that particularly protect from heart disease, osteoporosis, type-2 diabetes and Alzheimer's. But some scientists are starting a more ambitious project – a search for mutations that

provide complete protection. However the difficulty is to be able to figure out whether disease resistance is due to a "good" gene mutation or a good environment or simply good luck, defying the odds when a disease is likely but not inevitable. And if there is a "good" gene mutation involved, searching for it among the 25,000 human genes can be daunting. It is easier to find mutations that cause diseases – those appear to be many times more common (Kolata, 2014b).

According to Thomas Caskey of Baylor College of Medicine, Houston, the 25,000 known human genes correspond to 3.2 billion nucleotide pairs (3.2 Gb); the coding space for proteins amounts to 64 million nucleotide pairs or bases (64 Mb): *ca*. 2,500 genes are associated with human disorders; the mutations occurring in an individual affect some 4 million nucleotide bases (4 Mb), those which result in a change of proteins occur on some 12,000 bases (12 Kb), the number of mutations known to cause human disorders is about 100 Kb (personal communication, 2014).

### *Search for "good" gene mutations*

With fast and inexpensive methods of sequencing DNA and with massive and evergrowing databases of study subjects whose genomes have been sequenced, it has become possible to seriously contemplate a search for rarer good genes. One attempt being led by Eric E. Schadt and Stephen H. Friend, director of Sage Bionetworks, a not-for-profit organization based in Seattle, that promotes open science started because both scientists had become frustrated with the failures of drug development. S. Friend had worked with the Massachusetts Institute of Technology and Harvard, then founded a biotechnology company, Rosetta Inpharmatics, and later helped run the cancer drug discovery effort at Merck. All too often disease-causing mutations destroy or disable genes and drugs would have to restore what was lost, which can be difficult. Therefore E.E. Schadt and S.H. Friend decided to search for a good gene mutation that counteracts the bad one and, in an easier process, mimic that with a drug. They gave their plan a name, The Resilience Project, and decided to search databases that held genetic and clinical information, looking for healthy people with mutations for fatal diseases that strike early in life (Kolata, 2014b).

They analyzed data from more than 500,000 people and found 20 who seemed to be protected from a fatal disease. But because of privacy issues there were no names attached to the data. Four of the subjects were in China and it was very difficult to be in touch with them. The American researchers are now looking at other databases that might make easier to approach subjects, but they also decided to try different approaches. One will be to simply ask healthy people to let them sequence their DNA; people who agree would be contacted only if they appear to be protected from a fatal disease. Another approach is to collaborate with researchers studying extended families with a very severe genetic disease to see if they came across anyone who seemed protected (Kolata, 2014b).

When they called on researchers at Washington University in St. Louis, Missouri, who were studying families with a gene, presenilin, that causes early Alzheimer's disease, they discovered Doug Whitney, a 65-years-old resident of Port Orchard, Washington State, who has this gene; he could still get Alzheimer's, but it would have been substantially delayed. This man had been waiting for Alzheimer's symptoms, starting

when he turned 40. He knew he had a 50-50 chance of inheriting the Alzheimer's mutation, but nothing happened. In 2011 he joined a study at Washington University, led by Randall Bateman, that recruited people from families with an early-onset Alzheimer's gene mutation. D. Whitney had finally concluded he did not have the gene mutation – he was 61, after all, and his memory and thinking were fine. On 31 May 2011, his 62nd birthday, he decided to have the genetic test. A month later the result was that he had the gene. D. Whitney who retired at the end of 2014 was still ready to help the researchers, not only R. Bateman, but also Thomas D. Bird, a neurogeneticist at the University of Washington, as well as S.H. Friend and E.E. Schadt, who approached him. There is therefore hope that such kind of study may lead to unravel the genetic causes of Alzheimer's disease and perhaps to a more efficient treatment of the illness (Kolata, 2014b). See also *The Economist* (2016).

## Understanding diseases through unravelling the switches and genes they control: the Human Epigenome Project

Researchers have long known that genes are only a small part of DNA – the rest contains switches that control gene expression. And researchers suspect that changes in these switches may have as much to do with diseases and with traits, like height or weight, as changes in genes themselves; 90% of DNA alterations associated with diseases are turning out to be in switching areas, not the genes themselves. Scientists stated they urgently needed a map for understanding those circuits (Kolata, 2015a).

### *A road map to the human epigenome*

To find the switches and figure out the circuits that control genes researchers examined cells taken from healthy people and from patients suffering from a range of diseases including multiple sclerosis and diabetes. They also studied cells from different stages of life, including fetal cells and stem cells, which are present at the very earliest stage of development. Using those cells investigators found millions of switches that control genes. The results were published in 24 papers in *Nature* and the *Nature* journals (Roadmap of Epigenomics Consortium et al., 2015). "We now have an unprecedented view of the living human genome," stated Manolis Kellis, a computer scientist at the Massachusetts Institute of Technology (MIT) and a leader of the federally funded project (Kolata, 2015a). He used the following metaphore to explain the concept behind the Human Epigenome Project: "The Human Genome Project has given us the book of life that characterizes an individual. All our cells have a copy of the same book, but each cell reads distinct chapters and underlines distinct chapters. The Human Epigenome is the sum of all the switches in the genome of each cell type, which are chemical modifications of the DNA and the ways in which the DNA is arranged at a large scale" (Sampedro, 2015). The figures relating to the Epigenome Project are impressive: more than 100 tissue and cell types; 2,800 experiments, each one at the scale of the entire genome; 150 billion DNA probes or fragments that overlap the genome 3,000 times. With all these data in hand scientists will be able to understand which genes are active, or switched off, or those which can be activated in each cell or tissue type (Sampedro, 2015).

Thus they may be able to understand what is the real difference between a liver cell and a heart cell. They could also compare the cells or tissues of patients, thus understanding which are the switches that control diseases or some traits. For instance they have realized that the genetic change associated with a high or low stature was active in stem cells. Also researchers had previously found DNA alterations associated with high blood pressure, for example, but the alterations were not in genes. Presumably they were in switches. The new research found that these switches were only active in the heart and, it seems, in the heart muscle of the left ventricle. It was also realized that the genetic variants associated with type-1 diabetes, rheumatoid arthritis and multiple sclerosis were found in the cells of the immune system; this is logical in so far as these diseases belong to the autoimmune-disease category (Kolata, 2015a; Sampedro, 2015).

An example of how the human epigenome can help physicians is that of Alzheimer's disease. Being a disease where the neurons are progressively destroyed, it was suspected that the genetic switches which are associated with the onset and progression of the disease would be active in the neurons themselves. This is not the case, because these switches function in the cells of the immune system. This completely changes the prospects for searching preventive or palliative treatments of the illness (Sampedro, 2015). By all means and as it has been the case with the sequencing of the human genome in 2003, the epigenome will be very useful as a tool for understanding gene regulation, cellular differentiation and human disease, and will show its benefits in the years to come.

## The boom of synthetic biology: advances and public acceptance

### *Scope of the debate*

As occurred with genetically modified organisms (e.g. transgenic crops) which are widely accepted in the United States (as foodstuffs) and which are generally rejected in Europe, public or social acceptance is just as important when one deals with synthetic biology. E. Pauwels, an associated researcher at the Woodrow Wilson Center in Washington, D.C., has been analyzing the public perception in this fast-growing area of life sciences and biotechnology (Lesnes, 2015a). See also Sasson (2013). She found through a poll carried out in April 2015 that 23% of Americans interviewed (compared with 17% of Europeans) have heard about synthetic biology, but they did not really know what it is exactly. Lacking such knowledge they keep in their mind science-fiction scenarios. The modification of the genome seems potentially interesting, but people do not know how to communicate on that subject. Even the scientists prefer not to speak too much about it, while J. Craig Venter preferred to speak bluntly and provoke a shock among the public (Lesnes, 2015a).

In March 2016 J. Craig Venter and his co-workers published their results about designing a synthetic genome containing the minimum number of genes enabling a living cell to divide and multiply. This was the achievement of 20 years of work and the new synthetic genome was christened JCVI-syn3.0. (Service, 2016 ). In 2010 the American researchers had created a synthetic genome, named JCVI-syn1.0 and built from the bacterium *Mycoplasma mycoides*; it was thereafter introduced into another species, *Mycoplasma capricolum*, whose genome had been withdrawn. The introduced artificial genome allowed the new hybrid species to reproduce (Pennisi, 2010).

JCVI-syn3.0 contains 473 genes and is the simplest form of life known up to now. It divides every 180 minutes. The "dispensable" genes have been eliminated after three cycles of "design-manufacture-test." "It is a functional approximation of a minimum cell genome," commented J. Craig Venter and his colleagues. "A compromise between a small genome size and an acceptable growth rate for an experimental organism," they added (Morin, 2016b; Service, 2016). The artificially-created bacterium – an excellent example of synthetic biology – is considered a good experimental tool aimed at studying the basic functions of life. Of the 473 genes of JCVI-syn3.0, 41% encode the expression of the genetic code, 18% are involved in the synthesis of the membrane, 17% contribute to the cell metabolism, 7% preserve the genetic code, while 17% have no known functions (Morin, 2016b).

Besides this important achievement in microbial synthetic biology, the debate in this area is often drifted towards such subjects as the modification of gametes, babies with desired traits, etc. The fact is that we do not focus on the basic question: if the human genome is modified, what is the part that would not be under control? What is the sequence that is going to be modified, while it was not the target? The debate, according to E. Pauwels, tends to reduce uncertainty and complexity. The DNA is presented like a software and the cell like a computer that can be programmed. Scientists think of themselves as engineers and they are mainly interested in the paradigms of directed construction and reconstruction of the cell. But when these issues are debated with scientists, they often reckon that there are still many aspects they do not know or grasp, that there are many mechanisms of adaptation and mutation in biology and there is most probably a function in this uncertainty of the cell. Besides the laboratory this analogy with the world of informatics does raise a problem, because it is too reductive and relatively dangerous. It strengthens the description or the narration of a control on the cell system. There is a need to find out how to raise and discuss the issues within a democratic dialogue (Lesnes, 2015a).

### *Endeavours aimed at informing citizens*

There is an ongoing endeavour aimed at explaining cell biology and genetics as well as the bases of synthetic biology. For instance a course was designed in San Francisco by SynBioBeta, a network of enterprises and investors, and it aims to reach scientists and programme designers belonging to other areas of knowledge, who would be able to bring in their expertise in this fast-growing area of synthetic biology. The course's mentor is John Cumbers, a cell microbiology specialist, who left NASA in order to become an expert in synthetic biology. The other main teacher of the course is Josiah Zayner, who also comes from NASA: he is developing plastic-degrading microorganisms with a view to preparing a spatial expedition to Mars (Lesnes, 2015a).

The programme of the course includes the basic mechanisms of cell function; the promises of synthetic biology – such as the production of very resistant materials, like spider silk, the design of viruses that allow antibiotics to overcome bacterial defence systems; and the ways to use the tool of genome-editing CRISPR-Cas9. The students participate in an experiment of DNA modification. Such kind of scientific popularization course is not an exception. In San Francisco Bay three laboratories propose teaching sessions and practical work (in groups) to "scientific citizens", who can thus extract

DNA from a strawberry, produce luminescent plants and even imprint living cells in 3D. "This kind of initiatives has been considered with scepticism from the bioindustry," stated John Cumbers. But now biotechnology companies look at citizens' participation in a different way. None of these companies wants to have the public against it and become the "Monsanto of synthetic biology" (Lesnes, 2015a).

Biological engineering does increasingly attract the interest of the private sector in the United States. In 2005 there were 45 companies operating in that sector, while their number rose to 103 in 2010 and 200 in 2014. Private investments reached US$560 million (or €493 million) for the first nine months of 2015, indicated J. Cumbers, and that was more than the combined investments for 2013 and 2014. And J. Cumbers added that since 2013-2014 the high-tech billionaires have been attracted by biotechnologies: from Peter Thiel, co-founder of PayPal, to Eric Schmidt of Google, who personally invested money in Zymergen, a startup which has robotized DNA production. The public sector is not absent from the area of synthetic biology: according to a report published in September 2015 by the project on synthetic biology at the Woodrow Wilson Center, the United States government had invested US$820 million in research on synthetic biology between 2008 and 2014. And in this regard a fact should be highlighted: investments made in defence were higher than those made in agriculture or health (Lesnes, 2015a).

The research arm of the Pentagon, the Defence Advanced Research Projects Agency (DARPA), invested US$100 million in 2014 (compared with almost 0 in 2010), and is by far the leading investor, ahead of the National Science Foundation (NSF), the National Institutes of Health (NIH) or the Department of Agriculture (USDA). Actually the DARPA contributes almost 60% of the public funding for synthetic biology. When the investments of other branches of the Department of Defence are added, the overall contribution reaches 75%. Such disbalance worried the researchers because the areas where DARPA is working remain imprecise willingly, or they are labelled as "defence-secret" in the requests for subsidies that are reviewed by the Wilson Center. In 2012 the DARPA launched the project called "Living Foundries," aimed at building DNA blocks. By the end of September 2015 the DARPA allocated US$32 million to the synthetic biology laboratory of the Massachusetts Institute of Technology; with the following objective: to develop "new products that are of key importance for human health-care, agriculture and chemistry, and become a mechanism that should come to grips with some global key issues" (Lesnes, 2015a).

### *Addressing regulatory and ethical issues*

As a consequence of the larger proportion of investments made by the Department of Defence, the public funding of research on the risks of synthetic biology has been decreasing, to reach less than 1% of total funding. Not only the impact of DNA engineering on the environment or public health is not dealt with, but also legal and ethical issues receive little attention: also less than 1% of total funding. David Rejeski, director of the programme on science and technological innovation at the Wilson Center, was worried by the fact that the United States were not well prepared to the implications of the flow of more than US$1 billion (from both the public and private sectors) into such a young research area (Lesnes, 2015a).

In July 2015 President B. Obama's administration launched a review of the legal framework that had been regulating biotechnology since 1992. The current system is, according to John Holdren – President B. Obama's scientific advisor – too complex. The public "does not understand how the safety of biotechnological products is assessed," he explained. And the biotechnology startups cannot easily interpret the regulations in order to determine whether they depend from the agriculture department, the Environment Protection Agency (EPA) or the Food and Drug Administration (FDA). Also in Europe the European Commission is in the process of revising its evaluation procedures regarding "biologically improved" plants or crops (Lesnes, 2015a).

The American Congress has made a few hearings. A draft law is being studied. In the United States experts are not expecting a real progress before the November 2016 presidential election, because of the sensitivity of the issues concerning GMOs and of the strong opposition of the Christian wing of Republicans to any kind of manipulations regarding "life". "We are in a strange situation where there is more money and a regulatory framework that is becoming obsolete," summarized David Rejeski (Lesnes, 2015a). In 2015-2016 there were 115 distinct products and applications derived from synthetic biology, and *ca*. 50 among them are being commercialized or ready to be on the market, such as genetically modified microalgae (under the EPA review) or a fast-growing salmon (finally authorized by the FDA after 15 years of review) [Lesnes, 2015a].

## Human Protein Atlas

By early November 2014 the Human Protein Atlas was completed. It consists of photographic maps, 13 million of them, of tissues from all bodily organs. It shows which proteins are found there. The maps were made by creating antibodies to 17,000 individual proteins, attaching staining molecules to those antibodies and then applying the combination to thin slices of preserved tissue to see, by what colour the tissue went, which antibodies had stuck to it. The atlas will be of great value to researchers trying to understand how tissues differ at the molecular level. Most biological functions depend on proteins, so it is the mix of proteins within a cell which defines what the cell is. The atlas will also (because 20 types of malignant tumours are included in it) help to explain how cancerous tissues differ from their healthy progenitors (*The Economist*, 2014f).

There are *ca*. 20,000 protein-coding genes in the human genome; so even though the atlas covers all parts of anatomy, it is not yet complete. Indeed the cellular machinery that translates the information contained in genes and uses it to construct proteins often applies tweaks to those proteins as it goes. This means that there are more sorts of proteins than genes, expanding the task of mapping what is happening still further. The researchers who created the atlas, led by Mathias Uhlen of the Royal Institute of Technology, in Stockholm, have nevertheless discovered that 3,500 human genes encode proteins specific to just one or two tissues. These are, presumably, crucial to what the tissues do. Many of these proteins are peculiar to the cerebral cortex – which is not surprising, as the brain is regarded as the body's most complex organ. But, proteinwise, it turns out that it is only the second most complex, behind the testis, with a third of those 3,500 genes (*The Economist*, 2014f).

# PART THREE

## *CURRENT ACHIEVEMENTS AND PROSPECTS IN MEDICAL BIOTECHNOLOGY*

# CONTENTS

# GLOBAL HEALTH CHALLENGES

## Bill and Melinda Gates Foundation's Grand Challenges in Global Health programme

In 2004 the Bill and Melinda Gates Foundation began divvying the money for what it hoped would be a novel approach to the task of solving the world's health problems. The new programme's designers, led by Bill and Melinda Gates themselves, had identified 14 "grand challenges" in the field – from "preparing vaccines that do not require refrigeration" to "development of a genetic strategy to deplete or incapacitate a disease-transmitting insect population" – and had invited suggestions from the world's scientists for specific projects of a kind that might not otherwise be funded, which might meet these goals. Not surprising, since the foundation had announced a year earlier that it was making US$200 million available to pay for all this, hundreds of research groups lined up to make proposals. In a tenth-anniversary review meeting of the Grand Challenges in Global Health programme, as it is known, which was held in October 2014 in Seattle, Bill Gates and his fellow board members had to examine whether their philanthropic version of venture capitalism should lead to breakthroughs in the search for vaccines and other treatments for widespread and destructive diseases such as malaria. In fact, a decade – and US$1 billion – later, neither the original project nor its offspring, Grand Challenges Explorations (which gives seed money to young researchers rather than relying, as the original did, on established teams), has thrown up any of the blockbusters that real venture capitalism requires to counterbalance the numerous, inevitable failures. Nevertheless Bill and Melinda Gates announced in Seattle a new set of challenges, this time spreading the range wider than the strictly science-based suggestions the programme had encouraged until then (*The Economist*, 2014e).

Grand Challenges in Global Health has enjoyed at least modest success. Of the 44 original projects, a fifth are moving towards fruition and another fifth have worked in part. Scott O'Neill of Monash University, in Melbourne, for instance, was one of the original challengers. He plans to control dengue fever not by killing the mosquitoes (*Aedes* sp.) which transmit it, but by making those insects immune to the virus that causes it. Such immunity is conferred by a bacterium, *Wolbachia*, which is sexually transmitted in a way that encourages it to become ubiquitous (it passes from mother to egg, and if an uninfected female mates with an infected male, its eggs will not develop – so the number of infected mosquitoes decreases with each generation). That might wipe dengue out, at least locally. Grand Challenges has therefore invested US$44 million in Scott O'Neil's project, and the Wellcome Trust, the Tahija Foundation and the Gillespie Family Foundation, three other charities, have added more.

S. O'Neil and his team have begun field trials in Australia, Brazil, Indonesia and Vietnam, releasing *Wolbachia*-infected mosquitoes to see if these can establish themselves as the assumption predicts they should (*The Economist*, 2014e).

Another promising project, led by James Collins of Boston University, is attempting to create a drink laced with bacteria that kill other, cholera-causing bacteria after they have become established in someone's intestines. J. Collins' genetically engineered microorganisms will produce anticholera drugs, and then disintegrate when their work is done. He created a company, called Synlogic, to develop this idea and to investigate whether it can be extended to controlling other diseases. One grand-challenges investment in neonatal health that is already paying off is a machine designed to stop the lungs of premature babies collapsing. Such machines have been present in rich countries for a long time but, at US$6,000 a machine, the poor cannot afford them. Rebecca Richards-Kortum, a bioengineer at Rice University, in Houston Texas, has developed a version that costs US$400. These machines were installed in 17 hospitals in Malawi, the country that has the world's highest rate of pre-term births. The survival rate for premature infants born in these hospitals has, as a consequence, risen from 24% to 65% (*The Economist*, 2014e).

## New strategic approaches

The new grand challenges (as spelled out in Seattle in October 2014) are rather different from the existing ones. "All children thriving," "putting women and girls at the centre of development" and "creating new interventions for global health" sound more like aspirations than proposals for action. Indeed, the third of them embraces 11 of the original grand challenges under a single heading. And this time the foundation is engaged in all sorts of partnerships, from the United States Agency for International Development (USAID) to the governments of Brazil, Canada, India and South Africa. The new challenges are, in part, a response to criticism that the original ones were too technocratic. It is true that public health depends on educating people and persuading them to change their behaviour, as well as on having the right medicines, as the example of HIV/AIDS eloquently shows. That kind of approach requires social change as well as appropriate technology. Children will not thrive by the invention of a new vaccine if mothers are not convinced of that vaccine's value – and these mothers are less likely to be convinced if they are poorly educated, which is why they need to be at the centre of development. The grand challenges' change of direction thus makes sense (*The Economist*, 2014e).

And yet, what made the Bill and Melinda Gates Foundation's original changes such an innovative approach was precisely their specificity. The whole bureaucratic machinery of global health and development, from foreign-aid agencies to charities to the World Health Organization (WHO), is signed up to the idea of children thriving and of women and girls being at the centre of development. No right-thinking person could disagree with those ideas. But if the Gates Foundation can bring to these ideas specific proposals to improve matters, as it has tried to do with disease, then its change of direction may become more than meaningful. The Grand Challenges in Global Health programme should not lose its specificity, so as to be a valuable and novel alternative approach to solving global-health problems (*The Economist*, 2014e). See also Sasson (2008, 2011).

## Funding biomedical research and innovation

Funding research and development and innovation in the biomedical sciences remains a global, regional and national challenge. For instance, in the United States, Francis Collins, director of the National Institutes of Health (NIH), has complained that the agency's budget of *ca*. US$30billion, when adjusted for the inflation in cost for medical research, reflected a drop of nearly 25% in purchasing power. "We have investigators in the United States who have great ideas, talent, creativity and energy, who are frankly at the point of giving up," he stated. The NIH hoped that the 2016 budget, as proposed by the President to the Congress, will increase its own budget by US$1 billion. But NIH was not the only institution that has been affected. In fact, the growth rate in medical-research funds has dropped nearly 10% on an inflation-adjusted basis since 2004. This innovation gap, nicknamed a "valley of death" by some in the field, has coincided with the launch of startups that rely on crowdfunding strategies to raise small amounts of cash from lots of different people instead of relying on hefty sums from one (Sifferlin, 2015).

The website *Experiment*, for instance, collects donations for projects like developing a better eye prosthesis or finding effective drugs for hookworm – which raised more than US$18,700. *Consano*, another crowdfunding site, is a non-profit that is popular with new scientists. And the newest research crowdfunder, *Give to Cure* (*GTC*), was launched in November 2014 with a focus on clinical trials that may accelerate new-drug discovery. Unlike other platforms that fund studies of all stripes at a given moment, GTC focuses on one disease at a time, and it is starting with Alzheimer's. Once GTC picks its area of focus, it invites scientists to submit clinical trials that have regulatory approach but not funding. Next, a scientific advisory committee picks the five most promising trials, and GTC begins raising cash. The hope is that funding numerous projects for the same disease will increase the chances of finding a cure. The studies are also audited along the way so donors can see where their money has gone and keep records on the scientists' progress. The co-founder of GTC, Lou Reese, said the hope behind GTC and projects like it is to attract people with a personal attachment to a cause, who want to see their donations go to specific research that might some day benefit their loved ones. That was in part what inspired L. Reese to choose Alzheimer's as GTC's first disease (Sifferlin, 2015).

# DISEASE PREVENTION : VACCINES AND VACCINATION ISSUES

## Vaccine-development milestones and vaccination policies

Since the late 18th century, vaccines have played a key role in the prevention of communicable diseases. They are part of countries' public health policies aimed at controlling those diseases since the early infancy, and even at eradicating them. There are many milestones in vaccine development, followed by successful prevention of disease. For instance, the vaccines against smallpox and rabies were developed in 1796 and 1885, by Edward Jenner (England) and Louis Pasteur (France), respectively. Vaccines against tuberculosis (BCG, Bacillus Calmette-Guérin, France), diphtheria, tetanus and whooping cough, poliomyelitis, measles, mumps, hepatitis B and papillomavirus, have been developed in 1921, 1923, 1926, 1952, 1963, 1967, 1981 and 2006, respectively (Cabut et al., 2015). See also Sasson (2008, 2011).

In France, for instance, vaccine policy is part of the strategy aimed at controlling communicable diseases. It is carried out by the health ministry, while taking into account the epidemiological data concerning the diseases that can be prevented through vaccination, the technical progress made in the area, the World Health Organization (WHO) recommendations and the organization of the French health-care system. Vaccinal policy is based mainly on the advice and proposals of the High Public Health Council (HCSP, French acronym), elaborated through the Vaccination Technical Committee (CTV, French acronym). Thus, in 1902, the vaccine against smallpox became mandatory; in 1938 the vaccine against diphtheria, after the introduction of aluminium salts in the development of vaccines in 1926; in 1952 against tetanus; in 1965 against poliomyelitis; in 1967 against yellow fever in French Guyana. In 1977 the last case of smallpox was officially detected in the world (WHO). In 1979 smallpox vaccine was not anymore mandatory; in 2007 the mandatory vaccination of the child and teenager, using the BCG, was lifted. Since 2008 the vaccine against diphtheria, tetanus and poliomyelitis (DTP) has been withdrawn. The French vaccination policy recommends many vaccines, taking account of the age, gender, lifestyle and profession of the vaccinees. The vaccines against diphtheria, tetanus and poliomyelitis remain mandatory for the children (2015) [Cabut et al., 2015].

In the United States, measles was eliminated in 2000, but 2014 saw 23 outbreaks. Measles still killed 145,700 people per year worldwide in 2014; most were under the age of 5. In 2015, all 50 States required schoolchildren to receive a broad spectrum of vaccines. Specific religious or philosophical objections, the United States Supreme Court ruled as far back as 1944, do not give parents the right to avoid mandates

imposed by the State. Vaccines, after all, are not just another means to protect an individual from an untimely end, they also protect others, by creating an immunity shield that stops pathogens from coursing through populations where they might target the most vulnerable, many of whom are unable to have vaccines on their own (Scherer, 2015).

Yet, ever since Boston first required smallpox vaccination for schoolkids in 1827, public backlash has lingered. When some see a public health benefit, others see a needle pushing foreign bodies into the bloodstreams of children. On 2 February 2015 measles jumped from an outbreak of unvaccinated kids in California's Disneyland; there were 102 cases of measles in the United States, including Disneyland employees. The measles outbreak went along with the age-old debate over parental rights, public health and government mandates. "The State does not own your children," Kentucky Senator Rand Paul followed up on the subject of vaccines. "Most of them ought to be voluntary," he said (Scherer, 2015). The fear of government-mandated injections remains. In 1900 leafletters warned against the "menace to personal liberty," and that language is once again ascendant, from the Tea Party (Republicans' far right) conclaves of the Deep South to the farmer's markets of Hollywood. A debate over whether States should require a new vaccine against the human papillomavirus, a cause of cervical cancer, broke out during the 2012 presidential race, when then candidate Michele Bachmann wrongly claimed it could cause mental retardation. By mid-February 2015 party leaders from around the United States rose up to end the debate. The mandated vaccines should remain mandatory, they said, almost without exception. Soon after his remarks New Jersey Governor Chris Christie clarified his support for measles mandates, and even R. Paul, who once described mandatory vaccines as a step towards martial law, did what he could to raise a white flag – inviting a reporter from *The New York Times* to photograph him receiving a booster shot during a doctor visit (Scherer, 2015).

But is there a lack of vaccine confidence among populations?

## "The State of Vaccine Confidence 2015"

This is the title of the report drafted by Heidi Larson, an anthropologist and lecturer at the London School of Hygiene and Tropical Medicine. She oversees there research on vaccine confidence and the implications of this lack of trust for vaccination programmes. She was interviewed by Paul Benkimoun of the French daily newspaper *Le Monde*, which published that interview on 1 July 2015 (Cabut, 2015b). H. Larson indicated that there is an increasing problem of confidence versus vaccines in the wealthiest population strata in the United States, Canada, Australia, Japan and Europe. The mistrust is also perceived in the least privileged population classes. In fact, the curve of lack of confidence is a U curve where the extreme parts in terms of income show the greatest mistrust, while the middle classes (at the centre of the curve) show more confidence. Social networks play a key role in the dissemination of the messages of the opponents to vaccines and vaccination. However, noted H. Larson, the acceptation of vaccination has made progress when the health authorities have done a good work on the health benefits provided by vaccines: for instance, in the United Kingdom, with respect to such vaccines as those against measles, mumps and rubella (Cabut, 2015b).

Two main reasons explain, according to H. Larson, the current vaccine confidence problem: first, ideological position that emphasizes nature; secondly, the defiance against health authorities. The "nature" ideological position predominates in those regions or countries, that are less or little affected by diseases against which vaccines provide protection. Those who oppose vaccination are also against new technologies, vaccine adjuvants, GMOs. These opponents are mainly found in the industrialized countries and also appear in the wealthiest social strata of emerging countries' populations. The second motivation is more widespread among poor and marginalized populations who can show a great distrust against governments and health authorities. Vaccines are the only top-down health intervention that is regulated by the government – it fixes its calendar – and that deals with the whole population. Anyone who has a problem with the government can become reluctant to vaccination, particularly during massive vaccination campaigns. Also those who have doubts about the behaviour of industrial pharmaceutical groups, would be more prone to question the benefits of their vaccines; they will underline their will to above all increase their sales and turnover, they will also highlight the collusion between big pharmas and health authorities (Cabut, 2015b).

## The situation in France: antivaccination mood versus experts' voices

In France the mandatory vaccine market is mainly supplied by two manufacturers: Sanofi Pasteur MSD (the vaccine branch of the big pharma Sanofi) and GlaxoSmithKline (GSK). They develop new vaccines and, in due course, elaborate their requests for vaccine commercialization (AMM, French acronym for Authorization for Marketing), which is authorized by the health ministry, on the basis of the recommendations made by the High Public Health Council (HCSP, French acronym) and the technical advice of the HCSP permanent working group, the Vaccination Technical Committee (CTV, French acronym). The CTV relies on a multidisciplinary expertise, as well as on the assistance of the various national agencies which are members of the CTV (Cabut et al., 2015). Furthermore the authorization for commercialization of vaccines can be delivered by the European Commission across all the European Union member states, after the advice given by the European Medicines Agency (EMEA).

The main opponents to vaccination in France come from a broad range of civic-society associations and opinion leaders. For instance, Henri Joyeux, an oncologist surgeon, 70-years old, has been able to collect 680,000 signatures underneath a petition that questioned the antihepatitis B vaccine efficiency; this vaccine has been suspected for a long time to cause outbreaks of multiple sclerosis among the vaccinees, although this was not demonstrated. H. Joyeux also denounced the presence of aluminium and other adjuvants in certain vaccines. He underlines that some vaccines that are mandatory and protect against diphtheria, tetanus and poliomyelitis (DTP) were not easy to find on the market, because they are being replaced by hexavalent vaccines which contain, according to him, "two dangerous, or even very dangerous, substances, and which are much more expensive." It should be mentioned in this respect that when the DTP vaccine was withdrawn in 2008 from the French market, it cost €6.70; the hexavalent vaccine cost €39.04 in 2015 (Cabut et al., 2015).

The National League for the Freedom of Vaccinations (*Ligue nationale pour la liberté des vaccinations*) requests the cancellation of mandatory vaccinations, the indemnization by the state of serious secondary effects of vaccinations. The Association E3M, while not agreeing on H. Joyeux's petition that is finds "questionable and not enough justified from the scientific viewpoint," is acting in favour of the use of vaccines without aluminium. The Association REVAHB includes patients who consider their suffering is due to the antihepatitis-B vaccine; they object to the "hidden" vaccination against hepatitis B, using the Infanrix Hexa. This vaccine, by contrast to tetravalent and pentavalent vaccines, is widely available: in addition to diphtheria, tetanus and poliomyelitis (DTP), it immunizes the human body against three other diseases, including hepatitis B (Cabut et al., 2015).

Despite the basic criticism of some obvious scientifically incorrect facts existing in H. Joyeux' petition, the message of the cancer surgeon seems to have been more echoed in the French population than the discourse of the health authorities and vaccine specialists. Maybe because, as indicated by Heidi Larson, the credibility of these health authorities is increasingly questioned for several reasons. They still continue to bear the consequences of their bad management of the antihepatitis-B vaccination, as well as that of the A (H1N1) influenza pandemia in 2009 – one of the vaccines used at that time had caused narcolepsia in some vaccinees. Their communication policy is not as effective as it should be. There are sometimes contradictory approaches: for instance, they carry out campaigns for mandatory vaccination, without anticipating the possible lack of some vaccines on the market. As also indicated by H. Larson, the experts' voice seems dubious to the larger public, because of the suspected hidden economic interests between them and the pharmaceutical industry (Cabut et al., 2015).

Sometimes, recently publicized secondary effects of a vaccine can fuel the antivaccination mood. For instance, still in France, in April 2015, a vaccine against rotavirus caused deaths in newborns. At the beginning of July 2015 a seven-month-old female newborn suffered from high fever and convulsions, that jeopardized her life, after the simultaneous injection of an hexavalent vaccine (Infanrix Hexa) and a vaccine against pneumococcus (Prevenar). By contrast, in Spain, also in July 2015, a child died from diphtheria, because his parents did not vaccinate him against the disease (Cabut et al., 2015). In France, the reply from the health minister, Marisol Touraine, on 29 May 2015, has been a firm one, when she responded to H. Joyeux's petition: "Vaccination should not be disputed ... There should be no doubt about vaccines, but this does not exclude transparency and more research aimed at improving the quality of our vaccines." On the other hand, on 25 June 2015, the National Council of Physicians (*Conseil national de l'ordre des médecins*) announced that it sued H. Joyeux before the first-instance disciplinary court of the Languedoc-Roussillon region, because of all his statements on vaccines (Cabut et al., 2015).

## Why there is a lack of some vaccines?

Uncertainties remain and deserve a public debate: Why mandatory vaccines are lacking and are there alternative solutions? What do we really know about adjuvants? Is mandatory vaccination still relevant?

In France, since the beginning of 2015, combined tetravalent vaccines (against diphtheria, tetanus, poliomyelitis and whooping cough) and pentavalent ones (additional protection against *Haemophilus* B) have been almost impossible to find in pharmacies. Only the hexavalent formula (pentavalent + antihepatitis B), that is commercialized by GSK and recommended for the vaccination calendar, is normally supplied. It seems that the situation is improving, at least for the pentavalent vaccines: according to the French National Agency for the Safety of Medicines and Health Products (ANSM, French acronym), the companies Sanofi Pasteur and GSK are planning to supply their vaccines, "so as to stabilize the market and decrease the tension period over the second half of 2015" (Cabut et al., 2015).

Since 2008 the simple trivalent vaccine, DTP (diphtheria, tetanus, poliomyelitis), that is a mandatory vaccine, has been withdrawn from the market, because of "allergic complications." Sanofi Pasteur proposes a kit of three mandatory vaccines (DTVax and ImovaxPolio). Initially developed for children who cannot be vaccinated with the antiwhooping cough vaccine, it became affordable freely upon request of the physician, to the families who only wished to confine themselves to the mandatory vaccines. DTVax has been replaced since May 2015 by a vaccine against diphtheria and tetanus that was produced for the American market. DTVax has also been criticized because it contains a conservative derived from mercury and called thiomersal. Regarding the boosters, there exists the Repevax of Sanofi or the Boostrix of GSK (respectively for the three-years-old and four-years-old children), which are more or less easily found in the pharmacies. The only difference with conventional tetravalent vaccines is a weaker dose of antigen against diphtheria and whooping cough. While Sanofi and GSK are suspected of reducing conventional vaccine production, so as to promote the sales of more costly vaccines, both companies underline that there is an increase in the global demand of antiwhooping cough vaccines, further to the fact that many countries have broadened their recommendations about this vaccine. Furthermore the companies had to discard batches of vaccines after a series of checks and that created a disbalance in the availability of some vaccines. GSK also explained that the yield of production of the antiwhooping cough vaccine has been lower than expected (Cabut et al., 2015).

## Safety of vaccine adjuvants

Vaccine adjuvants, used in order to boost the immune response and consequently to reduce the quantity of antigen per vaccine dose, are jeopardizing the safety of vaccines because of their secondary effects. The opponents to vaccination, and some researchers, claim that their presence in the vaccines should be reviewed extensively. However, the Global Advisory Committee for Vaccine Safety (GACVS), which is associated with the World Health Organization (WHO), has come to the conclusion that these adjuvants are safe, particularly the squalene which is found in some antiinfluenza vaccines and those against human papillomaviruses (HPV). But during the vaccination campaign against the A(N1H1) influenza pandemia, two types of the vaccine, including one without adjuvant for pregnant women, have been used, and that was troublesome regarding the innocuity of adjuvants (Cabut et al., 2015).

Aluminium-based adjuvants may cause inflammation on the site of injection. Aluminium hydroxide may provoke muscle pain, fatigue and even invalidity; this disease, called macrophagic myofasciitis, has been described for the first time in 1998 by Romain Gherardi (Henri-Mondor hospital, Créteil, south of Paris) and Michelle Coquet (Pellegrin hospital, Bordeaux) and their results were published in *The Lancet* (Gherardi et al., 1998). Far from being an antivaccine person, R. Gherardi has made a correlation between this disease and aluminium hydroxide that most vaccines contain. The Association E3M (*Entraide aux malades de myofasciite à macrophages*) is struggling to help those suffering from this illness, and also to make the health authorities recognize the correlation between the illness and the aluminium hydroxide adjuvant. Requests for indemnization of the patients have been made. Didier Lambert, president of E3M, who has signed the petition presented by H. Joyeux, requests the commercialization of a DT-Polio vaccine without aluminium adjuvant. A substitute could be calcium phosphate, used in the 1960s at the Pasteur Institute, Paris, in antidiphtheria and antitetanus vaccines, and thereafter abandoned in the 1980s. Didier Lambert declared: "We wish to see more transparency and research on this whole issue of adjuvants." While the French Academy of Medicine, in 2012, as well as the Society of Infectious Pathology and the High Public Health Council (HCSP) in 2013, did not conclude in their deliberations that adjuvants should not be used in vaccines, the WHO had underlined in 2004 that "the safety of adjuvants in an important issue, that is neglected," and required that their evaluation was "indispensable." In 2010, after the A(N1H1) influenza outbreak and vaccine campaign a report by the French Senate concluded that new research work was needed in order to "better understand the effects of such adjuvants as mercury, aluminium or squalene on the human body," and to determine the possibly dangerous concentrations (Cabut et al., 2015).

## Are mandatory vaccinations still justified?

France is with Italy and Belgium one of the five European countries that maintains the principle of mandatory vaccination. Three mandatory vaccines (against diphtheria, tetanus and poliomyelitis) are a societal requisite, e.g. for the admission of children to nursery and primary schools. There are also mandates in some regions (e.g. vaccination against yellow fever in French Guyana) or professions (vaccination against hepatitis B for medical staff, or typhoid for some laboratory personnel). This vaccination or strategy is being questioned not only by the opponents to vaccination, but also by professional or constitutional bodies. For instance, in September 2014, the HCSP, when it has been requested to propose a vaccination policy, estimated that mandatory vaccination or not is a "societal choice", that should deserve a debate to be organized by health authorities. The HCSP view was that if vaccination remains mandatory, "the list of mandatory vaccines should be revised." The French Constitutional Council has even been involved, when a couple was sued before a correctional court after refusing to vaccinate their children. The Council gave the result of its deliberations on 20 March 2015: the mandatory character of the vaccination, as it is stated in the code of public health, is not anticonstitutional (Cabut et al., 2015).

Another criticism concerns the likelihood in the people's mind of a hierarchy between mandatory and recommended vaccines, such as those against hepatitis B, whooping cough, meningitis, papillomavirus or influenza. "Mandatory *and* recommended vaccines are *all* useful and important," stated the National Institute for Prevention and Health Education (INPES, French acronym). This viewpoint is shared by the National Agency for the Safety of Medicines and Health Products (ANSM) and of course by the industrial pharmaceutical manufacturers (Cabut et al., 2015).

Heidi Larson of the London School of Hygiene and Tropical Medicine observes that the number of vaccines and infections has increased considerably during the last decades, and efforts should be made in order to try to rather rationalize vaccinations, rather than to pile them. From the social viewpoint the anthropologist thinks the health authorities and medical community have not taken for granted the acceptance by society of their decisions concerning vaccination. One should therefore take the time to explain the usefulness of vaccination and its benefits for disease prevention and better health. When a new vaccine is introduced, it is absolutely necessary to take into account the historical, societal and political factors that may have an impact on the people's reaction. It is therefore necessary to invest into social-science research in order to better understand the modalities of vaccine confidence and to channel the relevant results or information to those in charge of setting up a national vaccination policy or strategy (Cabut, 2015b).

## Improving the quality and administration of vaccines

The public debates on vaccination, prevailing in industrialized countries and the privileged social classes of some emerging countries, cannot lead to an oversight of the real benefits of vaccinations across the world regarding the prevention of infectious diseases, or in avoiding the risk of cancers in some cases. Despite the economic shortcomings and the paucity of vaccine manufacturers (particularly in the developed countries), despite the lack of economic attractiveness of the vaccine market (drug discovery is much more lucrative), research is still being carried out with a view to developing new vaccines (see from p. 195) as well as to improving their administration to the vaccinees. For instance, the vaccine against meningitis, Nimenrix, developed by GSK, was considered a "major" breakthrough by the French High Public Health Council. The latter had screened 169 new medicines in 2013 and reported, on 1 July 2014, that only this vaccine could be regarded as the most significant advance.

Researchers also explore new means for the replacement of the conventional intramuscular injections of the majority of vaccines, and subcutaneous injections as well. A nasal spray of an antiinfluenza vaccine (by AstraZeneca) and an intradermic one (by Sanofi Pasteur) have been commercialized. Cutaneous administration is thoroughly studied. "The main advantage is to trigger a good immune response with a low dose of vaccine and without a needle; henceforth, an economic gain. This can meet a real need in developing countries, and this explains the important investments

made in this respect by the World Health Organization and the Bill and Melinda Gates Foundation …," underlined Behazine Combadière, a research director at the National Institute for Health and Medical Research – INSERM – Immunology and Infectious Diseases Centre, Paris. In 2008 this researcher has been among the first in the world to carry out a clinical trial with a transcutaneous vaccine. The principle of the trial is to target Langerhans cells, present in the epidermis, which play a key role in the initiation of a good immune response. As these cells are concentrated around the hair follicles, the researchers apply a dermatological glue that withdraws the hair on a surface of 16 $cm^2$. Thereafter a very small dose of the vaccine is applied on this hairless skin area. "Using an antiinfluenza vaccine, we have shown that the means of administering the vaccine is well tolerated and that it induces a better immune response than the intramuscular injection. By contrast it does not induce the production of protecting antibodies," explained B. Combadière. This result led the research team to test its technique in the case of infections where the cellular immune response prevails (compared with antibody production). Clinical trials are being carried out with several anti-HIV candidate vaccines, the results of which were expected in 2016 (Cabut et al., 2015).

A nanopatch made of 20,000 nanoneedles covered with the vaccinal antigen has been developed by Mark Kendall in Australia. The system would be stable at room temperature, which would make a cold chain unnecessary. The first clinical trials were to be carried out in 2016, with a view to testing this patch with three vaccines produced by Merck. At the Georgia Institute of Technology, Atlanta, the team of Mark Prausnitz has also devised a patch with *ca*. 100 microneedles which are resorbed a few minutes after the patch application. Clinical tests were expected in 2017 with an antimeasles vaccine (Cabut et al., 2015).

Another challenge of vaccinology is to be able to propose customized vaccinations, taking account of individual profiles (genetic features, immune status, environmental factors). On 5 June 2015 an international team presented in *Science* the system Virscan, that can determine the viruses to which an individual has been exposed during his/her lifetime, through the detection of the antibodies produced by the organism as a protection means (Xu et al., 2015). Such test needs less than a drop of blood and costs US$25 (or €22.30). This kind of system would permit an early detection of certain infections, but would also indicate whether a vaccination or a booster are needed (Cabut et al., 2015).

## Redesigning genes to produce antibodies (immunoprophylaxis by gene transfer, IGT)

Conventional vaccines prompt the body's immune system to learn to make antibodies by exposing it to attenuated or dead pathogens, or even just their molecular fragments or subunits. In some cases these antibodies provide strong defences. Vaccinations against diseases like smallpox and measles can lead to almost complete protection. But against other diseases, conventional vaccines often fail to produce effective antibodies. The Human Immune Deficiency Virus (HIV), for instance, comes in so many

different strains that a vaccine protecting against one of them will not work against others. In a few people, however, some antibodies against HIV turn out to be very potent. So-called neutralizing antibodies can latch on to many different strains of the virus and keep them from infecting new cells. Yet most people do not synthesize these powerful molecules (Zimmer, 2015).

So Philip R. Johnson, a virologist at the University of Pennsylvania, wondered whether it could be possible to give broadly-neutralizing antibodies to everybody. At the time, P.R. Johnson and other researchers were experimenting with gene therapy for genetic diseases like hemophilia. They were figuring out how to load genes into viruses and inducing them to invade cells. Once inside, the viruses can deliver replacement genes for defective ones and cells begin making proteins they had been unable to produce. P.R. Johnson thought he might be able to use that strategy to introduce the gene for a powerful antibody into a patient's cells. After the latter began producing antibodies, the patient would in effect be "vaccinated" against a disease. In their experiment the American researchers sought to protect monkeys from SIV (Simian Immunodeficiency Virus), a primate version of HIV. To do so, they used viruses to deliver powerful antibody-encoding genes to the monkeys' muscles. The latter produced SIV antibodies. Then, they injected SIV into the animals, which produced enough antibodies in their muscles to protect themselves from SIV infections. The control animals (without the IGT) died after being infected with SIV (Zimmer, 2015).

In 2011 David Baltimore, a Nobel Laureate of Medicine or Physiology (1975) and virologist at the California Institute of Technology (Caltech), and his colleagues showed that antibodies produced by cells which were engineered with viruses like in the case of SIV, could protect mice against infections with HIV, suggesting that IGT could protect people against HIV in contaminated needles. But most infections by HIV occur through sexual intercourse. So D. Baltimore and his colleagues also injected female mice with HIV through their vaginal membrane. In 2014 they reported that the technique also protected mice from infection in this way. D. Baltimore concluded: "So what we are doing is pretty fundamentally different from vaccination, although the end result is pretty similar." By delivering synthetic genes into cells of laboratory animals (muscles or vagina membrane), the scientists are re-engineering the animals to resist disease. At the Scripps Research Institute, La Jolla, California, Michael Farzan and other immunologists are increasingly hopeful that this technique may be able to confer long-term protection against diseases for which vaccines have failed. The first human trial based on IGT was underway by early 2015 and several new ones were planned, in particular to see whether IGT could be helpful not just against HIV, but also Ebola, influenza, hepatitis viruses and malaria (Zimmer, 2015).

Gary Ketner, a microbiologist at the Johns Hopkins Bloomberg School of Public Health, Baltimore, was intrigued by D. Baltimore's results and wondered whether IGT could be marshalled against malaria. They succeeded in producing a potent antibody in engineered mice, using IGT. In August 2014 they reported that when malaria protozoan-laden mosquitoes bit the mice, up to 80% of the treated animals were protected. G. Ketner is searching for better antibodies that provide more protection against malaria in a smaller dose. In 2013 James M. Wilson, a pathologist at the University of Pennsylvania, and his colleagues reported that virus carrying antibody

genes into airway cells could enable mice and ferrets to fight off a wide range of influenza strains. The scientists have been working on gene therapy to treat cystic fibrosis by delivering genes into the cells lining patients' airways. Many fast-spreading viruses, such as influenza and SARS (Severe Acute Respiratory Syndrome), also infect the same cells. Consequently J.M. Wilson and his colleagues have tested IGT against several viruses causing deadly outbreaks – including Ebola. They teamed with Mapp Biopharmaceutical, a company that has developed an antibody against Ebola, called ZMapp (see p. 217). They synthesized a gene for this antibody and delivered it into mouse muscle cells. By mid-2015 the experiments were in their early stages, but the data collected were encouraging (Zimmer, 2015).

In February 2015 P.R. Johnson began the first clinical trial of IGT in human volunteers to see whether the treatment is safe. The researchers expected to finish gathering the results by the spring of 2015. D. Baltimore is collaborating with the National Institutes of Health (NIH, Bethesda, Maryland) to start a similar trial against HIV, while J.M. Wilson is willing to test IGT against flu before the end of 2015. Human immune system may attack the artificial antibodies or the viruses delivering them, destroying their protective effect. Or re-engineering muscle cells might make too many antibodies, because they do not have the built-in regulation that immune cells do. Researchers therefore need to gauge the safety and effectiveness of IGT in humans. Regarding the bioethical aspects, there should not be major hurdles, because IGT is based on gene therapy which has been developed for more than 30 years (Zimmer, 2015).

## Eradication of smallpox in Africa: illustration of an effective vaccination programme

The last case of smallpox in the United States occurred in 1949, but from 1880 to 1980 the viral disease killed a half-billion people worldwide. The United States National Communicable Disease Centre (which became the Centers for Disease Control and Prevention – CDCs) began its overseas eradication campaign in West and Central Africa in 1966. From the center offices in Atlanta, Georgia, J. Donald Millar, a physician and public-health official, led the global smallpox eradication programme (from 1966 to 1970) and oversaw the training, deployment and support of dozens of health workers in *ca*. 20 countries. Many, like Sierra Leone, Guinea, Niger and Togo, then had some of the highest rates of smallpox in the world. Operating under the aegis of the World Health Organization (WHO), J.D. Millar's programme focused on locations, like marketplaces and festival sites, where inhabitants of remote rural settlements came together in large numbers. There, local workers trained by his staff vaccinated as many people as possible against smallpox. Eventually, *ca*. 4,000 Africans were trained by J.D. Millar's staff to administer the vaccine. *The New York Times* reported, J.D. Millar's programme had helped vaccinate 100 million people in the region (Fox, 2015).

"This was considered to be the most difficult area of the world, because of communications and transportation and so forth," William H. Foege, a former director of the CDC who in the 1960s worked under J.D. Millar in Nigeria, stated on Thursday

3 September 2015, after the announcement of J.D. Millar's death on 30 August 2015. "The objective was to stop smallpox within five years, and the goal was actually reached in three and a half years." The Africa programme became a model for smallpox eradication campaigns in countries such as India, Pakistan, Bangladesh, Afghanistan and Brazil. "Over the years, the CDC became the largest contributor of people to global smallpox eradication around the world, most of them deployed through the WHO," W.H. Foege said (Fox, 2015).

The world's last case of naturally transmitted smallpox was recorded in Somalia in 1977. The following year, two cases resulted from the accidental release of the smallpox virus at a laboratory in Birmingham, England. In 1980 the WHO declared the disease eradicated. As early as 1969, in the *New England Journal of Medicine* (*NEJM*), J.D. Millar maintained that smallpox vaccinations, long an American childhood ritual, were no longer necessary in the United States. With his co-author, J. Michael Lane, he argued that by then the vaccine potential complications – fatal in *ca*. one case per million – outweighed its potential benefits for most Americans (Lane et al., 1969). The routine vaccination of Americans against smallpox ended in 1972 (Fox, 2015).

John Donald Millar, after receiving a bachelor's degree in chemistry from the University of Richmond in 1956, earned an M.D. in 1959 from what was then the Medical College of Virginia and did his internship at the University of Utah. Assigned to the Communicable Disease Center, J.D. Millar began his career as a member of its epidemic intelligence service. In 1966 before assuming the leadership of the smallpox programme he earned the equivalent of a master's in public health from the London School of Hygiene and Tropical Medicine. In later years with the CDC, J.D. Millar led its public-health delivery programme, which helps States administer services like tuberculosis prevention, dental hygiene and the tracking of sexually transmitted diseases. He served as the director of the National Center for Environmental Health, part of the CDC, from 1980 to 1981. From 1981 to 1993 J.D. Millar was the director of the National Institute for Occupational Safety and Health, also part of the CDC. A retired assistant surgeon-general of the United States Public Health Service, J.D. Millar died on 30 August 2015 at the age of 81, at his home in Murrayville (Fox, 2015).

## Developing a universal anti influenza vaccine

Today's available antiflu vaccines raise immunity against a molecule on the virus surface, haemagglutinin or HA, a key protein in influenza viruses such as A(H5N1), which causes bird flu, and A(H1N1), better known as swine flu. The "head region" of HA is a good place to target, because it is easily accessible, but it has the major drawback of recurrent genetic mutation. Finding a "universal vaccine" that eliminates the need for annual flu shots and protects against new pandemic strains is one of the highest priorities in clinical virology and vaccination. Two teams have been working on trying to resolve this challenge. One team is a collaboration between the Scripps Research Institute in California (La Jolla, near San Diego) and the Janssen Prevention Centre in the Netherlands, part of the Johnson & Johnson pharmaceutical group; the other is working at the United States National Institutes of Health (NIH, Bethesda,

Maryland). The approach of both research teams is to target, instead of HA head region, the less accessible HA "stem region" which undergoes little or no mutation. Their results, published in 2015 in the journals *Science* and *Nature Medicine*, showed that in mice, ferrets and monkeys such vaccines could prevent infection by a wider range of influenza viruses, including A(H5N1) (Cookson, 2015; Yassine et al., 2015).

"If the body can make an immune response against the HA stem (region), it is difficult for the virus to escape," stated Jan Wilson, professor of biology at the Scripps Research Institute. "We are moving in the right direction for a universal influenza vaccine. This was the proof of principle… The ultimate goal, of course, would be to create a life-long vaccine," he added. Sarah Gilbert, professor of vaccinology at Oxford University, said: "This is an exciting development, but the new vaccines now need to be tested in clinical trials to see how well they work in humans. This will be the next stage of research which will take several years." The implications of developing or designing a universal antiinfluenza vaccine will be outstanding in terms of disease prevention and drastically decreasing (or even nullifying) its impact on human populations. In 2009 the A(H1N1) flu virus caused the death of between 150,000 and 575,000 people worldwide and there are fears that many millions would die of an exceptionally virulent new variant of the virus, such as the 1918-1919 Spanish flu which left more than 50 million people dead, swept across the globe. According to the United States Centers for Disease Control and Prevention in Atlanta, seasoned flu sends *ca*. 200,000 people a year to hospitals in the country and kills *ca.* 36,000 a year. When a virus strain emerges that is not dealt with by an existing vaccine, the toll can be greater (Cookson, 2015).

## Mutant influenza viruses: a major concern for researchers and health-care authorities

### *Development of mutant H5N1 and H7N9 influenza viruses*

A series of experiments have been initiated in 2011 on two influenza viruses, H5N1 and H7N9, which have become widespread in Asia. Both viruses are killers. The infected persons show high fever, and thereafter suffer from pneumonia and respiratory ailments that are often lethal. Between one-third and one-half of *ca*. 1,000 humans who have been infected with these viruses, generally died in *ca*. ten days. In January and February 2014 the recorded deaths caused by H7N9 in China were 72. These viruses are found naturally and massively among birds and fowl, e.g. ducks and chicken. Considerable concentrations of viruses (i.e. very close contact with fowl) are needed for human contamination. The receptors, which serve to hook both viruses, are slightly different in mammalian and fowl cells. However these viruses do not infect humans from humans (contamination between humans). Until when? And this is where the research work initiated in 2011 is of some relevance. A few mutations in these influenza viruses can make them communicable to humans. They can circulate through the little drops emitted by cough and sneeze, and thus they can move from a person to another person, without any physical contact ("aerosolization"). Five minutes in a bus are sufficient to be infected, if one of the passengers has the virus (Sciama, 2014).

Such mutant viruses may have been produced and stored at Amsterdam Erasmus University and the University of Wisconsin (Madison, WI.) Department of Pathobiological Sciences, which are at the forefront of this kind of research. The teams are led by Ron Fouchier and Yoshihiro Kawaoka, respectively. They have been able to make these viruses communicable, not between humans (this is prohibited by the current ethical norms), but between ferrets. "The ferret is a laboratory animal of which the respiratory system is very close to the human one, " explains Vincent Racaniello, a virologist at Columbia University, New York. "It shows all the symptoms found in humans: it coughs and sneezes, which is not the case of mice and even of monkeys" (Sciama, 2014).

The researchers published their results in *Nature* and *Science* in 2012: their work consisted of introducing into the virus, via genetic engineering, a few mutations; thereafter, of throwing a concentrated solution of these viruses into the nostrils of a ferret. The following experimental protocols used by the Dutch and the American scientists differed slightly: R. Fouchier contaminated in sequence ten animals, one from the other (this is a practice known to increase the virulence of some viruses); Y. Kawaoka, on his side, did not want to create more mutations than those introduced by his team into the ferrets. But the end result was the same: these "ferrets of apocalypse," as they were nicknamed, put in a cage set up at some distance from another healthy ferret, have been able to infect it. Without any physical contact! (Herfst, Fouchier et al., 2012). The new mutant viruses, analyzed by the researchers and immediately stored in a safe place, are still H5N1 and H7N9, but they are communicable, at least among ferrets (Herfst et al., and Fouchier, 2012; Imai et al., and Kawaoka, 2012).

### *Risk implications*

The risk associated with these " Frankenviruses ", as qualified by Americans, is thought even higher, because these experiments were carried out in Biosafety level (BSL)-3 laboratories and not in BSL-4 ones, i.e. those where are manipulated the most dangerous viruses, like Ebola. A report published in January 2014 revealed that in the United States, between 2004 and 2010, four accidental infections with dangerous viruses had occurred in BSL-3 laboratories. The likelihood of an annual accident, per laboratory and per year, is 0.2%. In other words, if ten laboratories are working for ten years on these viruses, the risk of infection reaches 20%. Furthermore there have been laboratory infections with Ebola, Marburg and SRAS viruses in several cities across the world, and even a few serial contaminations outside laboratories. To sum up, the likelihood of an accident is far from negligible, while the consequences would be terrifying (Sciama, 2014).

"It is really a folly," said Roberto Kolter, a director of a renowned laboratory at Harvard University and former president of the American Society of Microbiology (ASM). Another 56 scientists, including three Nobel Laureates, signed at the end of December 2013 a public letter addressed to the European Commission, demanding "a real risk analysis" for this kind of experiments which expose humankind, warned Marc Lipsitch, an epidemiologist at Harvard who co-signed the letter, to a possible "pandemia … that can kill hundred million people." Confronted with the concerns expressed by the scientific community the European and American virologists imposed a moratorium on

their work in January 2012. But in January 2013 they lifted the moratorium, unilaterally and despite the lack of consensus. The reaction by many scientists at the end of 2013 tried to re-establish a moratorium, apparently without success (Sciama, 2014).

### *Reactions of the scientific community*

In January 2012, in *Nature*, many researchers justified the need for pursuing their work, extending and diversifying it (Fouchier, Kawaoka et al., 2012). Yoshihiro Kawaoka announced in March 2014 that funding of his work had been maintained. The scientists concerned argued, in the first place, that their experiments would be useful for the production of new vaccines, treatments and even to put in place early warning systems. But they had to make a backstep, since then, in front of the criticism by specialists in the areas of virus infections, spread and immunological response. Y. Kawaoka retorted that "nature creates continuously this kind of things" and that it was therefore "indispensable to study them, in of course the best safety conditions." R. Fouchier admitted that "in the first place, this work aims at increasing our fundamental comprehension." He made a strong plea for a science "motivated by the curiosity" that in many cases has led to scientific breakthroughs. Such statement exasperated Simon Wain-Hobson, a virologist at the Pasteur Institute and one of the leaders of the movement against these experiments (Sciama, 2014).

It is true that the scientific community was divided about the experiments carried out on mutant viruses that could be very dangerous pathogens for humankind. On the one hand, the letter addressed to the European Commission brings the evidence of a radical opposition to these experiments. The opponents are often researchers with a brilliant career behind them. On the other hand, R. Fouchier and Y. Kawaoka have supporters. But there is some kind of divide among disciplines and research fields: physicians, epidemiologists and other public health specialists are generally cautious, while virologists and molecular biologists, and particularly influenza-virus specialists, tend to be reckless. For instance Vincent Racaniello of Columbia University defends this work on influenza viruses and estimates that "there are already too many regulations. The society has become too cautious; if there are dangerous experiments, they are not supported, while at the same time people want vaccines against influenza, drugs, treatments … all that without taking any risk!" Peter Palese, one of the main influenza specialists in the world, member of the American Academy of Sciences and researcher at New York Mount Sinai Hospital, deplores an "antiscience and antiresearch hysteria." He considers that the dangers associated with the experiments carried on the influenza viruses were extremely low, because of the very high safety of the laboratories where they are conducted, and also because the virus strains have small pandemic risks. The opponents nevertheless insist on the unpredictability and dangerosity of the influenza viruses (Sciama, 2014). See also Fouchier et al. (2012).

Malcolm Dando has recalled that "it is difficult to think of an experiment which all scientists would agree to prohibit." Some fear that a wide-ranging debate would become a Pandora box that would threaten any kind of research on dangerous viruses. "We will end killing virology in our countries and run the risk to see its development in China," stated Peter Palese. That may explain the silence of most scientific associations,

while the American authorities which allocate most of the funds needed for the experiments via the National Institutes of Health (NIH), made public in February 2013 a series of guidelines which were summarized by Roberto Kolter as: "Above all, be careful." Beyond its specificity and related risks, the research work on the two main strains of influenza viruses H5N1 and H7N9 and their mutants, raises issues that may reflect a crisis within life-sciences research. In this regard, the following dates should be recalled :

- 2005: an American team of researchers succeeds in rebuilding the 1918 influenza virus and shows that it is still functional;
- September 2011: Ron Fouchier announces in a congress that he has been able to make transmissible the H5N1 virus;
- April 2012: debate at the Royal Society in London on the relevance of R. Fouchier's research work;
- summer 2012: Ron Fouchier and Yoshihiro Kawaoka published their first research results on H5N1 in *Nature* and *Science*;
- February 2013: American authorities published guidelines on the research carried out on highly pathogenous influenza viruses;
- August 2013: twenty-two researchers, including R. Fouchier and Y. Kawaoka, announced they will extend their research work to another lethal influenza virus, H7N9;
- December 2013: letter addressed by 56 renowned researchers to José Manuel Barroso, president of the European Commission, requesting the suspension of these experiments (Sciama, 2014).

### *Ethical debate and the need for scientific autoregulation*

Some analysts consider that there is not enough ethical debate and reflection on the implications of the achievements and possible developments or risks of biological research. Simon Wain-Hobson, head of the retrovirology department at the Pasteur Institute in Paris, president of the Foundation for Vaccine Research and member of the Academia Europaea, recalled that physics, often described as the "queen" discipline of the 20$^{th}$ century, had generated a harvest of outstanding innovations, "but also the atomic bomb." S. Wain-Hobson also warned that biology, considered as the discipline of our century, "may create its own bomb, if one does not take care" (Sciama, 2014). In an interview given to the French daily newspaper *Le Monde* and published on Wednesday 12 March 2014, S. Wain-Hobson recalled that controversial research on influenza viruses was being carried out for a dozen years. In 2005 an American team could reconstitute the 1918 influenza virus (called the Spanish flu which killed *ca*. 50 million people), using tissues of corpses buried in the permafrost. A few years later, a team working in Villejuif, a suburb south of Paris, has reconstituted a functional influenza virus from degraded DNA segments, dispersed in the human genome. A virus that had most probably disappeared millions of years ago. Regarding research guidelines S. Wain-Hobson stated that they exist, but they must be better known and disseminated, and strictly applied. In 2005 a common declaration on biosafety

drafted by 72 academies of sciences across the world stipulated that scientists ought to cause no harm, to take into account the likely implications of their activities and to keep away from any research whose consequences would be harmful for humankind. But very few scientists are aware of this declaration, said S. Wain-Hobson (Sciama, 2014).

S. Wain-Hobson added that his institution, the Pasteur Institute in Paris, wants to stand at the forefront of these issues, which must be discussed extensively, because they will not disappear by themselves. In his view, a very open debate should take place with the participation of scientists, of course, but also of lawyers, bioethicists, representatives of the civic society and journalists. Because this is the biggest problem that science has to deal with since the Manhattan project (that led to the atomic bomb), we must be able to decide where to draw the yellow (or red) line. Otherwise, if an accident happens, the public would make the whole scientific community responsible for it, and, in all likelihood, science itself (Sciama, 2014).

Referring to another example of scientific autoregulation, the Asilomar guidelines on DNA research, another former researcher of the Pasteur Institute, Agnès Ullmann, who was 88 years old in 2014 and a co-researcher of Jacques Monod, a Nobel Laureate of Medicine or Physiology in 1965 (shared with François Jacob and André Lwoff), stated that since the Asilomar conference in 1975 "the growth rate of technologies has been exponential, but not the conscience of biologists." In 1974 Paul Berg, a biochemist who was to be awarded the Nobel Prize for Chemistry in 1980 (shared with W. Gilbert and F. Sanger), crossed for the first time a simian virus (SV40), probably carcinogenic, with a virus that can infect bacteria living in the human digestive tract. But before exposing a bacterium to this virus hybrid, P. Berg, who was concerned about the powerful potential of the tools he invented himself, decided to interrupt his research work. He therefore proposed a moratorium on what was not yet called "genetic engineering", until the organization of a conference where all the members of the life-sciences community would agree on a common ethics. This conference, held in February 1975 in Asilomar, California, with the help of the National Academy of Sciences of the United States, was attended by 140 biologists, lawyers and physicians. The conference issued practical guidelines and principles, such as the prohibition to clone DNA of organisms highly pathogenous for humans. Despite some lack of clarity in the conference recommendations, many biologists still refer to Asilomar as a highly significant episode of scientific autoregulation (Sciama, 2014).

## West Africa Ebola epidemics and control of the disease

### *Discovery of the Ebola virus*

In the 1970s Peter Piot a Flemish student who was to become the co-discoverer of the Ebola virus, decided to abandon his engineering studies and to initiate a medical career. He was interested in science, people and society, and belonged to the generation who was struggling against colonization and for the self-determination of peoples. He became a medical doctor at the service of the poorest populations. He was trained in

microbiology and worked as an assistant-researcher at Antwerp Institute of Tropical Medicine (Benkimoun, 2015e). On 29 September 1976 Peter Piot received two glass tubes of blood from Zaire (now the Democratic Republic of Congo), that were sent by a physician caring for a Flemish nun who was dying of fever and loss of blood. One tube was intact, the other was broken; its contents mingled with melted ice in the to form a red soup. A bloodstained note explained the nun was among 200 people, including physicians and nurses, dying in an outbreak that had raged for three weeks in remote Yambuku, not far from the Ebola River (Altman, 2014).

The prime suspect was yellow fever that P. Piot's laboratory was equipped to detect. P. Piot who was at that time just 27, and his colleagues emptied the thermos, wearing only thin latex gloves – none of the high-security masks and moonsuits that are now standard. They removed small amounts of blood from the intact tube to carry out routine tests for known microbes and special tests for yellow fever virus, along with hemorrhagic-fever viruses like Lassa, Marburg and dengue. They also injected the nun's blood into cells grown in the laboratory and the brains of mice. Those tests excluded all known infectious agents, but P. Piot assumed that whichever one had caused the nun's illness must have been destroyed during the airplane transport from Zaire. Still, he and his colleagues checked the mice each day. After a week all the mice were dead, strongly hinting that the infectious agent had not been destroyed after all. An autopsy showed that the nun's liver had microscopic lesions that Stefaan Pattyn, P. Piot's boss, knew to occur in Lassa virus-infected patients. But because Lassa virus had already been ruled out, S. Pattyn steered the research towards identifying a new one (Altman, 2014).

At that time, only three laboratories outside the Soviet Union were equipped to handle deadly viruses safely: Porton Down, near London; Fort Detrick, a military base in Maryland; and what is now known as the Centers for Disease Control and Prevention (CDCs), in Atlanta, Georgia. The World Health Organization (WHO) instructed the Belgian researchers to immediately send the samples in tightly sealed containers to Porton Down, which, in turn, forwarded them to the CDCs because it was the world's reference laboratory for hemorrhagic viruses. The Belgian scientists kept some of the biological material and looked at it through an electron microscope. It showed that the virus was a new one – wormlike and huge as viruses go – that resembled Marburg virus. CDC scientists then confirmed the Antwerp team's discovery and named the virus Ebola. P. Piot wanted to travel to Zaire to undertake the epidemiologic investigations to determine how this new virus spread. As American, French and South African scientists who knew about the findings headed to Zaire, the Belgian government realized it would be shutting out its own scientists, and it rushed P. Piot to its former colony to study the virus (Altman, 2014).

Two Belgian nuns with Ebola had been evacuated from Yambuku to Kinshasa, Zaire capital, and a possible uncontrolled outbreak loomed on the horizon. P. Piot joined an international team of scientists who flew to Yambuku and mapped Ebola spread, determining that the principal route was through unsterilized needles and syringes, contact with infected patients and touching bodies at funerals. They collected blood from patients for later testing and prepared for the arrival of a larger and better-equipped

team to control the outbreak. In four months of field operations, other scientists also discovered a second outbreak of Ebola in Sudan. P. Piot said he expected the discovery of more viruses like Ebola. And despite today's much safer laboratory procedures, he added, such virulent pathogens could prove as dangerous. "We were lucky not to be infected, not only in the laboratory but later on when I was drawing blood from patients and touching them," he stated from England where he is now dean of the London School of Hygiene and Tropical Medicine. He was lucky in another way. In 1976 ordered to fly in a waiting helicopter to meet with Belgian officials in Kinshasa, he smelled alcohol on the pilot's breaths and refused to go. Soon after it took off, the helicopter crashed. A few days later, P. Piot led a team to retrieve the corpses from the jungle (Altman, 2014).

After the end of the first Ebola virus epidemic, P. Piot "absorbs like a sponge epidemiology, molecular biology, but also political science," and is very much interested in sexually-communicable diseases. This knowledge was an important asset when the epidemic caused by the human immunodeficiency virus (HIV) slowly emerged. He went to Zaire to investigate cases soon after the AIDS (Acquired Immunodeficiency Syndrome) virus was first recognized in 1981. In his books titled *No Time to Lose : A Life in Pursuit of Deadly Viruses* (W.W. Norton ed., 2012, 388 pp.) and *Une course contre la montre: Mes combats contre les virus mortels, sida et Ebola* (Odile Jacob ed., Paris, 2015, 432 pp.), P. Piot explains: "It was therefore my second trip to Zaire which changed my life." He indeed returned in October 1983 to Kinshasa in order to develop a research project on HIV infection, without being afraid of meeting populations who were living with the virus and for this reason were discriminated (Benkimoun, 2015e).

In August 1992 he started working in Geneva in the framework of the World AIDS Programme of the World Health Organization. There, he felt he was useful. When the United Nations decided to create the UNAIDS in 1995, with a view to coordinating the struggle against AIDS/HIV, he was selected to lead the new United Nations body. During the 13 years of his leadership at the helm of UNAIDS, his behaviour was both that of a diplomat and an iconoclast. One had to know how to speak of seropositive persons, prostitutes or drug users before the leaders of the Chinese Communist Party or at the Vatican; or to try to convince the G7 leaders that they should increase their financial contributions to combating the pandemic. One had to dare invite the representative of an association of Brazilian prostitutes to participate in the governing board of UNAIDS. In fact Peter Piot has been able to put the struggle against AIDS on the international community's agenda, while combating discriminations and insisting that persons infected with HIV should have their say. He nevertheless reckoned that he had doubts about the feasibility of access of developing countries to anti-HIV drugs, when in 1996 these drugs started to drastically change the fate of patients. But the response to that issue came through the international mobilization and the availability of the relevant drugs, further to the production of generics by Indian manufacturers (Benkimoun, 2015e).

In 2008 P. Pilot stepped down from his post at UNAIDS without misfeelings except that about "useless quarrels that generate such a loss of time while thousands of people were dying from AIDS every day." That was also the moment when he went to live in New York, after his divorce; his second wife is an American anthropologist,

Heidi Larson, who studies the public's trust in vaccines. Both are now working at the London School of Hygiene and Tropical Medicine, of which Peter Piot is the director. He made the following comment on his new position: "For me, it is a platform aimed at promoting global health and I do still travel a lot and meet persons infected with the HIV. It is my family" (Benkimoun, 2015e).

### *The Ebola epidemic in West Africa in 2014-2015*

On 26 May 2014 a woman was admitted to the Kenema Government Hospital maternity ward in Sierra Leone capital Freetown, after a bloody miscarriage. Augustine Goba, director of the hospital diagnostic laboratory and a long-time collaborator of Pardis Sabeti, a geneticist and infectious-disease researcher at the Broad Institute and Harvard University, Boston, ran a series of molecular-biology tests and detected the first case of Ebola infection in the hospital, and one of the first confirmed cases in the country. Sheik Humarr Khan, Sierra Leone leading virologist, and other medical staff members isolated the patient and wore gloves, gowns and masks while treating her. She survived and made a full recovery, and no one else was infected (Sabeti, 2014).

Had this patient been one of the first cases of Ebola-infected persons in West Africa, the hospital rapid diagnosis and expert handling might have helped control the outbreak quickly. But by May 2014 the epidemic had already been spreading for six months, with hundreds of cases in neighbouring Guinea and Liberia. When the hospital outreach team travelled to the patient's village, they found that 14 people had already been infected, with what turned out to be two distinct strains of the virus. With each passing week the number of confirmed cases in Sierra Leone grew to 31, then 92, then 147, until the virus emerged in nearly every district of the country (Sabeti, 2014). In July 2014 P. Piot sounded one of the first alarms that the Ebola epidemic in West Africa was out of control. He publicly called it "a mega-crisis" that required military-like logistics and control measures like isolation and quarantine of infected individuals and their contacts. He worried that his message about the world's tardy response to control Ebola might be hyperbolic, he said, but that he "would rather be accused of overreacting than responding too late or not doing enough." In fact he believed he was "on target." Earlier on, in February 2014, P. Piot returned to Yambuku to celebrate his 65th birthday, a month before Ebola was first identified in Guinea. Since then he has not gone to the affected areas in West Africa because of his duties at the London School of Hygiene and Tropical Medicine (Altman, 2014).

In Sierra Leone Kenema Government Hospital was well positioned to detect and treat Ebola-virus-infected persons because of its experience in combating another deadly virus, Lassa, a project Pardis Sabeti started working on in 2008. As the hospital reputation for treating Lassa fever spread, more patients with unexplained fevers began to travel there. Rapid diagnosis of more people not only helped treat individual patients, but it could also uncover other unexpected pathogens hiding in the population, thus warning of outbreak before they became global threats. Just weeks before Ebola fever appeared in Sierra Leone, Sheik Hummar Khan and P. Sabeti joined colleagues from around the world in Nigeria to inaugurate the African Center of Excellence for Genomics of Infectious Diseases, a new venture that would

enable monitoring of dangerous microbes across West Africa. The Ebola outbreaks put these plans on hold. Soon after S.H. Khan returned to Kenema Government Hospital, the latter became overwhelmed. He and the nurses were treating up to 80 patients at a time, working 16-hour days. The constant flow of severely ill patients made it impossible for hospital staff members to protect themselves. Over a few months *ca.* 40 contracted the disease, including S.H. Khan, who died by the middle of the summer of 2014 (Sabeti, 2014).

Pardis Sabeti (2014) thinks it did not have to be that way. If diagnostic facilities like those at Kenema Government Hospital had been more widely available, the virus could have been caught as it emerged. A team of trained individuals, perhaps 50 people, could have been sent to the site of the outbreak before it spread, and stayed for 50 days after the last infection. Working with the local community they could have isolated and treated the sick, buried the dead, traced each person's contacts over the incubation period, performed outreach and education, and ensured food supplies, all while keeping themselves safe from infection. With the virus confined to one village this 50-50 approach would have been a modest investment for the world. Instead the virus could escape the first village and then spread into four more countries. This resulted in the death of a large number of people, including medical staff (Sabeti, 2014). In this respect Peter Piot stated that "the mortality to medical staff and nurses has been enormous and devastating." "Our colleagues are the most affected of all professions, paying a high price for their work, and also infecting others," he added (Altman, 2014).

Based on WHO numbers, more people became infected with the Ebola virus in August 2014 than in all the earlier months of this epidemic combined. The WHO predicted that Ebola might afflict 20,000 people before the outbreak ends, but that was only if the world mounted a prompt and considerable response. Otherwise the death toll will increase a lot, and meanwhile the virus is evolving as it spreads from human to human, raising the risk of it becoming even more contagious (Sabeti, 2014).

## *The toll of Ebola epidemic in West Africa*

The worst epidemic of hemorrhagic fever since the identification of the virus in Central Africa in 1976 has spread in West Africa since December 2013 without drawing much attention. The French NGO *Médecins sans frontières* (MSF, Doctors Without Borders) which was at the forefront since the outbreak discovery rapidly realized that this was an unprecedented outbreak, with a lot of stricken spots and an extension outside forest zones towards the large urban centres; this spread was enhanced by transborder movements of populations. By mid-April 2014 both MSF and the United Nations' staff were worried by the magnitude and the unusual characteristics of the epidemic, and they requested the help of WHO assistant director-general, Keiji Fukuda, in charge of health security at the Geneva-based international organization. The reaction of WHO has been slow: in an e-mail dated 5 June 2014, revealed by Associated Press on 20 March 2015, Sylvie Briand, head of the pandemic and epidemic diseases department at the WHO, indicated that "it might be more efficient to use other diplomatic means for the

time being," while one of her colleagues said that one was dealing with "a public-health emergency of an international magnitude." Five days later, in a memorandum addressed to Margaret Chan, WHO director-general, K. Fukuda and other staff members stated that initiating a global alert or even call a meeting to discuss the issue "might be interpreted as a hostile act." Finally the WHO waited until the 8th of August 2014 to declare the on-going Ebola epidemic an international emergency which led to the mobilization of governments (Benkimoun and Barroux, 2015).

To sum up, the outbreak would have started on 6 December 2013 in the Guinean village of Meliandou, near the border with Liberia and Sierra Leone. Thereafter, from the epicentre of Guéckédou in Guinea, other outbreaks occurred at rather long distances and they affected the three countries (from 5 May to 3 August 2014). Between 4 August 2014 and 4 January 2015 the WHO launched an alert, while the three capitals were stricken (Freetown, Sierra Leone; Monrovia, Liberia; and Conakry, Guinea) and that more than 1,000 Ebola-infected patients a week were counted. In Sierra Leone the peak of the epidemic was reached between early November and early December 2014 with *ca*. 550 cases a week. In Liberia that occurred between mid-September and mid-October 2014 with *ca*. 450 cases a week. In Guinea the number of cases was lower: *ca*. 125 cases a week by the end of December 2014. The cumulative death toll was 1,013 on 9 August 2014, 5,147 on 12 November 2014 and 10,299 on 23 March 2015, according to the data provided by the WHO and national health authorities (Benkimoun and Barroux, 2015).

Liberia has been the most affected western African country with 4,808 deaths, followed by Sierra Leone with 3,955 deaths and Guinea with 2,536 deaths. It has been nevertheless Sierra Leone which recorded the highest number of cases (14,089), almost half of the 28,571 cases recorded in the three countries. Another 36 cases, of which 15 fatal, were recorded in seven countries. A total of 11,299 deaths were caused by this Ebola outbreak (Lepidi, 2015). Between 5 January and 15 March 2015 the Ebola epidemic declined, but population movements help maintaining the disease, especially in Sierra Leone. In fact West Africa is still reeling from efforts to stop the Ebola epidemic, officially declared on 25 March 2014 by the WHO. In Liberia, on 5 March 2015, the last infected patient had left the hospital after two tests showed that he was free of the virus. This country hoped to proclaim it had got rid of the Ebola virus, after a lapse of 42 days without detecting any new infection – this was twice the maximum incubation period of the pathogen, estimated at 21 days. Unfortunately a new infected patient, identified on 20 March 2015, died on 28 June 2015 (Benkimoun and Barroux, 2015).

On 3 September 2015 Liberia has been declared Ebola-free. Sierra Leone was also to be declared Ebola-free on 7 November 2015 by the WHO. This occurs when not a single case of Ebola has been declared across the whole country for 42 days. Sierra Leone inhabitants, and in particular in the health-care centres, will be singing "Ebola bye bye!" However surveillance must be maintained, because "there may be reservoirs of the virus which we do not know," explained Jacob Maikere, head of Doctors

Without Borders in Sierra Leone. In particular epidemiologic surveillance should be pursued and physicians must continue to be in touch with former patients (Lepidi, 2015). In this county measures had been taken in March 2015 with a view to confining its population, so as to carry out field work, knocking at every house's door, in order to slow down the transmission of the virus. On 21 March 2015, Sierra Leone's president, Ernest Bai Koroma, announced that the country's six million inhabitants "will have to stay at home from Friday 27 March, 6 am, to Sunday 29 March, 6 pm" (Benkimoun and Barroux, 2015).

Those who survived the epidemic in Sierra Leone have been estimated at 5,000. Despite wide-ranging campaigns across the country, songs written by popular singers (I'm An Ebola Survivor) and speeches by the country president with a view to facilitating the re-insertion of these survivors into the social fabric, many of them are discriminated, and even rejected. The great majority of these survivors suffer from psychiatric disturbances; they speak of a deep sadness, sometimes associated with the feeling that they should not have survived the disease. Their ailments are also physical: joint inflammation, migraine, decrease in their hearing and vision capacity. "There is no miracle treatment of the side-effects of the disease," explained Caroline Scholtes, medical coordinator of Doctors Without Borders, who opened at the end of August 2015 a clinic for survivors in Freetown's Aberdeen borough. "We request that the former patients be protected and taken care of. This is essential," she stated (Lepidi, 2015).

A decontamination phase, approved by the WHO, was initiated. It includes the disinfection of the red zone (high risk zone), where patients have been treated and where authorized staff can enter with an individual protection equipment; as well as that of the green zone (lower risk zone), where medical staff is stationed. After a detailed inventory, the equipment that must be disinfected or destroyed was classified into three broad categories: equipment that can be disinfected and thereafter reused, such as beds, matresses, shelves; equipment that must be destroyed, such as wooden apparel in the corridors, altered or porous material; and those items which must be disinfected and thereafter buried, such as syringes, bed parts (Lepidi, 2015).

On Thursday 14 January 2016 the WHO declared the end to the deadliest Ebola outbreak on record. The announcement in Geneva came after recent cases in Liberia were snuffed out, marking the first time since the start of the epidemic two years earlier that Guinea, Liberia and Sierra Leone – the three countries hardest hit by the Ebola virus – had reported zero cases for at least 42 days. Margaret Chan, WHO director-general, hailed the "monumental achievement" in curbing the outbreak, which, according to the United Nations data, killed more than 11,300 people and infected more than 28,500. In a statement released in Geneva M. Chan added that "our work is not done, and vigilance is needed to prevent new outbreaks." Just after the announcement made on 14 January 2016 another patient died in Liberia!

The immediate threat indeed stems from the persistence of the virus in body fluids, notably in the semen of male survivors, up to a year after they are free of the disease and show no symptoms, said Rick Brennan, the WHO director of emergency risk management, in Geneva. Ten flare-ups had been reported across the three countries in the past nine months, four of them in Liberia and three each in Guinea and Sierra Leone, "and we are anticipating more," R. Brennan stated. The risk, though significant, was low he estimated (Searcey et al., 2016).

"People of course want to return to a normal life, but it is a new normal," underlined Peter Graaff, a WHO director who is in charge of Ebola response. "Ebola has been added to a number of their diseases that affect the population." The three West African countries now have the world's biggest pool of expertise in handling Ebola, and greater professionalism, confidence and resources for dealing with it, he stated. Their approach, P. Graaff said, is that "it is a problem, a big problem, it is going to affect us again, but we know how to handle it" (Searcey et al., 2016).

### *Economic and social impact*

The Ebola epidemic had a serious economic impact on Guinea, Liberia and Sierra Leone, three nations already at the bottom of global economic and social indicators: airlines have cancelled flights to these countries, prices of staple foods rose and food supplies dwindled; border posts have been closed; expatriate workers went home and national economic growth rates plummeted. Aggravating both the economic and social consequences, these countries and their frightened African neighbours enacted concentric circles of quarantines, cutting off neighbourhoods, regions and even whole nations. International medical authorities have warned against such practices, arguing that they worsened suffering and deprivation and did little to stop the spread of the viral disease. But many African nations have gone ahead anyway, sealing borders, barring entry to residents of the affected countries, stopping their national airlines from flying to them, and even, in some cases, refusing to allow humanitarian flights with urgently needed supplies and medical personnel. For the worst-hit countries, "isolating and stigmatizing them and making it difficult to transport supplies, personnel and other resources" can only make things worse, the WHO regional director for Africa, Luis Gomes Samba, stated at a meeting in Ghana by the end of August 2014 (Nossiter, 2014).

Guinea, Liberia and Sierra Leone had only recently emerged from decades of war and political upheaval. With sections of Liberia and Sierra Leone under quarantine and the borders of Senegal and Guinea sealed, the movement of goods slowed. National budgets were under strain, health-care expenditures rose, government revenues dropped and agricultural production, especially in Sierra Leone, took a hit. "Food insecurity was poised to intensify," the Food and Agricultural Organization of the United Nations (FAO) regional representative for Africa, Bukar Tijani, said by early September 2014. The United Nations reported that the price of cassava, a staple starchy food, increased 150% in Monrovia in the first week of August 2014. In Sierra

Leone, rice, fish, palm oil and other basic foodstuffs all rose in price, according to the finance ministry there. Fear of Ebola fever added uncertainty, recalling the worst period of the civil wars in West Africa in the 1990s. "This is going to be as bad as the war," said Rupert Day, manager of Tropical Farms, a British cocoa and coffee trading company in eastern Sierra Leone, at the heart of the Ebola outbreak. The World Bank projected a drop of at least one percentage point in Guinea's economic growth rate (Nossiter, 2014).

In **Liberia** health-care expenditures accounted for 25% of the government's annual budget because of Ebola, instead of 8%, the finance minister announced. Arcelor Mittal, which runs a major iron-ore mining operation in Liberia, delayed an expansion because contractors evacuated 645 employees (Nossiter, 2014). In this country of 4 million people, and after having been declared safe for the second time, on 3 September 2015, schools and markets are open, people have food to eat and locals are back to their old ways of greeting visitors with hugs and holding hands. Yet not all the hard-won lessons have been forgotten. At the height of the outbreak, Redemption Hospital, a labyrinthine market-turned-medical facility in Monrovia slum, was receiving hundreds of patients a day. Chlorine supplies ran dry, protective equipment was scarce and refuse piled up behind the wards. Twelve of its workers lost their lives. Today staff carefully wash their hands and patients are not forced to share beds. Rubbish is destroyed in incinerators and cleaners douse the wards with disinfectant (*The Economist*, 2015e).

The resources that were channelled to Liberia's health-care system to stem the disease are continuing to do some good. A measles outbreak erupted after 2014 vaccinations were suspended. When immunization shots were finally dispatched they reached more than half a million children, according to Liberia's deputy health minister – one of the highest coverage rates to date. But the aftermath of the pandemic is a battle in itself. Hospitals which are applying better standards of infection control now turn patients away for lack of space. There were fewer than 60 Liberian physicians in the country before the viral disease broke out and some 10% of them died after catching it. Donors worry about what will happen to the health system when the flow of aid stops. Longer-term social issues are also coming to the fore. Families that lost their supporting members faced poverty, and there was scant assistance for the 5,000 orphaned children now living under caregivers' roofs. Street Child, a charity, estimated that child labour was on the rise as struggling families take children out of school and send them to work (*The Economist*, 2015 l).

In Liberia annual economic growth rate should rebound to 2%-3% in 2015 from less than 1% in 2014, the World Bank reckoned, but that looks paltry in comparison to a pre-Ebola rate of close to 9%. Not all of this is due to the viral disease: falling commodity prices have played their part, too. Iron-ore revenues have fallen and rubber exports have dropped. International companies that were prospecting for oil a few years ago have now stopped work. Liberia has easily lost three years in its development agenda, stated one diplomat. He also added: "The real issue is not rebuilding Liberia after Ebola, it is building it from scratch" (*The Economist,* 2015 l).

In **Sierra Leone** the Ebola outbreak occurred when the country was finally on the road of development and recovery after having suffered from a civil war that left 120,000 deaths behind it between 1991 and 2002 In 2013 Sierra Leone economic growth rate reached 11.3%. Even though life expectancy was very low (46 years), it increased by eight years over a decade. "Ebola came at the worst moment when investors trusted the country because all economic standards were promising and because of political stability," underlined Moses Sichei, economic adviser at the United Nations Development Programme (UNDP). "The virus had an impact on all sectors," he added. For instance, Mark Reading, director of Terra Nova Solutions, a firm specialized in logistics and founded six months before the beginning of the outbreak, stated that his company's losses were around 50%. "Before the outbreak, *ca*. 48 planes used to land every week at Freetown airport. At the peak of the outbreak, there were only five planes… Everybody was afraid. Expatriated persons, often employed by international corporations, went back home. Only independent employees stayed in the country," he added. Between September 2014 and June 2015 all major airline companies decided not to fly to Sierra Leone, with the exception of Brussels Airlines and Royal Air Maroc – the Kingdom of Morocco's airline (Lepidi, 2015).

Agriculture (rice, cassava, sweet potato, which are staple foods) which makes up half of the country's resources, was stricken dramatically, because the harvests could not be made on time, due to a precautionary measure that has forbidden gatherings of more than four persons. The inflation rate reached 10% by the end of 2015, while the gross domestic product (GDP) was to plummet by 21.5%. "The mining sector (iron, gold, diamonds) has also been severely affected, because in addition to the impact of the Ebola outbreak, there was a slump in the global economy and in the prices of raw materials," explained David Tinel, in charge of investments in an international bank, member of the World Bank group. Because of the lack of manpower gold exports amounted to only one-third of the usual figure during the first half of the year (2015) and diamond exports were halved (Lepidi, 2015).

There was "scaling down" at the country's three biggest manufacturers: a brewery, a bottling company and a cement plant, the finance ministry indicated. Construction on major road projects has been suspended following the evacuation of foreign staff members overseeing the projects (Nossiter, 2014). Tourism has a real potential in Sierra Leone: white-sand beaches, preserved forests and a cheerful population are real assets for developing tourism in a country that has become epidemiologically safe. But during a year and half, there was not a single touristic venture. In Freetown, along Lumley Beach, many hotels closed down during the peak of the outbreak, because no shop or business could remain opened after 6 pm. "It will take time and efforts to bring back European visitors who were so numerous during the 1980s," reckoned Yassin Kargbo, director-general of the Tourism Department (Lepidi, 2015). The World Bank announced that an amount of US$1.62 billion or €1.49 billion was to be allocated to the three western African countries affected by the Ebola outbreak, in order to help them control the disease and support their economic development: US$385 million to Liberia, US$318 million to Sierra Leone and US$260 million to Guinea (Lepidi, 2015).

The Ebola epidemic had a major social impact in these countries hit by the viral disease: "Ebola is scaring, it strikes population without distinction, it affects physicians and health-care staffs, it leads to the isolation, or even the rejection of the patients, while there is not an efficient treatment. Furthermore the measures taken to control the virus interfere with the behaviour towards dead people – a very important tradition in Africa," commented Jean-François Delfraissy, director of the French Thematic Institute of Microbiology and Infectious Diseases. Laurent Vidal, an anthropologist and researcher at the French Research for Development Institute (IRD, French acronym), who works in Cameroon on the access to health care in the case of AIDS, made some comparisons between both viral diseases: "Politicians have quickly reacted by issuing and applying quarantine measures. The patients in boroughs of Monrovia, Liberia, were considered more 'guilty' than 'victims'. There were even some rumours highlighting a responsibility of the West in the epidemic," stated L. Vidal. This trust crisis is not irreversible. "One has to go to the grassroot level – with social scientists – and repeat that these are exceptional measures taken to respond to an exceptional crisis. The involvement of persons that have been treated and cured can make the sanitary measures more acceptable," suggested L. Vidal (Benkimoun and Barroux, 2015).

## *Treatments of Ebola hemorrhagic fever*

Since the emergence of the Ebola fever in 1976 in Zaire (now the Democratic Republic of Congo) and later on the discovery of the virus, and for almost 40 years, there has been no efficient treatment of the disease. According to Jean-François Delfraissy, "the development of treatments and vaccines against such viruses as Ebola was not a public-health priority; there were few research teams and paltry funding" (Cabut and Hecketsweiler, 2014).

### Competition in research

The 2014-2015 Ebola epidemic has triggered research-and-development activities and even led to a rivalry among researchers and drug companies, with respect to the development of drugs and vaccines against the Ebola virus. On 5 September 2014 after a two-day seminar involving 150 experts, the WHO announced several priorities: firstly, to immediately use the serum of patients who are feeling better, in order to treat those who are ill; vaccinate since November 2014 the exposed medical and health-care staff, if the first vaccination trials are positive; and evaluate in humans available experimental drugs as quickly as possible. "With respect to basic research work, we saw a competition between the journals *Nature* and *Science*; and regarding the clinical research, between *The Lancet* and the *New England Journal of Medicine* (*NEJM*)," stated Hervé Maisonneuve. Like in the 2009 flu pandemia, there is a race for publication: "Reviews publish on line with an accelerated approach called fast track, implying a minimum peer review and an offensive communication service," underline H. Maisonneuve. "Their priority is that their title be present in the medias, particularly on the television, while they are a little laxist about quality and the scientific matter," he added. Jean-François Delfraissy has also stressed the fact that "some renowned journals tend to publish articles which they may not have accepted in other circumstances."

He incidentally mentioned that the results of trials carried out in monkeys should be examined with caution, and he insisted on the lack of data concerning trials in humans, tolerance to the virus and the effect of candidate or potentials drugs on viremia. That was the case of an experimental cocktail of three antibodies called ZMapp, which was administered to seven patients for humanitarian reasons. Two of them died, four were being cured, and the health of the seventh was still in jeopardy. Such results are difficult to interpret, according to specialists. Instead J.F. Delfraissy thinks that a phase-1 study which would have allowed the collection of useful data, should have been carried out (Cabut and Hecketsweiler, 2014)

United States' edge

The United States have an edge over other countries in the international competition concerning the search for treatments against Ebola hemorrhagic fever. It is true, however, that the strain of the Zaire Ebola virus (EBOV) which caused the epidemic, was isolated and identified in a French laboratory, with an article in the *NEJM* (Maganga et al., 2014). But most publications are made by teams with a strong North American membership. For instance scientists of the vaccine research centre of the National Institutes of Health (NIH) announced in *Nature Medicine* published on line on 7 September 2014 that they had obtained, for the first time, a durable immunity in *Macacus* monkeys againt Ebola virus challenge, further to an injection of a vaccine derived from a chimpanzee adenovirus (Stanley et al., 2014).

Several factors explain the advancement of North American researchers compared with scientists from other regions of the world. In the United States dangerous viruses are considered as weapons and they are a threat to American soldiers stationed in "high risk" regions. Also the re-emergence of tropical diseases like dengue stresses that the country is not any more protected from epidemics which were up to now striking the countries of the South. Several recent science-fiction movies dealt with the dangers of contamination with lethal viruses. In 1998, Ken Alibek, one of the Soviet scientists most involved in the development of bacteriological weapons, stated that the Soviet Union had worked on Ebola. Since then the threat of bioterrorism has been taken very seriously as shown in an article published in May 2013 in the renowned American journal, *Global Policy Essay*. Authored by Amanda M. Teckman, the article expresses worries about the presence of terrorist movements in East Africa and their capacity to use the Ebola virus. To use the virus as a weapon implies an expertise, but scientists may work for terrorists for ideological or financial reasons, and A.M. Teckman quoted the example of anthrax which a Japanese sect was able to obtain (Teckman, 2013).

To counter these threats, the United States army created in 1969 the US Army Medical Research Institute of Infectious Diseases (USAMRIID) which employs *ca*. 200 high-level scientists. It is based in Fort Detrick, Maryland, near to the National Institutes of Health (NIH) research centre on infectious diseases. Both institutes have very high-security laboratories (four level), which are the only ones where the most dangerous microbes are studied, e.g. Ebola virus, anthrax, botulin toxin (*Clostridium*

*botulinum*). In addition to their own research programmes the NIH and the United States Department of Defence (DoD) are the major providers of funds for the struggle against Ebola: *ca*. US$65 million subsidies allocated since 2004 and another US$50 million in the form of contracts. Among the first beneficiaries, the Canadian Tekmira, which signed a US$140-million contract with the DoD in order to accelerate the development of its candidate vaccine; the Californian firm Mapp Pharmaceuticals received US$32 million from NIH to develop an antibody-based treatment (ZMapp); the Dutch Crucell which received US$20 million in order to develop another candidate vaccine. All these amounts were considered highly significant for a disease that caused more than 11,000 deaths in 2014-2015, compared with the 600,000 deaths caused annually by malaria or even with the 20,000 annual lethal cases of dengue – two diseases that also affect the poorest countries of the world (Cabut and Hecketsweiler, 2014).

As participant in this global competition led by the United States is found, for instance, the University of Texas with in Galveston a laboratory carrying out advance research on Ebola. Thomas Geisbert, who works in this laboratory and has devoted his career to the study of Ebola, received a subsidy of US$26 million over five years from the NIH, with the aim of advancing three major programmes on vaccines and treatments against Ebola hemorrhagic fever. T. Geisbert's team had obtained promising results in monkeys against the Marburg virus which is close to Ebola (Cabut and Hecketsweiler, 2014).

Role of pharmaceutical companies

Also the pioneering firms which focused their R&D efforts on Ebola are now coveted by the big pharmas. For instance, in 2011, Johnson & Johnson acquired the Dutch firm Crucell, specialized in vaccines, for US$2.4 billion; moreover it built in 2014 a partnership with the Danish laboratory Bavarian Nordic that has been working for several years on Ebola. In May 2013 the British pharmaceutical group GlaxoSmithKline (GSK) acquired for US$324 million a small Swiss firm specialized in vaccines, Okairos. Both GSK and Johnson & Johnson, due to their in-house expertise and that acquired through buying specialized firms, are the top leaders in the search for a vaccine against Ebola (Cabut and Hecketsweiler, 2014).

Europe's contribution

French scientists were the first to identity the virus of the 2014-2015 Ebola epidemic: Sylvain Baize, director of the Pasteur Institute National Reference Centre for Hemorrhagic Fevers, isolated that strain (Baize et al., 2014). He complained about the difficulty of obtaining funds for their research on primates. For instance, in the case of the Lassa fever, which causes *ca*. 5,000 deaths a year, government funding has been obtained after ten years. In the context of competition between the United States and Europe, S. Baize regrets that his American colleagues are reluctant to share their strains. He cites the following example: "During the Ebola epidemic in Uganda in 2007, it was a Belgian team of Doctors Without Borders (MSF) who took

samples from the first patients. For practical reasons the samples were delivered to the United States Centers for Disease Control and Prevention (CDCs, Atlanta) that showed the presence of a new strain, *Ebola bundibugyo*. Despite the requests from European scientists, no European team could receive that strain in order to carry out the relevant investigations. This was unacceptable. A similar case concerned a microbe identified in 2008 in South Africa, *Arenavirus lujo*, which killed four of the five infected patients." The race with the United States does not seem to be lost, because more European publications are expected. France is also carrying out studies, such as a programme aiming to identify among thousands of molecules already commercialized to treat other diseases, those which could be efficient against Ebola; thereafter rapid clinical trials would be conducted (Cabut and Hecketsweiler, 2014).

Blood transfusions

The World Health Organization (WHO) estimates that transfusion of blood or of blood derivatives from patients who are overcoming the disease are "culturally acceptable". They have been used in a former Ebola epidemic in 1995 in Zaire. However these treatments need the facilities and human resources that allow the medical staff to check if the donors are not infected with HIV or an hepatitis virus (Cabut, 2014b).

## *Vaccines*

Two promising candidate vaccines that have given good results in trials carried out in monkeys (*Macacus*), have received the approval of the WHO. One is developed by GSK and the other by the Canadian public health agency (with a license granted to the American firm, NewLinkGenetics). Both candidate vaccines are recombinant vaccines that contain components of the Ebola virus which induce the immune reaction, without the risk of causing the disease (Cabut and Hecketsweiler, 2014).

GSK candidate vaccine

According to Emmanuel Hanon, who is responsible for vaccine research at GSK, "the vaccinated animals which were thereafter exposed to the Ebola virus, were protected against it." These results led to the decision by the British pharmaceutical company to accelerate the trials in humans. GSK received financial support from the British government and the Wellcome Trust. Clinical trials on healthy volunteers (a few dozens) were carried out in the United Kingdom, the United States and in Africa – Mali, a country that has not been stricken by the Ebola epidemic – with a view to checking the innocuity of the vaccine (phase 1). A second phase (phase 2) aimed at checking the efficacy of the vaccine and evaluating the necessary doses of the latter, was carried out in December 2014 in Africa. "A vaccine is generally developed in ten years," underlined E. Hanon. "But in exceptional circumstances such as the Ebola epidemic, we work hand in hand with the health authorities in order to accelerate the whole process". Regarding the production capacity, GSK should produce 10,000 doses of the vaccine for the clinical trials, and E. Hanon stated that "we must evaluate the capacity of our plants to produce many more." "We obviously cannot stop the production of

other viral vaccines, particularly the pediatric ones, in order to make room for the Ebola vaccine," he added. On the other hand, when an efficient vaccine is available, it must be decided who would be vaccinated (medical staff, persons in contact with the patients and the African populations concerned). The appropriate funding of these vaccination campaigns has also to be resolved (Cabut and Hecketsweiler, 2014).

Canada candidate vaccine VSV-ZEBOV

On 3 August 2015 *The Lancet* published the interim results of the vaccine trial conducted in Guinea (where the Ebola epidemic started in December 2013) by the WHO and the country's health ministry. This trial, named "Ebola ça suffit" (Ebola it's enough), aimed to test the candidate vaccine rVSV-ZEBOV, which, according to the published results, can protect up to 100% of the persons that have been in close contact with an infected patient, as well as the people living near these persons. These clinical trials were initiated on 23 March 2015, thanks to a wide-ranging international cooperation involving, in addition to the Guinean government and WHO, the NGOs, Doctors Without Borders and Epicentre, as well as the Norwegian Public Health Institute, with funding from the British charity foundation Wellcome Trust, and also from the Norwegian and Canadian governments. The trial aimed to test the efficacy and innocuity (safety) of the vaccine (Henao-Restrepo et al., 2015).

The rVSV-vectored vaccine expressing Ebola surface glycoprotein (rVSV-ZEBOV) has been developed by Canada Public Health Agency and the company NewLinkGenetics, which signed a collaboration agreement with Merck. It was tested in 7,651 persons in Lower Guinea, following the approach of ring vaccination cluster-randomized trial, a technique which contributed to the eradication of smallpox in 1977 through the interruption of the pathogen's chain of transmission. When an Ebola virus-infected individual is identified, vaccination (using rVSV-ZEBOV) was proposed to all persons in direct contact with the patient, as well as to the persons of the second ring who were rather close to those in close contact with the infected individual. All adults, 18-years old or more, were vaccinated, with the exception of pregnant and lactating women (Henao-Restrepo et al., 2015).

All the persons who agreed to participate in the trial were randomized and distributed in two groups: 4,123 immediately received one single injection of the vaccine, whereas 3,528 received the vaccine 21 days later – i.e. the incubation period of the Ebola disease. This kind of trial aimed at making a comparison, without having to use a "placebo" injection, deprived of any immunological effect. Then periodic visits have been made during the period elapsing from the vaccination up to 12 weeks. The end result was that no infection by the Ebola virus had been observed among the vaccinees of the first group (immediate vaccination), compared with 16 cases in the second group (vaccination delayed by 21 days). Furthermore the overall efficacy of the vaccine among the people living around the infected individual and including vaccinated and non-vaccinated persons, or persons that could be vaccinated or not, has been estimated at 76%. Therefore the ring-vaccination strategy seemed to significantly reduce the risks of infection, even among non-vaccinated persons (Henao-Restrepo et al., 2015).

Bearing in mind these encouraging results, the promoters of the vaccine trial authorized on 26 July 2015 all the persons exposed to the risk of being infected, to be vaccinated immediately. The trial was to be pursued so as to involve volunteers, 13 to 17 years old, then children, 6 to 12 years old, depending on the data concerning the safety of the vaccine. Among all participants in the vaccination trial, 43 serious undesirable effects were recorded. Does that mean that an antiEbola vaccine is finally available on a large scale? John-Arne Røttingen, of the University of Oslo and co-author of the publication in *The Lancet*, is cautious: "It is not yet certain that this candidate vaccine can become a vaccine to be used on a large scale against Ebola outbreaks. More evidence is necessary with respect to the safety and efficacy of the vaccine, before using it beyond the framework of a clinical trial." Bertrand Draguez, medical director at Doctors Without Borders, added: "At the same time as the vaccination trial, we are also carrying out a trial with the same candidate vaccine on health-care staff who are at the forefront of the struggle against Ebola disease." The French NGO vaccinated 1,222 staff members. On her side, Marie Paule Kieny, WHO assistant director-general and co-author of the study published in *The Lancet*, underlined that "this work carried out in a very short period is a turning point in the history of research and development in health" (Benkimoun, 2015 g; Henao-Restrepo et al., 2015).

EBOVAC 2 vaccination project

A phase-2 clinical trial was launched in Europe and Africa by an international consortium coordinated by the French National Institute for Health and Medical Research (INSERM) and funded by "Ebola +" programme of the European Commission Innovative Medicine Initiative 2. The clinical trial started in July 2015 in England (Oxford University) and was also expected to be conducted in Africa. This project was called EBOVAC 2 and it should also involve *ca*. 300 French volunteers in order to lead to significant results. Since the beginning of 2014 INSERM researchers – especially those working at the P-4 laboratory in Lyon (centre-east of France) which is highly protected so as to manipulate pathogenic viruses – as well as those of the Pasteur Institute (Paris) have contributed to the characterization of the Ebola virus strain, causing the outbreak in Guinea, and to trace the virus during the first months of the outbreak through the affected areas. The INSERM was thereafter the promoter of the clinical trial JIKI carried out in Guinea by the end of 2014 with a view to testing the efficacy of an antiviral drug (see p. 217). Other trials are being carried out with the National Institutes of Health (NIH). Yves Lévy, INSERM president director-general, recalled: "We have the expertise acquired over the last 30 years in the struggle against pandemics (HIV/AIDS, tuberculosis, malaria), but also in the study of emerging infectious diseases (SARS 2003, chikungunya since 2005, A(H1N1) influenza in 2009 and H7N9 in 2012, MERS-CoV in 2013." It is therefore quite natural that INSERM research teams stood at the forefront against Ebola virus outbreaks, because, even though the peak of the epidemic seems now behind us, research is still necessary in order to find preventive solutions against the virus."

INSERM researchers have therefore designed an innovative vaccination approach to control the virus, with their partners from the London School of Hygiene and Tropical

Medicine, Oxford University, the Muraz Centre in Burkina Faso and Janssen – a pharmaceutical subsidiary of the global big pharma Johnson & Johnson. Rodolphe Thiébaut of INSERM and coordinator of the EBOVAC2 project, explained: "Our approach consists of injecting two different vectors so as to increase the overall efficacy. We test the validation of this hypothesis and we study at the same time the most efficient time lapse between the two injections." This vaccination approach, called prime boost, uses therefore two vectors developed by Janssen and Bavarian Nordic. The vectors are derived from known viruses that have been modified in order to be totally innocuous; the first one is derived from adenovirus 26 (virus of cold), while the second is derived from smallpox (MVA). Both vectors contain the genes encoding the proteins of the Ebola virus strain Zaire, that is circulating now. The second vector contains genes encoding proteins of other virus strains (Sudan, Tai Forest and Marburg).

Using two different vectors has the advantage of conferring to the vaccinees a better immunity and probably a long-lasting one. A phase-1 clinical trial started with a small number of volunteers in the United Kingdom, the United States and a few African countries. At this stage, it was demonstrated that the vaccination test was safe and well tolerated. Phase-2 clinical trials therefore followed and French volunteers were involved in this second stage. R. Thiébaut underlined that "in these clinical trials the efficacy of this vaccination approach is assessed through measuring the quantity of antibodies produced by the vaccinees' immune system." He stated that in 18 months the data will be available, and the researchers will be able to study the expression of modified genes in the cells of the vaccinees as well as the dynamics of the immune response, and thus understand how the vaccine works and how to optimize it. In France an appeal has been made to volunteers who should be in good health and between 18 and 65 years old; their residence should be near to one of the hospitals where the clinical trial is performed (Créteil, Paris-Cochin, Lyon, Marseille, Rennes, Saint-Etienne and Strasbourg). They also should accept to be monitored by the researchers for about a year. During this period the vaccinee, after a first medical visit to check the health status, receives two injections of the vaccine, and thereafter he/she attends six to 12 follow-up medical visits in order to assess the tolerance of the vaccine as well as the immune response. The volunteer receives a fee of €760 to €1,240, depending on the number of medical visits or checks.

In addition to the academic and pharmaceutical industry partners, Y. Lévy underlined that non-governmental organizations or associations played a key role in the EBOVAC2 vaccination project, within a North-South cooperation; e.g. Doctors Without Borders, Alliance for International Medical Action (ALIMA), the French Red Cross. Regarding the North-South partnerships, the INSERM has benefited from the collaboration of partners already in place for many years, such as the network Research and Action Targeting Emerging Infectious Diseases, the group Aviesan Sud, as well as from the local Pasteur Institutes, the French Research for Development Institute (IRD), the laboratories of the Mérieux Foundation and the French International Agricultural Research Centre for Development (CIRAD, French acronym). Also the INSERM had access to the P-4 laboratory in Lyon and to the European Clinical Trials Service Platform & Development (EUCLID). The overall mobilization of all these actors was necessary to effectively fighting the Ebola virus.

### *Potential drugs*

The National Institutes of Health (NIH, United States) and companies like Mapp Biopharmaceuticals (the creator of the ZMapp medication) are accelerating the development of drug candidates. The WHO has selected "5 to 10", including the ZMapp, a cocktail of three antibodies, RNA-based products and small antiviral molecules. On the other hand Pardis Sabeti and his colleagues of the Broad Institute and Harvard University have publicly released Ebola-virus genome sequencing data so groups around the world could use them for better diagnostics, vaccines and therapies (Sabeti, 2014). Several teams announced promising results with candidate drugs that were tested in monkeys (*Macacus*). The ZMapp has been prescribed to a small number of patients for humanitarian reasons, but no clear conclusions were drawn regarding the efficacy of the treatment. WHO experts recommended that "these candidate therapies should be evaluated by standard clinical trials, in order to draw clearcut conclusions with respect to their efficacy in humans." Production capacity of the efficient candidate drugs is another issue; for instance several months were needed for the ZMapp stocks to be replenished after they were completely used by early September 2014 (Cabut and Hecketsweiler, 2014).

A drug to treat Ebola hemorrhagic fever showed promising signs of efficacy in people participating in a trial in Guinea. The drug favipiravir interferes with the virus ability to copy itself; Toyama Clinical Company, the drug's maker, announced in October 2014 that it had 20,000 courses of treatment in stock. It seemed to have halved mortality – to 15% from 30% – in patients who had low to moderate levels of Ebola in their blood, researchers found. It had no effect in patients with more virus in their blood, who were more likely to die. The drug was generally well tolerated. "The results are encouraging in a certain phase of the disease," Sakoba Keita, director of disease control for the Guinean health ministry, said in a telephone interview. Researchers and the health authorities have been debating whether and when to publicize the preliminary results of the study. The dilemma they faced echoed those from the early years of the AIDS epidemic. Because mortality was so high in a disease with no proven treatment, there was demand to provide experimental therapies to everyone. Independent boards charged with monitoring the drug trial detected the encouraging findings and recommended that they be released. Results were expected to be submitted for review to the Conference on Retroviruses and Opportunistic Infection in Seattle at the end of February 2015. An abstract of the findings was described in the *New York Times* by Susan Shepherd, who served as medical coordinator at a treatment unit run by the Alliance for International Medical Action (ALIMA), one of the two sites where favipiravir was tested. The other was a Doctors Without Borders (MSF)-run facility (Fink, 2015a).

The results for favipiravir were based on an analysis of 69 patients over the age of 14 who have received the drug at two sites in Guinea since December 2014. The survival rates of those with low to moderate viral loads (viremia) were significantly better than those of patients previously treated at a centre run by Doctors Without

Borders in Guéckédou, Guinea. In a typical drug study participants would be randomly assigned to take the drug or not, and the outcomes compared to see if the drug uptake made a difference. Because Ebola virus is so deadly and there is no known treatment aside from supportive care, all patients in the study were provided with the treatment. The initial goal was to test 60 adult patients early in their illnesses. The drug was expected to be most efficient in patients within two to three days of showing symptoms, similar to antiviral treatments for influenza. But most study participants arrived at the Ebola treatment units later in their illness, a median of five days after they showed the symptoms. The trial was sponsored by the French INSERM with support from the European Union (the European Commission had allocated €140 million to help the countries hit by the Ebola epidemic), and was run by a consortium of organizations and the Guinean government. The drug, also known by the trade name Avigan, was developed by Toyama Chemical Company, part of the Fujifilm Group, and approved as an antiinfluenza treatment in Japan in March 2014 after safety testing. Toyama stated it will produce more doses of the drug in anticipation of the trial. It also provided the tablets on an emergency basis to several Ebola patients in Europe, said a company spokeswoman (Fink, 2015a).

The positive findings about favipiravir were the main factor complicating the start of a trial of serum transfusions, also known as convalescent plasma therapy, in Guinea's capital Conakry, said Roeland Scholtalbers, the head of communications for the Institute of Tropical Medicine (Belgium), which was the study's main sponsor. If patients receiving serum extracted from the blood of Ebola survivors were also given favipiravir, it will probably be more difficult to discern whether the serum had an effect. Both trials are all the more important, according to Xavier Anglaret – the researcher overseeing the favipiravir trial in Guinea – because of the abrupt cancellation of a study testing a third therapy, the antiviral drug brincidofovir, due to the small number of Ebola patients in Liberia where that drug was being tested. X. Anglaret stated researchers had expected to have results from all three studies around the same time. Instead one study advanced ahead of the others, with interim results that were encouraging, but not definitive. As of 3 February 2015 X. Anglaret indicated the favipiravir trial had enrolled 101 patients (Fink, 2015a).

### *Difficulties in handling an international public-health emergency*

Sixteen months after the Ebola epidemic began tearing through three of the world's most fragile countries – the longest and with the heaviest death toll since the discovery of the virus in 1976 – an independent panel of experts published a report on 7 July 2015, where it was concluded that the WHO remains unfit to handle an international public-health emergency. It also highlighted the lack of a decisive response by the international community. The panel, led by Dame Barbara Stocking, the former head of the British aid organization Oxfam and president of the Murray Edwards College of Cambridge University, stated: "WHO does not currently possess the capacity or organizational culture to deliver a full emergency public health response." While the agency itself has acknowledged the need for change, the panel added "it will need to be held accountable

to ensure that this transformation is achieved." The panel faulted the agency for being sluggish, financially unprepared and overly reliant on "good diplomacy." It pointed to a lack of "independent and courageous decision-making by the director-general," Margaret Chan, in the early days of the Ebola epidemic (Sengupta, 2015).

The report was in fact requested by WHO director-general in order to "evaluate all the aspects of the action carried out since the outbreak of the Ebola virus." The report's conclusions have been severe for the agency. The panel recalled the delay taken by the WHO before declaring the epidemic a public-health emergency of international dimension. This declaration was made on 8 August 2014, while the French association Doctors Without Borders (MSF) had been alerting the international community for several weeks. According to the panel, this "important and unjustifiable delay" was to some extent due to "the desire of not hurting the governments." "The World Health Organization has a normative culture and is not well prepared to handle urgent actions of such magnitude and duration, in several countries and simultaneously; it is not well adapted either when its member States' responsibilities should be recalled" (Barroux, 2015). Because of all these deficiencies – delays, "bad quality of the partnership set up with other third parties", inappropriate training of staff not often coping with emergency situations – and also because of "the insufficient competence in terms of crisis coordination and leadership, gaps must be filled," as explained by the panel which recommended a change in the agency's culture and procedure (Barroux, 2015).

The report urged the agency's regional and country representatives to be independent and ready to speak out against recalcitrant governments that do not take sufficient action on their own. And it faulted donor countries for stripping the agency's funding, urging them to contribute immediately to a "contingency fund" designed to respond to diseases outbreaks. The experts recalled that the member states' financial contributions to the agency have been decreasing for several years, so that WHO "purchasing power has plummeted by more than 30% since 2000." During WHO general assembly in 2016, member states should "examine the need to move from zero growth to a 5% increase in their fixed contributions." Member states will also be requested to make pledges for the "contingency fund" of *ca*. US$100 million (Barroux, 2015; Sengupta, 2015).

Still on the chapter of member states, the panel recalled that many governments did not comply with the International Health Rules: "Violating such rules, almost one-fourth of member states have prohibited travels and took other measures not foreseen by the WHO" (Barroux, 2015). The panel said a rapid overhaul of the organization was needed. It proposed the creation of a "WHO centre for the preparation and reaction to emergency situations," which would merge two areas of work, "nowadays distinct": the struggle against epidemic outbreaks and humanitarian action. This centre, with independent council, would be under the direct responsibility of WHO director-general, and its head will enjoy "a full operational authority." In case of a major crisis, the panel also proposed that a "special representative" be appointed by the United Nations Secretary General. But it is still the WHO that should lead the international health policy. "WHO must re-establish its leadership regarding the protection of global health," said the panel, "but to that end there is a need for significant changes" (Barroux, 2015).

Ebola virus killed 11,235 people in West Africa, mainly in Guinea, Liberia and Sierra Leone. Despite the announcement by the WHO that the epidemic was over in Liberia on 9 May 2015, this country was again confronted with the viral disease: in Margibi county, east of Monrovia, two persons were tested positively on 1 July 2015; these were acquaintances of a 17-year-old teenager who fell ill on 21 June 2015 and died on 28 June. "Since the return of Ebola, the authorities have reactivated the surveillance system in the country's 15 counties," explained Alex Gasasira, WHO officer in charge of Liberia. Although there have been no explanations on the origin of the contamination of these two persons with the virus, this was not surprising in the country that had the heaviest death toll (4,806 deaths out of 10,666 cases, by 1 July 2015). "With neighbouring countries where the virus is always active, it could not be excluded that new cases be detected," testified Nicoletta Bellio, MSF representative in Liberia. The virus is still being transmitted, west of Liberia. According to WHO, more than 20 new cases appear every week, since the end of May 2015, in both Sierra Leone and Guinea. To rebuild the health systems of the three countries in 2016 and 2017, the WHO estimated that it needed more than US$2 billion. A donor conference was scheduled on 10 July 2015 (Barroux, 2015).

The WHO stated it welcomed the report of the panel of experts led by Dame Barbara Stocking, and that its "numerous recommendations echoed the changes which were being made, while a thorough study of these recommendations will be carried out," stated Richard Brennan, in charge of the response to Ebola at the WHO. These changes included the creation of "a global health emergency work force and the contingency fund to ensure the necessary resources are available to mount an initial response." Margaret Chan had previously conceded that she waited too long to declare Ebola a public-health emergency of international concern. Joanne Liu, president of the international arm of MSF, wondered aloud about the tangible impact of the panel's report: "The question is how will this translate into real action on the ground in future outbreaks?" she posted on Twitter (Sengupta, 2015).

### *Lessons learnt from the Ebola epidemic*

The complications of managing the Ebola epidemic and particularly the relevant clinical trials needed for testing the efficacy of therapies, are a sign that more needs to be done to prioritize research in future outbreaks, said Bernard Lo, a bioethicist and president of the Greenwall Foundation in New York City (Fink, 2015b). In addition to the Ebola virus, discovered in 1976, there are many other dangerous pathogens, and the infection risk is not something new; e.g. the epidemics of A(H1N1) influenza in 2009-2010, of SRAS in 2003, and the threat of bioterrorism in 2001. Dozens of expert committees did meet on these occasions and billions of dollars were invested in preventive measures. To possess vaccines and drugs against all dangerous microbes would be a colossal endeavour, but a midway approach would be to select the worst microbes and to carry out a pre-development stage. This would mean that the full development of vaccines or drugs will depend on the availability of serious indications on their likely efficacy, further to experiments on laboratory animals and to low-cost phase-1 trials; information on the possibility to produce the relevant vaccines or drugs

massively (via chemical synthesis or fermentation); and of a large-scale clinical plan that can be carried out in an emergency way, but ethically correct. Thereafter each one of them could be selected for an accelerated development, when it is appropriate to do so. The human body produces millions of antibodies which, in their large majority, will never serve. That is the price the human body pays for its protection. Similarly a large proportion of those products pre-developed with preventive goals may not be utilized, but this is the price our societies should pay in order to save time and lives (Kourilsky and Piot, 2015).

In the case of drug discovery – which is not an easy task – it is suggested to start by examining whether already approved products to treat other pathogens have an efficacy, even medium. For instance, nucleotide analogues that block the replication of a virus like HIV could have an effect (modest, but useful) on other viruses. As a result clinical trials, mass production and the authorizations for commercialization or use could be facilitated and accelerated. Another approach consists of isolating specific monoclonal antibodies that can neutralize the infectious pathogen. More than half of the drugs commercialized since 2005 (particularly against cancers) are monoclonal antibodies. Their isolation is rather fast – sometimes a few weeks – and they are a rather homogenous class of molecules about which a wide-ranging knowhow has been accumulated; and this makes their development easier (Kourilsky and Piot, 2015).

The case of preventive vaccines is different. Their action is not immediate and they need at least 15 days in order to protect the vaccinees. Furthermore their development may be very difficult: in the case of HIV/AIDS, 32 years after the discovery of the virus, there is not yet any vaccine against the virus, while antiretroviral (ARV) drugs have been made available since 1995. Pathogens often have several means that allow them to escape the attack by the immune system. Predictions drawn from the experiments carried out on animals such as mice and monkeys are not always reliable. A vaccine must be tested on healthy volunteers and clinical trials need up to 5,000 or 100,000 volunteers (Kourilsky and Piot, 2015).

When an epidemic starts, it is vital not to lose time. Therefore, why not doing that before the emergence of the outbreak, instead of using authorized accelerated protocols, after the outbreak, as an exceptional measure? Why not setting up, with the countries having the resources and the political will, a few platforms where would be stocked pre-developed products targeting the most dangerous pathogens? Is that feasible? The full development of a single drug takes seven to 12 years, with an estimated average cost of *ca*. €1 billion to €2 billion. With the help of public research the cost of a pre-developed molecule would cost *ca*. €20 million, i.e. €2 billion for a hundred, not including the necessary costs for the maintenance, extension and the updating of the product library. These costs are not excessive at the global level, compared with the expenses and losses due to uncontrolled epidemics (several billions of dollars for the SRAS in Singapore and Toronto in 2006, several points of GDP for the African countries stricken by the Ebola epidemic), and the resulting human tragedy (Kourilsky and Piot, 2015).

In the case of the United Nations Fund for the Struggle against AIDS, Tuberculosis and Malaria, set up in 2002, agreements and funding could be found at the international level, as well as efficient operating methods. The danger we may face in the future could be considered of a smaller magnitude: Ebola hemorrhagic fever has caused several thousand deaths, AIDS millions, but how about the outbreaks of A(H1N1) influenza in 2009 if these outbreaks had become a global pandemia? How about an eventual epidemic caused by the Marburg virus, less known than Ebola, but as dangerous and endemic in Africa? Philippe Kourilsky, a former director-general of the Pasteur Institute and emeritus professor at the Collège de France, Paris, and Peter Piot, co-discoverer of the Ebola virus and director of the London School of Hygiene and Tropical Medicine (2015), insisted that an appropriate and efficient policy to control new infectious pathogens is to anticipate their emergence and pre-develop drugs and vaccines that would be used when necessary.

## Control of dengue

### *Global spread*

This viral disease, transmitted by mosquitoes (*Aedes spp*.) and the Asian tiger mosquito (*Aedes albopictus*), is a global public health problem : the WHO estimates that there are between 50 million and 100 million persons who are infected by the virus worldwide, every year. The progression of the disease has been "spectacular" over the last decades, states the WHO which evaluates the total of patients at *ca*. 390 million across the world. The disease has spread rapidly through Latin America and South-East Asia, further to the expansion of international travel and tourism, as well as to the sprawling of urbanization. Before the 1970s only nine countries had been hit by severe dengue outbreaks; nowadays their number has risen to at least 100 (Hecketsweiler, 2014e). Sifferlin (2016) estimated that half of the world population is at risk for dengue, which can cause severe nausea, pain, rashes, bleeding and even death. For instance, in Puerto Rico, health authorities estimated that 80% of the population was infected.

Less dangerous than malaria, dengue has a low rate of mortality if the patients are well treated (less than 1% of the cases are lethal). In emerging countries the main risk is associated with the overburden laid on hospitals during the large outbreaks, the lack of medicines and blood (needed for transfusion in case of hemorrhagia). In Asian and Latin American countries dengue has become over the years "a major cause of hospitalization and mortality among children." For instance "Mexico is not the most affected country, but it has been associated with the spreading of dengue, since its inception, like the Philippines," stated Olivier Charmeil, the manager of the vaccine division of the pharmaceutical and research company Sanofi-Pasteur. In Brazil dengue has spread quickly: at least 693 persons died from the viral infection in 2014, most of them in the State of São Paulo, a record figure since 1990 when the cases of dengue had been recorded for the first time. In 2013, 674 deaths were registered and a total of 1.4 million or even 1.6 million Brazilians were infected by the virus in 2015 (Hecketsweiler, 2014e).

The global spread of dengue and its insect vectors is further illustrated by two events. The first one was the announcement, on 28 August 2014, by the Japanese health ministry that dengue cases had been detected in the heart of Tokyo, between the boroughs of Harajuku and Shibuya, in the well-known park of Yoyogi. Three young men were bitten by Asian tiger mosquitoes. This was a shock in the archipelago, when dengue had disappeared for 70 years. The second event occurred in France, where, on 22 August 2014, one case of dengue was detected in the Var department, south-east of France. And also the Americans are now learning to live with the virus since 2009, in Florida and Texas, where it had been eradicated half a century ago (Hecketsweiler, 2014e).

In France, in 2013, *ca*. 30 patients with dengue virus had been treated at the department of communicable and tropical diseases, La Pitié-Salpêtrière hospital, Paris, according to Eric Caumes, head of that department. He also added that the French Health Vigilance Institute had estimated the number of dengue cases at a little less than 200 across the country. These are called "imported" cases, because most of the patients had been infected in South-East Asia, Latin America and the Caribbean during their journeys. There was not a real public-health problem, until the virus vector arrived in the south of France: autochtonous cases are now being detected. The Asian tiger mosquito appeared for the first time in 2004 in the Alpes-Maritimes department, and since then it has been spreading northwards; it is now found in 17 departments and in such large cities as Bordeaux, Toulouse, Marseille and Lyon. Epidemiologists believe that it can be detected in Paris in 2016. It is not, however, the first incursion of the virus into temperate-climate countries (see p. 231). Formerly summer outbreaks were frequent and in 1927 there was a large-scale epidemic in Greece (Hecketsweiler, 2014e).

## *India*

Regarding Asia, a study of dengue by researchers at Brandeis University, Massachusetts, is part of a growing body of literature demonstrating that no country in the world suffers as many dengue infections as India. The first isolation of dengue in this country in Calcutta, now Kolkata, in 1945, and the first epidemics there were reported in the 1960s. Many researchers believe that dengue is now so endemic that nearly all Indians are infected at some point in their lives, often several times. Officially, the Indian government reports that an average of *ca*. 20,000 people are hospitalized annually with dengue infections. The Brandeis University study suggests that the real number of hospitalizations is closer to six million, and other studies suggested that the number of Indians infected annually is probably more than 30 million (Harris, 2014). The study methodology, which focused on one State in its clinical assessments and included broad assumptions about how patients seek care and are tested for the virus, probably underestimated the actual burden of the disease, explained Donald S. Shepard, a specialist in the economics of health, working at Brandeis University and a co-author of the study, which was financially supported by Sanofi-Pasteur. D.S. Shepard is also carrying out or supervising studies aimed at evaluating the cost: efficiency ratio of Sanofi-Pasteur treatments of the disease. Government officials have long acknowledged that official data vastly underestimated the burden of the illness in India, but explained that any change in the system would impede year-to-

year comparisons. That the annual dengue epidemic coincides with the beginning of India's busiest tourist season may also play a role in the government's decision-making (Harris, 2014; Hecketsweiler, 2014e).

Experts have long complained that India's underestimation of the disease's vast reach stops people from taking preventive measures, discourages efforts to clean up the sources of disease and slows research efforts for a vaccine. It is true that for *ca*. 80% of those infected dengue causes only mild symptoms of fatigue and a brief fever. The remaining 20% may be affected by more-serious flulike symptoms, with high fever, vomiting, searing pain behind the eyes, skin rash, and muscle and joint aches that can be so intense that the illness has been dubbed "breakbone fever". The acute part of the illness generally passes within two weeks, but symptoms of fatigue and depression can linger for months. In about 1% of cases dengue advances to a life-threatening cascade of immune responses known as hemorrhagic or shock dengue. This potentially lethal condition generally happens after a second dengue infection. There are four strains of the dengue virus, and infection with a second strain can fool the immune system, allowing the virus to replicate. When the body finally realizes its mistake, it floods the system with so many immune attackers that they are poisonous. Such patients must be provided intravenous fluids and round-the-clock care to preserve vital functions and avoid death. The mortality of dengue varies between 2% and 15%, depending on the violence of the outbreaks and the care provided to the patients. When the latter survive and are cured there is a life-long immunity against the virus strain that causes the infection, but not against the three others. A new contamination is therefore possible. But dengue has no after-effects, which is not the case with other tropical diseases, such as chickungunya – a viral disease which can be transmitted by the same mosquitoes as dengue, and particularly by *Aedes albopictus* (Harris, 2014; Hecketsweiler, 2014e). The major public health issue with respect to dengue is that there was not yet an effective means to prevent the disease and the physicians have to rely on symptomatic treatments.

## *Eradication of the dengue-virus insect vectors*

### Available techniques

The mosquito bites a healthy human, passing on the virus, then looks for others to bite. For each blood meal *Aedes aegypti* female mosquitoes bite four or five people. And they take three to four blood meals in their brief lives (about two weeks). They fly only a few blocks in a town during their lifespan. The idea to introduce in a population of mosquitoes some individuals that bear a gene that inhibits the development of the insect at the larval stage and spread gradually through the whole population of mosquitoes, is an old one. In the 1950s American entomologists, Raymond Bushland and Edward Knipling, had conceived the technique of sterile males. This consisted of drowning a population of insects using a huge number of sterile male individuals. As the females generally mate once during their lifespan the overall population plummets rapidly. Such a technique allowed in the three following decades the eradication in several African and American countries of the very harmful fly species *Cochliomyia hominivorax*, whose larvae develop in wounds of livestock animals (e.g. sheep and cattle) and cause severe infections (myasis) that can be lethal. The male sterility was obtained by irradiation of the insects and the sterile males are often unable to compete with their wild rivals in mating with females. Henceforth the need to produce huge numbers of sterile males so as to overwhelm the wild population of insects (Herzberg, 2016).

The biotechnology startup Oxitec, a spin-off of Oxford University, which was acquired in 2015 by the American biotechnology firm Intrexon, has proposed a similar technique, but derived from genetic engineering. The genetically modified male insects transmit to their offspring a gene that inhibits their development. Larvae die before their metamorphosis. The technique has been tried since 2010 on the main dengue-virus insect vector (*Aedes aegypti*) in Malaysia, the Cayman Islands and Brazil. It has many advantages, but also a serious defect: huge quantities of insects must be released, and therefore the overall cost is high. Andrea Crisanti, at Imperial College, London, and Tony Nolan of the same laboratory have been trying to find a method whereby a few dozens of genetically modified individuals could eradicate a whole population (Herzberg, 2016).

Their method is called gene drive which consists of spreading a trait in a population through the infringement of Mendelian laws of genetic transmission. During sexual reproduction, a trait on a chromosome of one of the two genitors, has only one chance out of two to be transmitted to the offspring. Gene drive consists of overcoming this threshold and transmitting the genetic trait to 100% of the offspring. A. Crisanti and T. Nolan have been working on how to reach this grail for 20 years. In 2003 their colleague Austin Burt demonstrated that using certain so-called "selfish" genes, one could modify the traits of a whole population. Tony Nolan was able to introduce a transmissible fluorescence gene into the genome of a mosquito; and thereafter, still with the help of fluorescence, to differentiate male and female insects. In 2011 the team of A. Crisanti succeeded in integrating into the genome of *Anopheles gambiae* – a vector of malaria in Africa – the very first element of gene drive. When they linked this element to the fluorescence trait they demonstrated that in 12 generations a whole box of mosquitoes was transformed. Their results were published in *Nature* (Windbichler et al., Burt and Crisanti, 2011; see also pp. 147).

When the new technique CRISPR-Cas9 was discovered and used in plant and animal cells (and even in human embryos; see p. 152), the genetic transformation of insect vectors of pathogens changed gears. In January 2015 Ethan Bier and Valentino Gantz of the University of California, San Diego, used the technique to transform germinal cells of fruit flies (*Drosophila*) and they informed A. Janes of University of California, Irvine, about their results. Eric Marois, a researcher at the French INSERM and the University of Strasbourg, who also works with the team of A. Crisanti and on the genetic transformation of *Anopheles*, stated: "The new technique has made an upheaval and we all tried to grab it." The team of Anthony A. James of the University of California, Irvine, was the first to publish at the end of November 2015 in the *Proceedings of the National Academy of Sciences* (*PNAS*) an article where they described how to integrate a gene for resistance to *Plasmodium falciparum* – a Protozoan causing the deadliest form of malaria – into the genome of *Anopheles stephensi* – the main vector of the disease in the Indian subcontinent (Gantz et al., and James, 2015). On 7 december 2015 A. Crisanti, T. Nolan and co-workers published an article in *Nature Biotechnology* : they targeted *Anopheles gambiae* and wanted to spread a sterility recessive gene. The trait is initially silently transmitted and thereafter it spreads at a huge speed. The insect vector is not just transformed, it is eradicated (Hammond et al., Crisanti and Nolan, 2015; Herzberg, 2016). The Bill and Melinda Gates Foundation had supported A. James' research and also invested US$40 million in A. Burt's work on mosquito eradication (Sifferlin, 2016).

Each research team – the American and British – defends its technique. In both cases there are possible negative impacts on the environment and on the development of resistance among the mosquito populations. In the United Kingdom the House of Lords published a report that supports the work of Imperial College researchers, while in the United States the National Academy of Sciences was requested by concerned citizens to state its viewpoint. The WHO and the European Commission are following the issue. In France the government requested an urgent study from the High Biotechnology Council which was expected to deliver its results by June 2016 (Herzberg, 2016).

Kevin Esvelt, assistant professor at the Massachusetts Institute of Technology and also a pioneer in gene-drive research, made the following statement about the newly available techniques of eradication or reduction of insect vectors of pathogens: "This bestows on us a formidable responsibility. We must assess all the risks and take all measures and precautions in order to minimize them." He developed a system that can reverse the dissemination of a genetic trait (gene drive). He published in *Science*, with several other scientists from the main international laboratories working in the area, a catalogue of recommendations concerning the use of gene drive, from the confinement of the laboratories to a set of rules. In his own view there is no question to reject in principle gene drive (Oye et al., and Esvelt and Church, 2014). He was aware of the possible negative effects of this genetic technique, but, while he fully supported more research on these effects and ways to mitigate them, he recalled that working on the eradication of mosquito populations is a very relevant goal: "Do we forget that malaria still kills 1,000 children a day, despite the progress made?" (Herzberg, 2016).

In a way K. Esvelt responded to the doubts of Florence Fouque, in charge of vector-borne diseases within the Tropical Diseases Research Programme (TDR), under the aegis of the World Health Organization. While the number of deaths caused by malaria has almost been halved between 2000 and 2015, due to the use of antimalaria drugs, of protective nets (with insecticide) and to better care provided to the patients, F. Fouque wondered whether it was really necessary to promote gene drive. "I am not sure that public-health authorities would like to rely on heavy techniques or methods, costly and potentially risky, while with the current means we hope to reduce the impact of malaria by 95% in 2035," she commented (Herzberg, 2016). Austin Burt did not set out to commit mosquito genocide. "Our target is malaria, not mosquitoes," he stated. "Mosquitoes are a means to an end." But once unleashed, Burt's mosquitoes have no kill switch; they will carry out their mission until there are no females left. To some experts it is a small sacrifice. But others worry about the implications of leaving a biological niche empty (Sifferlin, 2016).

"There is a potential that we are in trouble if all mosquitoes are gone," said Cameron Webb, a medical entomologist at the University of Sydney in Australia. Mosquitoes are an abundant snack for many kinds of birds, bats, fishes and frogs, and they may also play an important role as pollinators for some plants. Still, he said, the selective elimination of a species like the Zika-carrying *Aedes aegypti* is not likely to do much harm, especially since it is largely an urban-dwelling creature. "If you were to eradicate *A. aegypti*, the ecological consequences are probably going to be quite low, and I think that is a fair trade-off given the incredible reduction in mosquito-borne diseases," said Cameron Webb (Sifferlin, 2016 ).

Eliminating malaria-carrying mosquitoes, on the other hand, might prove more difficult. There are more than 24 species of *Anopheles* mosquitoes that carry the parasites and their elimination could have a larger ecological impact. How should that impact be weighed against the health benefits? "If you are confident that you can save 1,000 humans and it will decimate two species of bats and cause great harm to a particular flowering plant, do you go ahead?" asks bioethicist and Stanford law professor Henry Greely. "What if it saves 1 million lives" (Sifferlin, 2016 ). As much as Anthony James would love to see a mosquito-free planet, he doubts we will ever get that far. "I just do not think there are enough, enough will, to do this. There will always be small, isolated pockets of mosquitoes that will persist." We cannot afford to let up on the workaday methods that may not offer the promise of total extermination but can still save lives – including clearing mosquito habitats, spraying walls and using bed nets (Sifferlin, 2016 ).

Experimental trials in Brazil

On 19 January 2016 the biotechnology startup Oxitec announced that its pilot programme on the release of "friendly" mosquitoes in the city of Piracicaba, São Paulo State, Brazil, had resulted in an 82% decrease in the populations of *Aedes aegypti* within a few months. *A. aegypti* is the vector of dengue, but also of chikungunya and Zika virus (see p. 232). Oxitec called its genetically modified *Aedes aegypti* mosquitoes "friendly", because only males are released and these do not bite humans. Oxitec also claimed that its solution is environment friendly because only one species is targeted, whereas chemical spraying can affect many types of organisms (Pollack, 2016c). This pilot programme was expected to be carried out until the end of the rainy season, in April 2016. Oxitec decided to announce intermediary results because of the severe outbreaks of Zika virus and their possible relationship with thousands of cases of microcephaly in newborns (see p. 233). "It is already a success," stated Andrew McKemey, a biologist and director of field operations at Oxitec (Herzberg, 2016).

The technique used for these experimental trials in Brazil consists of using genetically modified mosquitoes to act on their wild relatives as insecticides. From April to December 2015 Oxitec and its Brazilian partner Moscamed have released, three times a week, huge populations of "friendly" mosquitoes on a borough of Piracicaba, Cecap-Eldorado. These mosquitoes have been produced in a factory nearby, in Campinas: *ca*. 26 millions of genetically modified male mosquitoes have been released. They were supposed to mate with wild females (only female mosquitoes need a blood meal before laying their eggs and they are those which bite humans and transmit pathogens). When doing so they transmit a gene that inhibits the development of the insect at the larval stage. There is a need to produce huge quantities of genetically modified male mosquitoes, in order to increase the rate of mating (because the wild females prefer to mate with wild males): experiments have shown that between 20 and 100 transgenic male insects could compete with only one wild mosquito. The Cecap-Eldorado borough of Piracicaba (*ca*. 5,000 people) was heavily infested with *A. aegypti* and at least 133 cases of dengue had been detected there during the summer of 2014-2015 (in the southern hemisphere). After the releases of "friendly" mosquitoes, only one case of dengue was recorded in 2016. "We wait until the end of

the rainy season and of the trial, before claiming victory, but we are convinced that the figure concerning the drastic reduction of *A. aegypti* population will even be better," asserted Andrew McKemey (Herzberg, 2016). In other words there was by the end of 2015 a reduction in wild mosquito larvae – as opposed to larvae inheriting the lethal gene – of 82% (Pollack, 2016c). Earlier trials conducted since 2011 in three sites of Bahia State led to spectacular results: there was a 92%-99% difference between the treated areas and the control ones (Herzberg, 2016).

The mayor of Piracicaba did not wait for the end of the rainy season and announced it would pursue the trials and extend the project for another year. Furthermore it intended to expand these trials to the centre of Piracicaba, i.e. in a sector with a human population about 10 times higher (*ca*. 60,000 people). To that end a new plant for the production of transgenic mosquitoes was to be built in order to breed enough mosquitoes to cover an area with 300,000 people. A Brazilian commission that oversees genetically engineered organisms (GMOs) declared the Oxitec mosquitoes safe to be released into the environment in 2014. But Oxitec still did not have a license from Brazil's health regulators that would allow it to actively market its technique to Brazilian cities. Still, stated Haydn Parry, the company's chief executive, with the outbreak of Zika, "we have had a huge amount more interest from different municipalities" (Pollack, 2016c). In fact there were requests for such trials from several parts of Brazil and also from Latin American countries, according to A. McKemey. India and Sri Lanka were also negotiating cooperation agreements with Oxitec, while Malaysia decided not to pursue such trials because of the reluctance of the populations concerned (Herzberg, 2016).

For a city the release of tens of thousands GM mosquitoes on a weekly or twice-weekly basis to ensure that enough modified males mate with females, cost US$7 a person a year, on average – doable for a middle-income nation like Brazil but out of reach for the extremely poor African nations where the bulk of deaths from mosquito-borne diseases occur. By contrast Austin Burt (Imperial College, London) estimated that he would need to release a one-off "bucket or two" of GM mosquitoes – about 400 – per small village to create an enduring effect.

Another approach, being tested in one Rio-de-Janeiro neighbourhood, is to infect mosquitoes with *Wolbachia*, a bacterium that does not infect them naturally. Once infected, the mosquitoes do not pick up and transmit viruses as easily. The bacteria can be passed to the next generation through eggs, so they spread through the mosquito population. "The beauty of it is it is a sustainable method – once you put it out it sustains itself in the environment and gives on-going protection," stated Scott O'Neill, dean of science at Monash University in Australia. He is the leader of Eliminate Dengue, a *Wolbachia* project supported by the Bill & Melinda Gates Foundation and others. Tests are currently underway in Indonesia and Vietnam to see if this approach can reduce the number of people suffering from dengue. In Brazil, Paulo Gadelha, president of the Oswaldo Cruz Foundation, a renowned biomedical institute under the Brazilian health ministry, stated initial results were good and there were plans to try it on a large scale, in Niteroi, a municipality across Guanabara Bay from Rio (Pollack, 2016c).

The real challenge lies in the efforts made to convince human populations to accept the release of transgenic mosquitoes on their settlements. In Brazil an important information work has been done upstream and has resulted in much less reluctance. By contrast, in Florida, a strong opposition to a proposed test by Oxitec in the Florida Keys, resulted in a petition signed by more than 160,000 hostile persons, while public-health authorities of this American State wanted to prevent the return of dengue outbreaks and fears about the Zika virus (Herzberg, 2016). Oxitec will have to wait for the result of a referendum to be held on 8 November 2016 in the areas where genetically engineered *Aedes aegypti* might be disseminated. But the US FDA approved the assays by early August 2016.

### *Development of an antidengue vaccine*

The French pharmaceutical group Sanofi has been trying to develop an antidengue vaccine for two decades. It spent €1.3 billion for developing such a vaccine and invested €300 million in the construction of a plant at Neuville-sur-Saône, in the Rhône-region (centre-east of France). The annual production capacity of this plant is 100 million doses of vaccine, that are sufficient for vaccinating 33 million people. Sanofi was foreseeing an annual profit of *ca*. €1.4 billion from the sales of an antidengue vaccine, out of the company's annual turnover of €32.9 billion in 2013, including €3.7 billion for vaccines. This was a big bet taken by Sanofi CEO, Christophe Viehbacher, in 2009 (Hecketsweiler, 2014e).

One former antidengue candidate vaccine had been abandoned after disappointing clinical trials carried out in Thailand. Another candidate vaccine was still in a pre-development stage by a British vaccine-development company, Acambris, that has been acquired by Sanofi in 2008. Through the construction of the plant near Lyon (at Neuville-sur-Saône), C. Viehbacher thought his company could outpace its rivals, GlaxoSmithKline – GSK – and Merck. In order to check and validate the efficacy of Acambris'candidate vaccine, clinical trials have been carried out since 2010 at an unusual speed, with cohorts of several thousand patients, children and grown-ups, recruited in Latin America and South-East Asia. Each patient receives three injections of the candidate vaccine, at six-month intervals, and they must be monitored for 24 months. On 11 September 2012 the results were published in *The Lancet*: it was shown that the vaccine provided a protection against three of the four strains of the dengue virus causing the disease – a world first. But it also showed that the last strain which is the most widespread escaped the immunization process (Halsted, 2012). Sanofi decided to broaden its trials and the results were also published in *The Lancet* on 10 July 2014, showing this time that the vaccine was efficient against the four virus strains: more than 75% against types 3 and 4 of the virus; more than 50% against type 1; and 35% against type 2. The clinical trial carried out in Asia resulted in reducing the incidence of dengue by 56%, while the number of hospitalizations caused by the disease was reduced by 75% (Wilder-Smith, 2014).

On 9 December 2015 Sanofi announced that Mexico's health authorities had approved the commercialization of Sanofi antidengue vaccine. "This is a historical day," said Olivier Charmeil, head of Sanofi Pasteur vaccine division. "Because of its impact on public health, Dengvaxia – Sanofi-Pasteur new vaccine – is an innovation that can be compared

with the antirabies or antipoliomyelitis vaccines," he added. On 22 December 2015 the use of the vaccine was authorized in the Philippines; and on 28 December 2015 in Brazil. That was also the case in El Salvador, Costa Rica, Paraguay, Peru, Indonesia and Guatemala. Sanofi filed requests for approval and commercialization for its vaccine in *ca*. 20 countries. "We hope to be able to sell a few dozen millions of vaccine doses in 2016, before reaching our cruising speed of commercialization in 2017," explained O. Charmeil. The vaccine efficacy is more important among children, 9 to 16 years old (two-thirds have been immunized), than among individuals that have been infected. But the vaccine is not efficient in younger children and the reasons for that phenomenon are still unknown. Clinical trial results have not therefore led to elucidating the causes of the relative inefficacy of the vaccine, although these clinical trials had been carried out on 40,000 volunteers (Hecketsweiler, 2015aa).

The price of the vaccine was not advertised by the end of 2015. There were still discussions with Mexico's health authorities with a view to agreeing on an affordable price, according to Guillaume Leroy, vice-president in charge of the Dengue Programme at Sanofi. Also being discussed was the immunization programme, i.e. the choice of the persons who will be vaccinated. G. Leroy stated: "We estimate that if we vaccinate 20% of the population, we could halve the number of dengue cases." According to the most optimistic evaluations the potential annual turnover of Dengvaxia could reach €1 billion, which would be the best sales figure in vaccine history. In 2014 vaccine sales brought in *ca*. €4 billion, i.e. 11% of Sanofi total revenue (Hecketsweiler, 2015aa).

### *Economic impact of vaccination*

In order to convince the countries to buy its vaccine Sanofi underlined the economic burden of dengue across the world. "At global scale, the annual economic impact of dengue is estimated at €8.2 billion or US$9 billion. If we add the expenses relating to mosquito control, this amount should be doubled," stated O. Charmeil. "In a country like Brazil – the first large market to be conquered – it was estimated that a major dengue outbreak cost between 0.5 and 1 point of GDP," indicated Guillaume Leroy. In India the study carried out by Brandeis University estimated that the direct and indirect costs of hospitalizations caused by dengue outbreaks exceeded US$1 billion a year (Harris, 2014; Hecketsweiler, 2014e).

One of the studies carried out or supervised by Donald S. Shepard at Brandeis University and concerning Mexico has been published in March 2015 and estimated at US$170 million the annual cost of dengue outbreaks in that country. "If one takes into account the long-term sequellae of the disease, the impact on tourism and the disorganization of the health-care system during the outbreaks, the overall economic burden will be heavier," explained the authors of the study. And consequently, "these results must help the health authorities to make the appropriate decisions regarding the new ways of disease prevention so as to reduce the impact of dengue," they added. Mexican authorities have reacted accordingly and approved the commercialization of Sanofi-Pasteur antidengue vaccine. "Even using a vaccine with a 30% efficacy, will be economically worthwhile for the countries endemically affected by the disease," suggested G. Leroy. He also added: "This is the first time we are developing a drug specifically for emerging countries" (Hecketsweiler, 2014e; 2015aa).

## Climate change and expanding ranges of arthropod-borne diseases

Tropical diseases – some of them never seen in the United States or Western Europe, for instance – are marching towards the northern hemisphere as climate change lets arthropods like mosquitoes and ticks, which carry pathogens, expand their ranges. But whether a few cases explode into a full-fledged outbreak depends on a set of factors far more complex than the weather, scientists say. The list of arthropod-borne diseases seems to be longer every year: Lyme's disease (see p. 274), Chagas' disease, West Nile, dengue, chikungunya – and now the Zika – virus outbreaks, which occurred in Puerto Rico by the end of 2015 for the first time. Some factors in this spread are, for now, unstoppable, scientists state: the weather is hotter; cheap airfares mean humans travel more than they did a decade ago; and cities in tropical countries are becoming more crowded, creating reservoirs for each disease. But there are means to stop outbreaks: insects can be killed; patients can be cured before they are bitten again; vaccines can be developed; and simple measures like screens, air-conditioning and repellent sprays can play important roles (McNeil Jr., 2015).

West Nile which appeared in the northern hemisphere in 1999 exemplifies a virus moving independently of climate change. The outbreak began in New York City, and the strain was 99% identical to an Israeli one, so many virologists believed it arrived in the blood of someone on an aircraft. The first cases were in Queens, home to J.F. Kennedy International Airport (JFK), so the virus might also have been hiding in a stowaway mosquito or, less likely, a bird. West Nile spread slowly west, halting each winter as mosquitoes die off and the birds they fed on flew south. It took until 2015 to reach the Pacific Northwest (McNeil Jr., 2015). West Nile virus can cause inflammation of the brain and spinal cord, and outbreaks have been occurring in the United States every summer. Severe reactions are rare but can lead to death (Sifferlin, 2016 ).

In the northern hemisphere chikungunya was first detected in late 2013 on the island of St. Martin, in the Caribbean. It started on the island's French-speaking half and jumped to other francophone islands and to French Guiana. The vector of chikungunya is *Aedes albopictus* (so-called Asian tiger mosquito) [McNeil Jr., 2015]. In the continental United States most of the cases were imported by travelers, although there was some local transmission as well. Debilitating joint pain caused by the infection can last for months (Sifferlin, 2016 ).

Mosquito-borne pathogens are not the only ones on the move. Ticks can carry more than 30 pathogens, and some diseases, including Rocky Mountains spotted fever, can be fatal. That infection, once banished from northern Mexico, is surging again and moving into Arizona for the first time, stated Gerardo Álvarez, an epidemiologist at the University of Sonora. Children, especially those who sleep beside dogs, are the hardest hit, and some Indian reservations in Arizona have held "rodeos" of the semiferal dogs that roam from house to house, putting tick collars on them (McNeil Jr., 2015).

Regarding malaria (see p. 266) it once was widespread in the south of the United States and ranged as far north as Boston in hot summers. In this age of global travels almost 2,000 Americans return from overseas with malaria each year. But outbreaks

do not occur because the victims are usually treated quickly, killing the parasites in their blood. Furthermore most American homes have screens and air-conditioning, so their inhabitants are bitten only a few times a year, not the hundreds that a poor child living in a windowless shack in Rio de Janeiro or Mexico City might (McNeil Jr., 2015).

## *Zika virus outbreaks: another international public-health emergency*

On 28 January 2016 the WHO announced that it will hold an urgent meeting on Monday 1 February 2016 in Geneva, with a view to dealing with the outbreaks of the Zika virus which is spreading in an "explosive way", Margaret Chan, WHO director-general, stated. She underlined that "the level of alert is extremely high." Mainly because the gradual evidence of a link between the infection with the Zika virus and symptoms of neurological disorders and even congenital malformations, including microcephalies, has changed the "profile" of Zika, initially considered as not a big threat. In 2016 the Zika virus, transmitted by mosquitoes belonging to the *Aedes* genus (*Aedes aegypti* and *Aedes albopictus*), was present in 65 countries and territories, inchuding 23 in the Americas and the Caribbean, and imported cases had been identified in many European countries, including France, where five travellers showing the symptoms of the viral infection had been registered since the beginning of 2016, but none of them was suffering from the severe forms of the infection, indicated the French health ministry (Morin, 2016a).

### Origin of the Zika virus

The virus has been isolated in 1947 in the blood of a Rhesus macaque living in a forest in Uganda. It was later on detected in a mosquito in 1948 and in humans for the first time in 1952 in Uganda. The first outbreak that has been documented occurred in 2007 in the Micronesian island of Yap, where 75% of the population had been infected, but only 20% had minor symptoms. The virus was found in French Polynesia between 2013 and 2014. About 32,000 medical consultations and 42 hospitalizations were made during that period. Guillain-Barré syndromes consisting of gradual alteration, and often reversible, of nervous fibres, were recorded. Such neurodisorders do need sometimes a heavy respiratory assistance and a long reeducation. This syndrome rate was 30 times more frequent than during a "normal" period (Morin, 2016a).

### Patterns of outbreaks in South America and the Caribbean

Until May 2015 Zika had never touched South America, except on Easter Island, 2,200 miles off the Chilean coast. Erin Staples, an epidemiologist for vector-borne diseases at the Centers for Disease Control and Prevention (CDCs, Atlanta, Georgia) explained: Zika arrived on Easter Island in 2014. Although the island is Chilean territory, its original population was Polynesian. Zika had been island-hopping in the South Pacific since 2007. Easter Island holds an annual festival that attracts Polynesians who may have brought Zika with them. The virus probably reached Brazil by a different route, via the influx of tourists during the 2014 World Cup, or paddlers from French Polynesia who participated in a canoe race in Rio de Janeiro (McNeil Jr., 2015). By early December 2015

an official from the CDCs predicted that Zika would follow the pattern in the United States that dengue had: many cases in Puerto Rico, followed by outbreaks in Florida, Gulf Coast States and maybe Hawaii. In 2016 it was estimated that *ca*. 10,000 people were carrying the Zika virus in the United States, Porto Rico and Virgin Islands; this figure included 1,000 pregnant women. "Information is pretty limited," E. Staples commented about the Brazil outbreak of Zika that may be causing thousands of babies to be born with smaller heads and brains (microcephaly). "There has not been enough testing for Zika, so it is not known how many women have been infected" (McNeil Jr., 2015).

The virus was circulating in 14 Latin American or Caribbean countries and Puerto Rico in 2016. Prominent virologists stated that Zika plays a role in microcephaly but it could not yet be established it was the only cause; it may depend, for instance, on whether the mother was previously infected with dengue virus. How far the infection can spread to the north will depend on whether the Asian tiger mosquito, *Aedes albopictus*, turns out to transmit it as efficiently as *Aedes aegypti* transmits yellow fever. The yellow fever mosquito is not usually seen much farther north than Washington and Kentucky States, but the cold-tolerant Asian tiger mosquito is sometimes found as far as New York and Chicago. Zika's arrival in the northern hemisphere only one year after chikungunya is "probably a coincidence," stated Scott C. Weaver, the director of the Institute for Human Infections and Immunity at the University of Texas Medical Branch in Galveston. Both outbreaks demonstrate that illnesses do not inevitably follow weather patterns. They often follow linguistic or ethnic ones (McNeil Jr., 2015).

Regarding the relationship between the virus outbreaks and malformations of the central nervous system in newborns, French Polynesia may be an illustrative example: between March and May 2014, 18 cases of this kind of malformation have been identified in newborns or fetuses, "much more than the usual record". There are still nevertheless many unknown features regarding the transmission and effects of the virus. Can it be also transmitted via the blood or sexually? For instance the virus has been found in the semen of a patient and there have been cases of viral transmission during deliveries of newborns. We do not know whether the virus strain that is now circulating has mutated recently in order to become more harmful. Until recently its rather low virulence did not put Zika as a research priority, as this was the case formerly with chikungunya. That is why much more research will help design the appropriate therapeutic treatments (Morin, 2016a).

In the absence of a specific treatment and of a vaccine emphasis is laid on prevention of the outbreaks: reduce the population of mosquitoes and avoid them through various ways. For instance two dengue outbreaks in Florida, in 2010 in Key West and in 2013 in Martin County, were stopped by aggressive antimosquito measures, including aerial spraying, larvicides in ponds and neighbourhood sweeps for containers that hold water (McNeil Jr., 2015). Similarly, in 2016, in the Dominican Republic, the army and the police were pooling their forces to combat mosquito vectors of Zika. A military spokesperson stated that all soldiers were given the task to clean all the areas where mosquitoes can reproduce, along with the fumigations carried out by the public health ministry's staff (Bedinelli, 2016).

Zika-virus epidemic in Brazil

In Brazil the Zika virus epidemic is becoming a major public-health problem: since October 2015, 3,893 cases of patients who were likely to be infected with the virus, had been detected, but government sources mentioned that there were between 497,000 and 1,482,000 cases of Zika-infected persons in the country. The major concern of the Brazilian government is that the epidemic, as well as those of dengue (1.6 million cases in 2015, a record figure) and chikungunya, will reach a peak between the months of February and May, during the rainy season when the insect vectors of these viral diseases – *Aedes aegypti* and *Aedes albopictus* – find the optimal conditions for their proliferation (Bedinelli, 2016). The first symptoms of the three viral diseases – dengue, chikungunya and Zika – are quite similar: fever, joint ailments and rash.

Brazil and Colombia are particularly hit, but the virus is expanding across the Americas. On Monday 25 January 2016 the World Health Organization announced that the Zika virus will affect the whole continent, except Canada and Chile. By early January 2016 Brazil's health ministry, Marcelo Castro, reiterated what he had been saying since October 2015, when the first cases of Zika-infected persons were identified, that "Brazil was losing the war against the mosquito" (the vector of the virus). But by the end of January 2016 the Brazilian government and the President herself – Dilma Roussef – announced that all appropriate means will be used to combat the disease and the mosquito vector in the first place. That is why 220,000 soldiers were instructed to eradicate the mosquito by destroying all its sites of reproduction – water holes, sewage, watery wastes and any recipient of water that becomes a brooding site for the mosquito. The soldiers were expected to start their work as of 13 February 2016 and to support health workers engaged in the struggle against the disease-mosquito, e.g. helping them to enter houses where the presence of the virus was suspected. The soldiers will also distribute repellent mosquito nets to *ca*. 400,000 pregnant women who were at a high risk of developing the disease (microcephaly), as well as information pamphlets (Bedinelli, 2016).

The Zika epidemic in Brazil occurred at a very bad period. February is the month of Carnival and a large number of tourists visit the country on this occasion, particularly in Rio de Janeiro. Furthermore Brazil is the host country of the Olympic Games during the summer of 2016. A large proportion of the visitors have never been in contact with the viruses transmitted by both species of mosquitoes, including the Zika virus. The rate of infection could therefore be high and the health problem could become a major crisis. The health ministry seemed to be overwhelmed and had to focus above all on preventive measures, such as the destruction of the insect vector, because there is no cure. The health minister was criticized because of his controversial statements and his lack of experience in handling a huge health-care system. Marcelo Castro, a physician and health minister since October 2015, was chosen by President Dilma Roussef further to a political deal with the centre-right party (PMDB) which is part of the government coalition. In November 2015 when the first cases of Zika-infected persons were made public, the minister wanted to support a recommendation meant to reduce pregnancies whenever possible, and said: "Sex is for those who like it and pregnancy is for the professionals." Afterwards he pointed out that an antizika vaccine

could be developed, but it would not be accessible to all. And finally he added: "Hopefully, women may escape the Zika virus before their fecundity period. Thus, they will not need a vaccine" (Bedinelli, 2016). According to WHO, up to 21 July 2016, 1,700 microcephalies and congenital malformations of newborns'central nervous system, linked to Zika, were recorded in Brazil.

The Zika-virus epidemic was hitting Brazil at the worst moment of its economic and social situation. In 2016, according to the International Monetary Fund estimates, the economic crisis was expected to reduce by 3.6% the country's gross domestic product (GDP), due to in particular to a marked decrease in China's large imports of commodities. Inflation that has been mastered until 2014 and which is the Aquilles' heel of the Brazilian economy rose more than 10% in 2015, and made Brazilians fear a return to the hyperinflation years. The political situation was deeply suffering from corruption scandals, e.g. that of the national oil company Petrobras, which has tainted many politicians and some of the most important businessmen of the country. The credibility of Brazil among investors and global entrepreneurs was plummeting.

The president of Brazil, Dilma Roussef, has been enduring the process of impeachment since December 2015, amid a shrinking economy and spreading disillusionment. On Monday 18 April 2016 the Chamber of Deputies – the lower house of Brazil's Congress – voted overwhelmingly to approve the president's impeachment: 367 for, 137 against and 7 abstaining. The Chamber of Deputies sent the impeachment proceeding to the Senate. Its 81 members will vote by a single majority on whether to hold a trial on charges that Dilma Roussef, who was elected in 2010 to replace President Luis Inácio Lula da Silva and was reelected four years later, illegally used funds from state-owned banks to conceal a yawning budget deficit in an effort to bolster her reelection prospects. On 12 May 2016 the Senate suspended Dilma Roussef for six months and the former vice-president became Brazil's acting president. On Wednesday 31 August 2016 the Senate voted 61 to 20 to remove D. Roussef from office for the rest of her term (end of 2018).

Global transmission of Zika and international action

As mentioned above, WHO director-general, Margaret Chan, expressed her "profound" concern about the Zika epidemic. There were four reasons for that: the likely relationship between the viral infection and congenital malformations as well as neurological disorders; the likelihood of a global or international transmission of the virus because of the widening geographic distribution of the vector mosquitoes; the lack of immune protection in the population in the newly affected regions; and finally the absence of a specific treatment or vaccine, and fast diagnostic tests. The occurrence in 2016 of a strong El Niño climatic phenomenon could cause a proliferation of mosquito populations and make the overall situation even worse (Morin, 2016a).

On Monday 1 February 2016 the World Health Organization (WHO) declared the Zika virus outbreaks an international public-health emergency, a rare move prompted by the growing concern that the virus could cause birth defects. At a news conference in Geneva Margaret Chan stated that clusters of microcephaly in regions with Zika

cases "constitute an extraordinary event and a public health threat to other parts of the world." She added that "international response is needed to minimize the threat in infected countries and reduce the risk of international spread." M. Chan said case control studies on the connection between Zika and microcephaly would start in the next two weeks (Tavernise, 2016).

The WHO has declared a public-health emergency three times since 2007, when it first established the procedure – for the influenza pandemic in 2009; in 2014 when poliomyelitis seemed resurgent; and in August 2014 for Ebola. Some experts applauded the decision to add Zika to the list. For instance, in *The Journal of the American Medical Association* (*JAMA*) three American public-health specialists of University of North Carolina School of Medecine, Chapel Hill, NC, requested WHO to act swiftly; they explained that the measures to be taken were clear enough and they emphasized the high risks of transmission of Zika during the Olympic Games in Rio de Janeiro (summer 2016) [Lazear et al., 2016]. "This should be a global wake-up call," hammered Lawrence O. Gostin, director of the O'Neill Institute for National and Global Health Law at Georgetown University. But he added: "The main question on my mind is whether they will back up their worlds with decisive action" (Morin, 2016a; Tavernise, 2016).

In fact, now that an emergency has been declared, WHO can issue global travel advisories, and, crucially, coordinate global efforts to combat the mosquitoes that spread Zika, a role that is badly needed because mosquito populations are fluid and know no boundaries. The United Nations agency will also help coordinate surveillance efforts, including tracking and tallying cases of Zika and microcephaly. M. Chan in her news conference in Geneva on 1 February 2016 emphasized the urgent need to act swiftly: "Can you imagine if we do not do all this work now and wait until all the scientific evidence to come out, people will say, "Why did not you take action?" This meant that the official "emergency" designation of Zika can be the trigger for action and funding from governments and non-profit institutions around the world. It elevates WHO to the position of global coordinator and gives its decisions the form of international law. The agency is trying to cast itself as a global leader to revive its reputation after a faltering response during the Ebola hemorrhagic fever epidemic in West Africa (Tavernise, 2016).

One should point out that Zika and Ebola are very different. Ebola virus was incredibly deadly and it spread through contact with bodily fluids. Zika is not known to be fatal and it has mild symptoms for most people. Recent evidence is that the virus can cause brain disorders and induce microcephalies. Furthermore by early February 2016 a case of most likely transmission of Zika via sexual intercourse was mentioned. Zika had been found earlier in semen. This should lead to drastic preventive measures.

The August 2016 Summer Olympics and Paralympics took place in Rio de Janeiro, Brazil, which had over 1,700 cases of microcephaly. Nearly 800,000 international visitors, including 15,000 athletes from over 200 countries, attended Rio2016. Olympic organizers recommended that athletes do what they can to avoid mosquito bites. "In the case of Zika we need to inspect the venues every single day, especially for stagnant water," says Mario Andrada, spokesman for the Rio2016 committee. South Korea, for its part, announced that its athletes will wear uniforms with mosquito-repellent chemicals (Sifferlin, 2016 ).

More than 400 Americans have contracted the virus while overseas, but with an estimated 40 million Americans travelling to Zika-affected countries each year – 500,000 of them

likely pregnant women – Zika will almost certainly spread locally within the mainland United States during the summer of 2016. Puerto Rico has already reported more than 700 confirmed cases, including 89 pregnant women, and one person has died from the disease. It was estimated that an astonishing 20% of Puerto Rico's population will eventually contract Zika. It does not help that Puerto Rico is struggling with a debt crisis even as it tries to tackle Zika. The White House asked in February 2016 for US$1.9 billion in emergency funding to battle Zika. On 27 August 2016 President B. Obama urged the Congress to take measures aimed at controlling the virus, when it resumes its session on 6 September 2016 (Sifferlin, 2016 ).

People living in southern States and Hawaii – where the climate, geography and the presence of *A. aegypti* mosquitoes make eventual local transmission likely – are not currently at high risk of getting the virus in their home States, according to the Centres for Disease Control and Prevention (CDCs). However, by the end of July 2016, four cases of Zika infection were recorded in Miami-Dade county, just north of downtown Miami, one of the biggest ports of entry into the United States from countries where the Zika virus is in circulation. These cases were the first documented instance of local transmission in the continental United States. Another 13 similar cases were recorded on 9 August 2016 in the trendy and touristic borough of Wynwood, while a first newborn with microcephaly just died in Texas.

"If their partner has been traveling to an area of Zika transmission, there is a risk of sexual transmission," stated Margaret Honein, chief of the CDCs Birth Defects branch. Over time that risk may be enough to encourage American women of childbearing age to consider using birth control. Access to effective birth control is of particular concern in Puerto Rico where about two-thirds of all pregnancies are unplanned. "Abortion is a legal medical procedure in the United States, and in the context of Zika, couples need to make complex, highly personal decisions about pregnancies," stated Denise Jamieson, chief of the CDCs Women's Health and Fertility Branch (Sifferlin, 2016 ).

An exception is pregnant women who have traveled to one of the 65 countries where Zika has spread – all of them should be tested according to CDCs. For each test a doctor will send a sample to a state or federal laboratory. There is also a test that looks for antibodies in blood that show whether a person's immune system has ever fought the virus, but it is imperfect; it can mistake Zika for similar viruses like dengue and chikungunya. The CDCs have activated their Emergency Operation Center (EOC) to a Level 1 response for Zika, something that happened only three other times: during Hurricane Katrina, the A (H1N1) flue outbreak and the Ebola crisis. In the EOC in Atlanta scientists monitor cases of the virus, work on better diagnosis and run studies of pregnant women with Zika. The agency has sent better testing tools to state laboratories and recently concluded that the link between Zika virus and microcephaly was definite (Sifferlin, 2016 ).

New York City targets a type of mosquito that spreads West Nile virus but not the kind that spreads Zika. Though the city does not have *A. aegypti* mosquitoes right now, it does have *A. albopictus* which may also be able to spread the disease. The city is investing US$21 million over three years to modify its mosquito control and test travelers for Zika. If you are in an area with disease-spreading mosquitoes, wear long-sleeved skirts and pants no matter how warm they are – mosquitoes are more infectious when the temperature rises. Then, according to CDCs, use insect repellents that contain one of the following ingredients: DEET (20% to 30% concentration is best), picardin, oil-of-lemon eucalyptus, paramenthal-diol or IR3535. You should also make

sure windows have screens and the air conditioner is on if you are home during the day; the mosquitoes that spread Zika virus are day biters. And since a mosquito needs only a tiny bottle-cap-size pool of water to lay as many as 200 eggs, you should remove any standing water around your home and clean any vessels you find. Because even after the water source has dried out, the eggs can remain dormant and survive for months, sometimes even up to a year, on the inside of a container (Sifferlin, 2016). Aerial sprays of a strong insecticide have been carried out at the beginning of August 2016 on some areas of southern Florida. Prohibited in Europe, this product is highly toxical. But the health authorities in Florida stated that at small dose the insecticide was not dangerous for humans.

On 25 July 2016 in a Barcelona (Spain) hospital a woman, who was infected by Zika during a journey to Latin America, delivered a newborn with microcephaly. At the same date, five British and American researchers have estimated that 1.65 million pregnant women could be infected with the virus during the first wave of the outbreak; and that a total of 93.4 million people would be infected worldwide, including 37.4 million people in Brazil. France, while offering its assistance and experience to an international action under the WHO, has taken precautionary measures. For instance the health minister, Marisol Touraine, has "strongly" recommended to pregnant women to postpone their eventual journeys to the French Antilles and Guiana. It is almost impossible to prevent the arrival in France of asymptomatic carriers of Zika virus. On the other hand the Asian tiger mosquito may transmit the virus in the southern regions of the country as of May 2016. Since then small outbreaks may occur there, as this was the case with dengue and chikungunya. An epidemic is not expected to occur because the density of mosquito populations is much lower than in the tropics. But overseas, in the French Caribbean, the likelihood may be higher. The Pasteur Institute in Paris, a renowned international virus research centre, is working on Zika in order to initiate the development of a vaccine against the circulating strain of the virus (Morin, 2016a).

Development of an antizika vaccine

The zika-virus epidemic has led a few research-and-development institutions, such as the United States National Institutes of Health (NIH), and pharmaceutical and biotechnology companies to start developing a vaccine against this virus. Knowing the structure of the virus can help develop such vaccine. Researchers of the Markey Center for Structural Biology and Purdue Institute for Inflammation, Immunology and Infectious Diseases, Purdue University (West Lafayette, Indiana) and of the Viral Pathogenesis Section, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, (NIH, Bethesda, Maryland) have published in the 22 April 2016 issue of *Science* the 3.8 Å resolution cryo-EM structure of the Zika virus. It is a 20-facet polyhedron, made of 180 copies of two proteins that encase an RNA genome surrounded by a lipidic membrane (Sirohi et al., 2016). The same research team was the first one to determine the structure of the dengue virus, another flavivirus, in 2002. The American researchers hope that the detailed description of the virus envelope (capsid), from which protrude small "buds" or spikes of saccharide molecules, may help find how to neutralize the virus.

The French pharmaceutical group Sanofi and its vaccine division, Sanofi-Pasteur, announced that it was setting up a "task force" of *ca*. 80 scientists with a view to fostering research on the virus and its control through an efficient vaccine. "We have both the expertise and the production capacity to implement this task rapidly," stated Nicholas Jackson, the research director at Sanofi-Pasteur. This division has been

working for 20 years to develop a vaccine against dengue whose virus shares 60% of its genome with that of Zika and is transmitted by the same genus of mosquitoes, *Aedes*. The antidengue vaccine, Dengvaxia, was commercialized in 2016 in several countries, such as Brazil, Mexico, Salvador and the Philippines. Both viruses cause similar symptoms that recall those of a mild flu: moderate fever, headaches, muscle and joint pain. These symptoms generally disappear after a period of two to seven days, according to the WHO. As mentioned in p. 232, the Zika-virus infection can cause in a small number of people neurological disorders such as the Guillain-Barré syndrome – an illness of the nervous system that includes temporary paralyses, also found among a very small number of persons vaccinated against influenza. The zika-virus epidemic has been correlated in Brazil and the French Antilles with an increasing number of cases of microcephaly, where the skull perimeter is lower than the norm (Hecketsweiler, 2016c).

American researchers from various biomedical research institutions (Solomon Snyder Department of Neurosciences of Johns Hopkins University School of Medicine, Baltimore; Department of Biological Science, Florida State University, Tallahassee; Department of Biostatistics and Bioinformatics, Rollins School of Public Health, Emory University, Atlanta; Department of Biochemistry and Molecular Biology, Sealy Center for Structural Biology and Molecular Biophysics, University of Texas Medical Branch, Galveston; among others) have developed a miniaturized spinning bioreactor to generate forebrain-specific organoids from human iPluripotent Stem Cells (iPSCs), which are three-dimensional cultures that model organogenesis. These organoids recapitulate key features of human cortical development, including progenitor zone organization, neurogenesis, gene expression and, notably, a distinct human specific outer radial glia cell layer. They employed the forebrain organoid platform to model Zika virus exposure. Quantitative analyses revealed preferential, productive infection of neural progenitors with either African or Asian Zika virus strains. Zika-virus infection leads to an increased cell death and reduced proliferation, resulting in decreased neuronal cell-layer volume resembling microcephaly. The infection and its implications are more severe when the viral infection takes place early during the development of the fetus. At a stage corresponding to the second three-month period of pregnancy, the virus infection results in slowing down brain growth and the brain cortex becomes thinner. From the technical viewpoint the brain-region-specific organoids and the miniaturized spinning bioreactor, together, provide an accessible and versatile platform for modeling human brain development and disease and for compound testing, including potential antizika drugs (Qian et al., 2016).

On the other hand, Nicholas Jackson could rightly state that Sanofi-Pasteur had accumulated a great amount of knowledge on the flavivirus family to which belong the dengue, zika and yellow fever viruses. He thinks that it would be possible to adapt the antidengue vaccine (Dengvaxia) to a candidate vaccine against zika. This approach has been successfully followed by Sanofi-Pasteur to develop a vaccine against Japanese encephalitis, of which 1.7 million doses had been injected since 2013 – the year of its commercialization. Regarding another vaccine against yellow fever, more than 415 million doses had been injected since its approval in 1986. Sanofi-Pasteur's wide-ranging experience in developing vaccines against flaviviruses puts it in a good position towards the development of an antizika vaccine. Annual sales of Sanofi-Pasteur amounted to €4.7 billion in 2015, a 7% increase compared with 2014, and the prospects are good; due in particular to the sales in emerging countries: they rose 12% in 2015 and they make up almost one-third of Sanofi-Pasteur's annual turnover (Hecketsweiler, 2016c).

But the French group was not alone in the race towards developing an antizika vaccine. The Indian group Bharat Biotech announced in February 2016 that it would soon start its preclinical trials in animals. In the United States vaccine development is under way at the NIH: scientists are tweaking a vaccine that was initially developed for the West Nile virus and they expected to launch a safety trial for it in September 2016. "The need for a drug is less compelling than the need for a vaccine," stated Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases. "Since Zika is an infection that in most people is usually gone within a few days, it may be tough to have a major impact with a drug as opposed to prevention, with a vaccine," he added (Sifferlin, 2016). On 8 February 2016 President Barack Obama promised to release US$1.8 billion (or €1.64 billion) for the research on, and prevention of, zika. The European Medicines Agency (EMEA) decided to set up a group of experts in order to foster research on the virus. "We shall very much rely on the collaboration with the United States and Europe, because clinical trials on zika can be very costly," involving several thousands of patients, explained Elias Zerhouni, the president of research-and-development activities at Sanofi, during the presentation of the group's annual results on 9 February 2016. Sanofi-Pasteur is already allocating "several million euros" to a preclinical-research phase on the virus (Hecketsweiler, 2016c).

## Acquired Immunodeficiency Syndrome (AIDS) / Human Immunodeficiency Virus (HIV)

### *How AIDS first spread: the origins of the pandemia*

While some persist in revising the history of AIDS and claiming that the cause of the syndrome is not necessarily the HIV, an international team of researchers published its results on the origin of the pandemia – in space and time : the paper published in the 3 October 2014 issue of *Science* by a team led by Oliver G. Pybus of Oxford University and Philippe Lemey of the University of Leuven, Belgium, suggested that railways – one of humankind's most important inventions – played a crucial role in the early dissemination of HIV (Faria et al., Pybus and Lemey, 2014; *The Economist*, 2014d).

Most cases of AIDS are caused by HIV-1, originally a chimpanzee virus. HIV-1, however, exists in several types, a lot of which are rare, and only one of which, group M, has become pandemic. The researchers wanted to understand why HIV-1 evolves fast. One useful implication of this situation is that family trees can be drawn up which show not only what derives from what, but also (because the rate of genetic change is reasonably constant) when the various branches diverged. The other useful implication is that the virus genotype varies from place to place, depending on when it first arrived somewhere. This means it is possible to track its spread in some detail – which the international research team has done. They confirmed two suspicions. One is that the common ancestor of group M dates back to the 1920s. The other is that it originated in Kinshasa (then called Léopoldville) in what was the Belgian Congo and is now the Democratic Republic of Congo (DRC). The analysis ruled out other mooted points of origin, such as Brazzaville and Pointe Noire, in the Republic of Congo. In order to arrive to these conclusions, the team,

comprising European and North American researchers, analyzed the genetic sequences of several hundreds of samples of HIV-1, collected in the former Belgian Congo and its neighbouring countries during the 20th century and stored at the National Laboratory of Los Alamos, New Mexico (Faria et al., Pybus and Lemey, 2014; *The Economist*, 2014d).

The M group's ancestor seems to have come from southeast Cameroon, whose chimpanzees have the simian virus most similar to it. Some time before the first world war someone there (probably a hunter) was infected, most likely through close contact with chimpanzee blood. It then travelled south down the Sangha river, which was used to trade rubber and ivory between Congo and Cameroon. Martine Peeters, a virologist at the French Research for Development Institute (IRD, French acronym) multidisciplinary unit UMi233, in Montpellier (south-east of France), and a co-author of the *Science* paper, stated: "We have assembled the pieces of the puzzle in order to understand where and when the virus has been transmitted from its animal reservoir to humans. This transfer from apes to humans has occurred several times without giving rise to an outbreak, the virus remaining in the forest. But at one stage, the virus found itself at the right place and time, and that initiated the epidemic" (Faria et al., Pybus and Lemey, 2014; *The Economist*, 2014d).

Arriving in Kinshasa was group M's big break. Before Congo's independence in 1960 the city was at the centre of an extensive trade network. A mixture of river traffic (particularly along the Congo and Kasai) and railways spread it all the way to Kisangani (north-east) and Lubumbashi (south-east). In 1937 the ancestor of pandemic HIV-1 is being found in Brazzaville, the capital of the former French colony of Congo, that is located 6 km from Kinshasa, on the other bank of the Congo river. In 1939, after Lubumbashi in 1937, the virus is found at Mbuji-Mayi, along the railway and located up north-east from Lubumbashi. Since 1922 this railway has been transporting more than 300,000 passengers per year; running through the country, from west to south-east, its transport capacity increased to more than 1 million passengers in 1948. During the following decade, it was via the Congo river that the virus reached Bwamanda (1950s) and Kisangani (1950s), in the north-east of the territory (Benkimoun, 2014c; *The Economist*, 2014d).

Human activities, migrant workers, prostitution and use of non-sterile tools in injections of drugs against sexually communicable diseases (e.g. syringes and needles re-used for several persons) amplify the emerging epidemic. The amount of infection does not seem to increase faster than the human population until about 1960. Then group M took off – first in Africa (although it remained undetected by medical science) – and afterwards in the rest of the world. That happened in around 1964 when Haitian professionals who had come to Congo after independence took it at home, and it spread thence to the United States in 1981 (Benkimoun, 2014c; *The Economist*, 2014d).

What this geographical analysis does not address is why the rate of infection rose simultaneously in several places around the time of Congo's independence. In this group M differs from the next most-widespread type of HIV-1, group O. That also spread over the course of the 20th century and at a similar rate to group M until its 1960s growth spurt – but it did so in western Africa rather than central Africa. Two

hypotheses have been advanced to explain the discrepancy. One is that group M threw up a mutation which somehow changes its relationship with humankind, to its advantage. The other is that something people started doing back then gave group M a particular leg-up. The international research team believes it was the latter. They find it implausible that the necessary mutation could have happened simultaneously across group M range – which their data suggest would have to have been the case. Instead they suspect the change was human. One factor may have been the chaos that accompanied independence which encouraged impoverished women to turn to prostitution. The railways, though, did not seem to play a role in this part of the story. In the wake of independence the network broke down. Group M, by contrast, went on from strength to strength (*The Economist*, 2014c). Once adapted to its human host the virus can replicate in the latter, then it was transmitted from human to human, until a pandemia occurred. The first paper on AIDS cases was published in 1981 and the identification of HIV-1 was made in 1983. The HIV had infected 78 million people and killed 39 million of them (Benkimoun, 2014c).

## *Struggle against AIDS*

A few days before the opening of the 20th International AIDS Conference in Melbourne, Australia (20-25 July 2014), the United Nations AIDS published on 16 July 2014 its report on the status of the pandemia. In charge of coordinating the United Nations action against the spread of the viral infection, the UNAIDS underlined in its report the good results obtained since 2000 (13th Conference, Durban, South Africa), thanks to a high increase in the access to antiviral treatments. Thus the number of deaths worldwide in 2013 decreased by 11.8% in one year; this was the highest decrease recorded since the peak of 2005. The global number of new infections also slightly diminished, from 2.2 millions in 2012 to 2.1 millions in 2013 and *ca*. 2 millions in 2015-2016; it reached 2.9 millions in 2005. The reality is still somber: every day 5,700 persons are infected by the virus; in 2013 1.5 million people died because of AIDS; 35 million people were living with the virus, compared with 34.6 million one year earlier. This evolution is explained by the decrease in deaths, while infections are less numerous. Tuberculosis remains the main cause of death among people infected with HIV (Benkimoun, 2014a).

Africa remains the continent most affected by the disease: 24.7 million persons, including 58% women, are infected in sub-Saharan Africa. Almost 70% of the new infections are found there and 1.2 million deaths due to AIDS occur in Africa. This extraordinary proportion is partly explained by a still very insufficient access to antiretroviral drugs and treatments. In sub-Saharan Africa only 37% of people infected with HIV are treated: "In 2013, according to UNAIDS, 67% of men and 57% of women could not have access to these treatments." At global level there was a political will to make the antiretroviral treatments more accessible: in 2013 *ca*. 12.9 million persons infected with HIV had access to these drugs; this figure was close to the target of 15 million persons to be treated in 2015 thanks to UNAID's initiative "15x15" (Benkimoun, 2014a).

The two African countries most affected by the pandemia are South Africa and Nigeria, where live 18% and 9%, respectively, of the persons infected with the HIV, worldwide. In Nigeria the proportion of people needing antiretroviral treatments was estimated at 80%. During the presidence of Thabo Mbeki (1999-2008) more than 3 million seropositive South Africans had been deprived of antiretroviral drugs because of the

political will of the president and his government, who considered that HIV was not the real cause of the disease. Since then, with his successor Jacob Zuma, South Africans have represented one-third of the patients who started to use antiretroviral drugs during the period 2010-2013. And between 2005 and 2013 the number of deaths due to AIDS diminished by 39% in sub-Saharan Africa (Benkimoun, 2014a).

With 4.8 million people infected with HIV Asia and the Pacific is the second-most affected region of the world. However the number of newly infected people has decreased there, like in sub-Saharan Africa, Latin America and the Caribbean. By contrast the number of new infections rose in western and central Europe (+8%), in eastern Europe and central Asia (+5%). This was also the case in North Africa and the Middle East (+7%) [Benkimoun, 2014a].

The UNAIDS report recalls that since the beginning of the pandemia in the 1980s 78 million persons had been infected with HIV and 39 million were killed by the virus. These figures underline the magnitude of this catastrophe and stress the need to pursue the efforts aimed at decreasing the incidence of AIDS. In fact the UNAIDS set the objective to end the pandemia by 2030. That means a 90% reduction in the number of new infections (less than 500,000 per year) and also a 90% decrease in the discriminations linked to gender, sexual orientation, as well as against vulnerable groups of people such as prisoners, drug users and prostitutes. This is a huge task: among the 35 million persons infected with HIV the UNAIDS estimates that more than half (19 million) ignore they are seropositive, because they are discriminated or live at the margins of societies. Therefore UNAIDS wants that these persons who ignore they are ill be treated between 2015 and 2030 (Benkimoun, 2014a,b). The United Nations objectives imply political commitments and important funding pledges. In 2013 US$19.1 billion (or €14.1 billion) were available (supplied from all sources), according to UNAIDS. Annual funding needs were estimated at US$24 billion in 2014 and 2015, bearing in mind that in 2013 domestic investments from low or intermediary-income countries represented almost half of total expenses associated with the struggle against AIDS/HIV (Benkimoun, 2014a). UNAIDS and the Kaiser Family Foundation in their July 2016 joint report requested more funds from the big donors in order to reach the objectives set up for 2030.

### *Antiretroviral drugs*

When medical prescriptions including a cocktail of antiretroviral drugs are strictly followed, AIDS can be controlled and the life expectancy of patients is nowadays similar to that of healthy persons. These drugs have to be taken during the whole life. The first of these drugs, zidovudine or AZT, had been commercialized in 1987 by the British pharmaceutical group GlaxoSmithKline (GSK). Since then what was a niche market has become a cash machine for the company, which has broadened its portfolio of drugs. In 2013 antiretroviral drugs sales amounted to €1.8 billion, i.e. a little more than 7% of the annual turnover of GSK pharmaceutical division. In addition the profit margin was high: 64% compared with an average 37% for its other drugs. The last compound commercialized by GSK in 2014 in Europe, Tivicay, cost more than €7,000 per year in France and more than US$14,000 per year in the United States. Its sales should be over US$1 billion in 2017 according to Evaluate Pharma (Hecketsweiler, 2014i).

This should be a good prospect for investors, while GSK announced in October 2014 that it intended to introduce into the stock exchange a company called ViiV Healthcare, that has been in charge since 2009 of the group's antiretroviral drugs. This company whose value was estimated at *ca*. £15 billion should immediately become one of the 40 biggest firms listed on the London stock exchange, ahead of Marks & Spencer or the chain of supermarkets Sainsbury's. GSK was relying on this transaction (it will share only a small part of the company's equity) to increase its profits and meet the expectations of its shareholders. Like other big pharmas GSK is confronted with the expiration of patents protecting several blockbuster drugs and with the competition from low-cost copies of these medicines; the group must therefore find new sources of profits. Antiretroviral drugs could offer such an opportunity and would enable GSK CEO, Andrew Witty, who has been leading GSK since 2008, to carry on, while the group's share prize had decreased by 25% between May 2013 and October 2014 (Hecketsweiler, 2014i).

With 22% of the market share of antiretroviral drugs the American biotechnology company Gilead Sciences largely outpaces ViiV (13% of the market). Gilead was worth US$150 billion (or €120.6 billion) at the New York stock exchange, i.e. more than the whole GSK group (US$113 billion). Its sales were rocketing: from US$8 billion in 2011 to US$11 billion in 2013. Its blockbuster drug is Truvada, the annual sales of which amounted to more than US$3 billion in 2014. It cost between US$8,000 and US$14,000 per year per patient in the United States, and more than €6,000 in France. In addition to Gilead and ViiV Healthcare there are other firms present on the antiretroviral-drug market; for instance Johnson & Johnson, the sales of which are close to those of ViiV; Merck and AbbVie, from the United States, and the British Bristol-Myers Squibb (Hecketsweiler, 2014i).

Despite its obvious lucrative characteristics the antiretroviral-drug market is also fraught with difficulties. For instance, in 2012, ViiV sales decreased due to the expiration of patents protecting several molecules. Furthermore it is becoming more difficult to launch new therapies: the current drugs are very efficient, with lesser secondary effects and less constraining posologies (an increasing number of molecules are combined in a single pill). The only major change that may occur would be the discovery of a drug that could *cure* the patients infected with HIV. That explains why the pharmaceutical companies concerned turn to other areas of research and development: e.g. Gilead Sciences, Johnson & Johnson which developed and commercialized antihepatitis-C drugs, Sovaldi and Olysio, respectively; also AbbVie, which was authorized by the European Medicines Agency (EMEA) to commercialize two new drugs against the hepatitis-C virus. However ViiV has kept away from this market (Hecketsweiler, 2014i).

Another issue is that of the commercialization of antiretroviral drugs in poor countries, where most of the deaths due to AIDS/HIV occur (1.2 million in the African continent, i.e. 75% of the world's total number). Being aware of this challenge, in terms of both public health and image, ViiV granted 14 licenses to generic-drug manufacturers without right to royalties. In the other developing countries, ViiV set up "differentiated prices," based on the country's GDP (gross domestic product) and on the disease's incidence. "Our objective is to be able to improve our annual turnover as well as the

company's profitability, but at the same time to enable an increasing number of patients to benefit from our efforts," stated Dominique Limet, ViiV CEO (Hecketsweiler, 2014i).

It is worth mentioning in this respect that on 1 December 2014, the World Day of the Struggle Against AIDS, Medicines Patent Pool (MPP) and AbbVie announced the conclusion of a license agreement on Lopinavir and Ritonavir, two antiretroviral drugs recommended by the WHO for the treatment of children infected with HIV. This license will enable other manufacturers to produce low-cost copies of antiretroviral drugs, the main objective being to make these drugs accessible to low-income countries, where live the very large majority of the 3.2 million children infected with HIV. Created in 2010 MPP had already signed similar agreements with Bristol-Myers Squibb, Gilead Sciences and ViiV on 11 antiretroviral drugs. The WHO hopes that this kind of agreements can contribute to treating more patients suffering from AIDS in low- and intermediary-income countries, where only one-third of infected persons had received antiretroviral drugs in 2013 (Hecketsweiler, 2014i).

### *AIDS/HIV prevention through drug prophylaxis*

Since 2012 the WHO has endorsed the drug called PrEP (short for pre-exposure prophylaxis) as a way to prevent AIDS/HIV infection among those at risk. In 2012 the United States Food and Drug Administration (FDA) approved PrEP and since then critics have worried that the mere existence of such a pill would promote unsafe sex and cause HIV infections to surge. But a study published just before the World AIDS Day (Saturday 1 December 2015) proved them wrong. Reporting in *The JAMA Internal Medicine*, researchers showed that providing PrEP to men who are at high risk of contracting HIV dropped their rates of HIV infection dramatically. In the study, conducted at health centres in three cities, 437 men and transgender women took a PrEP drug called Truvada – a combination of two antiretroviral drugs with a fixed dose, produced by the California-based pharmaceutical company Gilead Sciences – for nearly a year. In that time all but two people remained HIV-free. Those who were infected showed extremely low blood levels of the drug, indicating that they took only about half their required doses. The people who reported engaging in the riskiest behaviours for being infected also showed the highest blood levels of the drug, meaning they were taking their daily doses. In that time the incidence of other sexually transmitted diseases (which Truvada does not treat) remained high but also did not go up (Liu et al., 2016).

Earlier studies have shown that PrEP can lower a person's risk of being infected with HIV by as much as 90%. But because the drug was tested in laboratory-based research settings, experts questioned whether it would work in the real world, where people are much less likely to dutifully take their pills at the proper doses. "These studies show yet again that PrEP works," stated Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases, which provided one of the grants to support the research. "The issue is, can we get PrEP to the people who really need it?" In their analysis the researchers noticed a worrisome trend: while all the PrEP takers showed protective levels of the drug in their blood, they were lowest in African Americans, suggesting that those men were not taking the drug every day. Albert Liu, one of the authors of the study from the San Francisco department of public health, believes that

cultural barriers – perceptions about HIV and mistrust of the medical system – may be contributing to that lower adherence (Liu et al., 2016).

A. Fauci and A. Liu both note that if it is not distributed in the right way, there is a danger that PrEP will further entrench disparities in HIV incidence and promote resistance to the drug. The Centers for Disease Control and Prevention (CDCs) recommend PrEP for anyone at increased risk of HIV infection, including HIV drugs users and sexually active people. PrEP was provided free to the duty subjects but costs US$8,000 to US$14,000 a year. And while cities like San Francisco make PrEP available at no charge to anyone who is HIV-negative but at high risk, most cities had not allocated such funds for HIV prevention. "We know if we prevent an infection, it saves more than US$350,000 over a lifetime for a person in health costs," stated A. Fauci. "So it is definitely an economically sound approach," he added. It is time, he said, to follow San Francisco's lead and figure out ways to make PrEP available to those who can benefit most. "We have enough data, so let us do it," he concluded. Human Rights Campaign, a civil rights group, called for insurers to cover PrEP for anyone who needs it (Park, 2015b). San Francisco's approach to controlling AIDS has been recognized as very effective: the number of infections with HIV has decreased by 30% in this city, while it has remained stable in the whole of United States. This is "a turning point", stated Barbara Garcia, director of San Francisco's public-health services, who supervises two hospitals, 20 clinics and manages a budget of US$2 billion (or €1.9 billion), with 8,000 employees in 2015. She considers that pre-exposure prophylaxis is a step that is as significant as the identification of the virus in 1983 or the first use of tritherapies in 1996. "A new era has begun," stated Steve Gibson, director of the Magnet Clinic, an outlet of the San Francisco AIDS Foundation. "We have reached the stage of the end of fear," he added (Lesnes, 2015b).

It is true that San Francisco feels free after 30 years of struggling against the virus. The Magnet Clinic receives between 40 and 70 persons daily. In 2014 10,000 patients attended the clinic, compared with 1,200 in 2003; 9,608 HIV-detection tests have been performed. And 700 seronegative people were being treated with PrEP; they receive during the first visit a box or flask of 30 pills of Truvada and they have to take one pill per day. At Kaiser Permanente (KP), the giant of private health insurers, which manages the main HIV clinic of San Francisco (3,000 patients), *ca*. 1,000 men were registered for PrEP (by the end of November 2015). Three weeks were necessary to make an appointment at this clinic (Lesnes, 2015b).

During the sexual revolution of the 1970s San Francisco has been "the epicentre of the AIDS/HIV epidemic," recalled Barbara Garcia: 25,000 deaths since 1982, 1,641 in 1992 – the worst year. Now, the city has the ambitious target of reducing the number of infections by 96% before 2020, while in 2015 there were 16,000 people living with the virus (of whom 58% are less than 50 years old). PrEP is a key tool in the city's policy of AIDS/HIV control. "It has become part of popular culture; it has even been mentioned in a television series," indicated Ifeoma Udoh, director of the Clinic Crush, in Oakland, California. This clinic received a public subsidy amounting to US$20 million in 2015 in order to promote prophylaxis against AIDS/HIV among teenagers belonging to minorities. *Ca*. 6,000 persons were receiving PrEP in 2015 in San Francisco – considered as a sort of global laboratory for the control of the viral

disease. As mentioned above the treatment (with Truvada) is very costly, but these expenses are almost entirely covered by the insurers. And for those who are not insured there is an aid fund provided by Gilead Sciences, Truvada's manufacturer (Lesnes, 2015b).

There have been critical comments on PrEP with respect to the increased risk of sexually transmitted diseases. Barbara Garcia did reckon the existence of that risk: "We save lives, but the sexually transmitted diseases are on the rise." Statistical data at the Magnet Clinic showed a 32% increase in the incidence of gonorrhea in 2013 and a 20% increase in the case of syphilis (a total of 1,014 cases for the whole city). But the main objective has always been the control of AIDS/HIV, while it should also be acknowledged that sexually-transmitted diseases do exist and must also be controlled (Lesnes, 2015b).

In France the health minister, Marisol Touraine, announced in November 2015 at the National Assembly (parliament) that she was in favour of the authorization of a temporary use of Truvada, as a prophylaxis means against AIDS/HIV. This was considered a "major progress" by Jean-François Delfraissy, director of the National Agency for Research on AIDS. By mid-November 2015 physicians, associations and researchers, including the Nobel Laureate for Medicine or Physiology, Françoise Barré-Sinoussi, had written an open letter to M. Touraine with a view to demanding such measure. PrEP was expected to start, according to the health minister, "probably during the first half of December 2015", and the cost of PrEP will be entirely borne by the social security" at the beginning of 2016. The cost of a 30-pill vial of Truvada is *ca*. €500. The drug must be taken daily, or before or after sexual intercourse (Lesnes, 2015b).

### *UNAIDS new approach to controlling AIDS/HIV*

The UNAIDS annual report, published on 24 November 2015, a few days before the World AIDS Day (Saturday 1 December 2015), makes a strong plea for another approach to controlling the viral disease: focusing action on the regions where populations are facing a high risk of infection by HIV. In other words, moving from global to local. There were more than 2 million new infected persons worldwide in 2016; this showed a 35% decrease compared with the number of persons infected with the virus in 2000. In 2014 1.2 million people died from diseases associated with AIDS, i.e. 42% less than the 2004 peak. Also in 2014 there were 36.9 million people living with the HIV across the world, compared with 33.3 million in 2010 (that is reflected in the decrease of deaths). By the time of the 21st International AIDS Conference held in Durban, South Africa (18-22 July 2016), 17 million patients had access to antiviral treatments, compared with less than 1 million in 2000 (Benkimoun, 2015k).

UNAIDS executive director, Michel Sidibé, stated that by targeting the regions with a high risk of infection, more people are reached, with a greater efficiency in resources distribution or allocation. "There are many successful examples of this approach. For instance, in Burkina Faso, the epidemic is concentrated in the largest cities of the country (Ouagadougou, the capital, and Bobo-Dioulasso), and particularly among prostitutes

with an HIV prevalence of 13% to 15%. The programme named Yerelon brings to these prostitutes a range of health-care and social services. "A study covering the period 2011-2014 showed that thanks to this programme there has been not a single new case of infection with HIV among the members of this population which was highly exposed to the virus," underlined M. Sidibé (Benkimoun, 2015k). UNAIDS executive director also mentioned "the training of 37,000 health-care workers in Ethiopia, whose action allowed a 92% decrease in the number of new infections during the last five years – 2010-2014." This initiative facilitated the acceptation of detection tests as well as the treatment of those persons infected with HIV; and this was carried out in order to reach the objectives determined by UNAIDS: in 2020 90% of people should be tested and know whether they are infected or not with HIV; 90% of seropositive persons should be treated; and among 90% of treated patients viremia should be suppressed (Benkimoun, 2015k).

Preventive measures should also be taken at the right place and the appropriate time. Among these measures the use of condoms was recognized as a successful means, but there seems to be a decreasing interest in condom use. Another measure, circumcision, was proposed to young men and had a 60% protection efficiency; by the end of 2014, 9 million men had been circumcised by physicians in the African countries considered as priority ones, and 3 million of them had been circumcised in just 2014. The use of antiretroviral drugs "has also been an effective prevention tool among those persons exposed to a high risk of infection with HIV and this can considerably reduce the number of new infections," underlined M. Sidibé. This is preexposure prophylaxis – PrEP. The UNAIDS annual report underlined that PrEP can be useful in the case of couples, where only one of the partners lives with HIV, as well as in the case of sexually active teenagers in countries where HIV prevalence is high, e.g. among female teenagers in southern Africa. The report also mentioned the very good results concerning the application of PrEP to prostitutes in Zimbabwe, homosexuals in Thailand or Brazil and to transgender persons in Brazil. M. Sidibé concluded that "we cannot win the struggle against AIDS/HIV by keeping our action at the global level" (Benkimoun, 2015k).

## Middle East coronavirus epidemic

The Middle East coronavirus (MERS-CoV) belongs to the coronavirus family, like the virus causing the severe acute respiratory syndrome (SARS). The virus is transmitted from animals to humans: the initial reservoirs of the virus are bats and thereafter camels and dromedaries. This occurred a long time ago. In the Gulf region 90% of dromedaries carry the virus. They probably are the origin of the epidemic which has been spreading through Saudi Arabia and Middle Eastern countries since 2012. The virus is also transmitted from human to human through direct contacts and via the respiratory tract. On 21 August 2015 the WHO indicated that 1,445 cases of infection by MERS-CoV had been recorded worldwide since 2012, and that 80% of these cases were registered in Saudi Arabia. Among these cases 512 have been lethal, the mortality rate being 35%. This rate was between 10% and 15% for the SARS, and it was *ca*. 50% in the case of the Ebola hemorrhagic fever (Benkimoun, 2015i).

The MERS-CoV epidemic in Saudi Arabia reached a peak in August 2015 with 98 cases recorded since the beginning of the month and 25 of them were lethal. Such an outbreak is explained by an active transmission of the coronavirus, since June 2015, in one of the main hospitals of the country's capital Riyad, the King Abdulaziz Medical City. Many patients and medical staff were infected by the virus. On Sunday 23 August 2015, 53 MERS-CoV cases had been registered in this very modern health-care centre, where members of the National Guard and their families are treated. Of these 53 cases, 17 deaths have been registered, according to the Saudi health ministry. A WHO expert was sent to Riyad by the end of August 2015 in order to evaluate this new outbreak of the virus, against which there is not yet a vaccine, nor a specific treatment (Benkimoun, 2015i).

Saudi health authorities have taken various measures in order to mitigate the effects of this new outbreak. A fast-reaction team, comprising specialists of communicable diseases, was sent to King Abdulaziz Medical City, immediately after the infections had been detected; also epidemiologists and public-health experts joined their colleagues at the hospital. More than 5,700 biological samples were analyzed with a view to identifying and monitoring the persons who may have been infected with the MERS-CoV. The emergency department of the hospital was closed. Since the peak of more than 300 cases recorded in June 2014, the transmission of the virus has continued in Saudi Arabia. It slowed down during summer and winter, but since mid-June 2015 the number of infections has been rising again (Benkimoun, 2015i).

Saudi health authorities' main concern is that the epidemic may spread swiftly among the population and to other cities, particularly during the great pilgrimage to Mecca, when several millions of Muslims arrive in the holy city from all the corners of the world and by 21 September 2015. It is however true that the pilgrimage (*hajj*) during the three previous years (2012, 2013 and 2014) had not provoked any outbreak of the virus. Furthermore sub-Saharan Africa has been spared up to now. In an editorial of the *International Journal of Infectious Diseases* a team of experts of infectious diseases expressed its concerns regarding this situation and called for the strengthening of epidemiological monitoring and vigilance (He et al., 2015).

In the Middle East, the MERS-CoV has infected people in nine countries: Saudi Arabia, Jordan, Qatar, United Arab Emirates, Oman, Kuwait, Yemen, Lebanon and Iran. Hong Kong and 17 other countries have registered isolated cases: Algeria, Austria, China, Egypt, France, Germany, Greece, Italy, Malaysia, Netherlands, Philippines, South Korea, Thailand, Turkey, Tunisia, United Kingdom and United States. The MERS-CoV epidemic had a significant impact in South Korea where 186 persons have been infected between May and July 2015; 36 patients died before the country announced the end of the outbreak. What may occur in sub-Saharan Africa if the virus reached this region, is dreadful; especially after the havoc caused in three West African countries (Sierra Leone, Guinea and Liberia) by the Ebola virus (Benkimoun, 2015i).

There is some hope to develop a vaccine against the MERS-CoV. On 19 August 2015 *Science Translational Medicine* published an article by David Weiner of the University of Pennsylvania Department of Pathology and Laboratory Medicine, Perelman School

of Medicine, and co-workers from the United States and Canada, on the induction of a protecting immunity against the MERS-CoV in primates. With a synthetic consensus anti-spike protein DNA vaccine the researchers were able to induce in mouse, macacus and camel protective immunity against the virus (Muthumami et al., and Weiner, 2015). "This is a preliminary work, the efficacy of the vaccine should be proved in humans, because we do not have a good animal model where can be transferred and studied the infection of humans by the MERS-CoV," stated Jean-François Delfraissy, director of the Institute of Immunology, Inflammation, Infectiology and Microbiology of the French National Institute for Health and Medical Research (INSERM). "In addition to neutralizing antibodies the experimental vaccine seems to have induced a cell immune response, but the North American researchers did not elaborate much on this aspect. Generally the efficient vaccines are those which trigger both kinds of immune response," he added (Benkimoun, 2015i).

A similar research work is being carried out by another team at the National Institute for Allergy and Infectious Diseases, Bethesda, Maryland, by Barney S. Graham and co-workers. They published their results in the 28 July 2015 issue of *Nature Communications*: rhesus macaques have been protected against the MERS-CoV thanks to neutralizing antibodies after vaccination (Wang et al., and Graham, 2015). Both teams' results are encouraging for the protection of humans through vaccination; they could also serve to vaccinate dromedaries so as to reduce the size of the animal reservoir of this lethal virus (Benkimoun, 2015i).

## Vaccines against human papillomavirus (HPV)

Since the two vaccines against human papillomavirus (HPV) – Sanofi Pasteur MSD Gardasil, by far the most utilized, and GlaxoSmith Kline Cervarix – have been commercialized in 2006 and 2008, respectively, with a view to preventing cervix cancer, there have been questions about their efficacy and their side-effects. For instance, in France, there were in 2015 some forty complaints before the courts, from young women who claimed they were suffering from multiple sclerosis, lupus, etc., after the injection of the antiHPV vaccine. At the same time a petition was being circulated with a view to obtaining a moratorium on the vaccination of female teenagers, that is currently recommended between 11 and 14 years of age, in the form of two injections. In these conditions the vaccination rate is decreasing: 17% in 2014 among 16-years-old female teenagers, compared with 30% in 2012. Worldwide and by 2014, 72 million persons had been vaccinated against HPVs and 215 million doses of the vaccine had been used (Cabut, 2015c).

In France 5.5 million doses of Gardasil had been sold between 2006 – the year of commercialization – and September 2013, while only 400,000 doses of Cervarix had been delivered since this vaccine had become available in 2008. Both vaccines are being strictly monitored. At the national level, in France, there is a reinforced pharmacovigilance, while at the European level there is a plan of risk management (PRM) concerning both vaccines (Cabut, 2015c). In order to analyze more precisely the eventual relations between the antiHPV vaccination and the occurrence of

autoimmune diseases, the French Agency for the Safety of Medicines and Health Products (ANSM, French acronym) and the social security (disease insurance branch) carried out an unprecedented study, with respect to its magnitude. A cohort of 2.2 million female teenagers, between 13 and 16 years of age, has been constituted and it has been followed during two years. It has been possible to compare the occurrence of 14 autoimmune diseases (including multiple sclerosis, type-1 diabetes, thyroiditis) among 840,000 vaccinees – one-third of the cohort – and among 1.4 million non-vaccinated persons. A total of *ca*. 4,000 cases were recorded, without an overall increase in the risk among the vaccinees. These results were made public on 14 September 2015. They were particularly reassuring with regard to multiple sclerosis, as this was also recorded in other countries (Cabut, 2015c).

The study revealed a significant correlation between the antiHPV vaccination and the occurrence of the Guillain-Barré syndrome (GBS), a neurological disease that is rare and regresses spontaneously, but potentially severe. The risk of suffering from GBS is multiplied by four after vaccination, which would mean 1 or 2 cases per 100,000 vaccinated female teenagers. The syndrome results in the paralysis of peripheral nerves; the lesions spread progressively during several weeks, but the patient recovers in the majority of cases in a few months. However, when the neck, head and respiratory-muscles nerves are affected by the GBS, the patients may be treated in intensive-care units with respiratory assistance. The overall result is that 5% of the severely affected patients die and 10% keep a mobility handicap. The GBS may occur during a viral infection; this is also a known side-effect of antiflu vaccines. In the French ANSM study 19 cases of GBS were recorded among vaccinated teenagers and 21 cases among non-vaccinated teenagers (two-thirds of the targeted population). Not a single death was registered. In the report it is underlined that the correlation between the vaccination and the GBS "was very strong during the first three months following the administration of the last vaccine dose; then it decreased over time, while remaining statistically significant." This side-effect of antiHPV vaccines was not found in other studies, but the GBS is mentioned in the Gardasil instructions-for-use pamphlet (Cabut, 2015c).

Another correlation, much weaker, has been found between the antiHPV vaccination and chronic inflammatory intestine diseases: the risk is multiplied by 1.19, or a 19% increase in the risk occurrence. According to the ANSM study this relationship is worth being investigated, but it may be a random effect or it could be due to some unknown factors that were not taken into account in the analyses (Cabut, 2015c). The French study was communicated to the European Medicines Agency (EMEA), which in July 2015 started an analysis of all the data concerning the antiHPV vaccines. With respect to the French National Cancer Institute (INCa, French acronym), the comforting results of the ANSM study should allow the promotion of the antiHPV vaccination as well as the campaigns of cervix-cancer detection relying on cervical smears. Agnès Buzyn, the INCa president at that time, recalled that although cervix cancers are not the most frequent in France (3,000 women per year and 1,100 deaths), "they are those which can be best prevented or avoided; they are also the only cancers whose survival rate in the five-year lapse has been decreasing during recent years, because they are discovered at an earlier stage." Such comments may

not be sufficient for convincing those who are sceptical. Another obstacle that must be overcome is an economic one: in 2015, for both vaccine injections the vaccinee had to pay *ca*. 80 euros (the rest of the cost being covered by the social security or disease insurance) [Cabut, 2015c].

To sum up, 3,000 cervix cancers diagnosed in France annually justify the vaccination of female teenagers as well as cervix smears among adults every three years so as to make a diagnosis and to start an early treatment. At the Lorraine Cancer Institute, north-east of France, Xavier Sastre-Garau developed a blood test aimed at detecting even earlier cancers related with HPV and at following the treated patients without making biopsies. X. Sastre-Garau and his co-workers showed that tiny amounts of viral DNA in the blood of the patient could be detected and isolated in order to correlate them with the HPV strain infecting the patient and therefore to evaluate the risk of cervix cancer.

# CONTROLLING COMMUNICABLE DISEASES

## Antibiotics resistance and the neglected antibiotics market

### *Antibiotics resistance: a scaring global trend*

In July 2014 David Cameron, the United Kingdom prime minister, appointed Jim O'Neill, former chief economist at Goldman Sachs, to head a panel of experts from the finance, research and industry arenas in order to look at ways to increase incentives for investment into research on, and development of, new antibiotics. This "task force" was funded by the Wellcome Trust – a powerful British foundation specialized in health – with a budget of £500,000 (€625,386). In May 2016 they published their final report and recommendations in the *Review on Antimicrobial Resistance*: "Tackling drug-resistant infection globally," under the chairmanship of Jim O'Neill. Also in the country of Alexander Fleming who discovered penicillin in 1928, the Longitude Prize, created in the 18th century to reward the person who would be able to find the way to locate a navigating ship thanks to its longitude, chose antibiotics as its theme for the year 2014; the laureate was to receive an allocation of £10 million (Hecketsweiler, 2014h).

In April 2014 the WHO warned that antibiotic resistance "threatens the achievements of modern medicine." Without urgent action, it stated, the world was "heading for a post-antibiotic era, in which common infections and minor injuries which have been treatable for decades can once again kill." Antibiotic-resistant infections kill *ca*. 50,000 persons a year (2013) in the United States and Europe, and this number is rising, the WHO reported (Ward, 2014b). Resistance to antibiotics can be the result of several mechanisms, e.g. the production by microorganisms of an enzyme that breaks down the antibiotic molecule, or the transformation of the microbe's membrane into a barrier against the penetration of the antibiotic. Bacteria can be naturally resistant or can become resistant after a repeated contact with antibiotics. A gene mutation could be the origin of resistance or the acquisition of one or several resistance genes from already resistant microorganisms.

Antoine Andremont, head of the Bacteriology Department at Bichat-Claude Bernard hospital in Paris, while making 100,000 bacterial tests a year, has expressed his concern about the increasing number of infections that are "difficult" to treat using current antibiotics and even when using those called of "last resort." This overall concern was expressed by the physicians who participated in the European Day of Information on Antibiotics, organized in Paris on 18 November 2014. A. Andremont published in 2014

a book titled *Antibiotiques, le naufrage* (Antibiotics, the Wreckage; Bayard publishers, Paris, 250 pp.) where he describes the unprecedented increase of pathogen resistance to a wide range of antibiotics. The French Institute of Health Vigilance has warned about the high number of infections where "superbugs" are involved, i.e. bacteria that are resistant to the most powerful antibiotic molecules: since early 2011 more than 1,200 cases had been detected in Paris hospitals. Mutant bacteria are discovered and they have sophisticated means of defence, designated by such acronyms as OXA-48, NDM-1 or KPC. With such mechanisms a cell *of Escherichia coli* (colibacillus) or of *Staphylococcus aureus* can become a killer (Hecketsweiler, 2014h).

These developments are associated with an increasing consumption of antibiotics that is not always justified from the medical viewpoint. In France this consumption remains above the average consumption in the rest of Europe and the United States. "Even though" it had decreased since the early 2000s, "an increasing trend has been noticed since 2010 and had been confirmed in 2013," according to the National Agency for the Safety of Medicines and Health Products. In fact, since 2010, the use of antibiotics has surged nearly 6%. In 2013 it was estimated that the consumption of antibiotics in France, in both hospitals and city private medical cabinets, reached 32.3 daily doses per 1,000 inhabitants (30.1 doses in the medical cabinets and 2.2 in hospitals). In 2012 the figures recorded for city medical cabinets were: 31.9 for Greece, 29.8 for Belgium, 29.7 for France, 27.6 for Italy, 20.9 for Spain, 20.1 for the United Kingdom and 14.9 for Germany. It was also calculated that the cost of antibiotic resistance for the health-care system amounted to €1.5 billion a year in 30 European countries (Hecketsweiler, 2014h).

### *Controlling antibiotics resistance*

To try to control antibiotic resistance and its impact on the health-care system the French health minister announced on 17 November 2014 the creation of a "working group" entrusted with the task of formulating proposals in June 2015 aimed at facilitating the discovery of new antibiotic molecules and new diagnostic tests. The working group was also expected to deal with those products, named "without market", because they target restricted populations. The working group's proposals will be part of the Plan "Antibiotics 2011", which aimed at reducing the consumption of antibiotics by 25% between 2011 and 2016. Such an objective was impossible to reach, according to the ANSM (Hecketsweiler, 2014h).

In the United States the same concerns exist and on 18 September 2014 President Barack Obama signed a decree that aimed to "strengthen the national surveillance capacities and to extend the whole range of diagnostic tools, antibiotics and other available means in order to combat antibiotic resistance." According to the White House antibiotic resistance had an annual direct cost for the health-care system in the United States that was estimated at US$20 billion (or €15.9 billion), while the induced loss of productivity was evaluated at US$35 billion in 2013. A working group which includes representatives of the health, defence and agriculture ministries has been requested to prepare before February 2015 a five-year programme of action. The presidential decree also requested the FDA "to pursue the efforts aimed at eliminating the use in agriculture of antibiotics whose medical value is important" (Hecketsweiler, 2014h).

The White House also created a US$20 million prize for the development of fast diagnostic tests targeting "superbugs" such as *Staphylococcus aureus* that is resistant to meticillin. In 2012 President B. Obama signed the Generating Antibiotic Incentives Now (GAIN) whose purpose was to encourage pharmaceutical companies to invest in the discovery of antibiotics through the increase of patent duration (Hetcketsweiler, 2014h). It is true that research on antibiotics has plummeted and physicians have increasing difficulties in combating "superbugs". About 20 new antibiotics had been marketed since 2000, but there has been "no outstanding discovery," stated Stephan Harbarth, specialist of infectious diseases at the University Hospitals in Geneva. "The best molecules – that kill the pathogenic bacteria without altering the human cells – have already been identified," underlined A. Andremont. Finding new molecules is becoming more complicated and a risky venture (Hecketsweiler, 2014h).

### *New research-and-development data*

Multiresistant bacteria cause *ca*. 160,000 infections annually in France and *ca*. 13,000 deaths. "This is a drama that goes far beyond the control of communicable diseases: it jeopardizes the progress made in two decades in surgery, cancerology and organ transplant (where many patients are immunodepressed)," deplored Patrice Courvalin, a professor at the Pasteur Institute, Paris. According to current recommendations physicians should try to treat most infections using available antibiotics. They should use the most-recently developed antibiotics in the treatment of severe or resistant infections. These "last-resort" medicines are broad-spectrum antibiotics that target multiresistant bacteria (Rosier, 2015e).

However an Israeli study questioned this dogma. Published on 25 June 2015 in *PLOS Computational Biology*, this study deals with a mathematical model that simulates the dynamics of bacterial resistance in hospitals (Obolsky, Stein and Hadany, 2015). Lilach Hadany (Tel Aviv University Department of Molecular Biology and Ecology of Plants) and her colleagues have compared the effects of different utilization strategies of three antibiotics: either (virtual) physicians prescribed firstly two or three molecules, reserving the third one to a last-resort utilization; or they treated at once the infection using one of the three antibiotics, with an equal frequency. In order to feed their model the Israeli researchers have estimated the frequencies of bacterial resistance on the basis of data gathered in hospitals. As expected it was found that a broader utilization of the last-resort antibiotic limits the number of badly treated patients. But a more surprising result was that it also sometimes reduces the dissemination of multiresistant bacteria. When therefore should the last-resort antibiotic be used at once? "When the bacteria already show a multiple resistance to current antibiotics," replied the authors of the study (Obolsky, Stein and Hadany, 2015). "That seems to be common sense," stated P. Courvalin (Rosier, 2015e).

"The main lesson is that one should not run the risk of a therapeutic failure, because this is when the resistance develops," summarizes Jean Carlet, a physician who has been appointed by the health minister, Marisol Touraine, to chair a task force on

antibiotic resistance, which was expected to deliver its recommendations in July 2015. "In the case of a severe infection, when the causative microorganism is not yet known, one should administer at once the most efficient antibiotic, even if it is a recent one. But as soon as the pathogen is characterized the treatment should rely on older antibiotics if the bacterium is not resistant to them," explained J. Carlet (Rosier, 2015e). According to Laurent Gutmann, a professor at the European hospital Georges-Pompidou, Paris, the Israeli study has the merit to present a mathematical demonstration of current clinical practices. He stated: "In the case of a seriously ill patient, one can effectively prescribe at once a last-resort antibiotic, if multiresistant bacteria can be involved in the infection: for instance when the patient returns from certain intertropical regions." This is in fact a major risk: half of the people travelling in those regions come back with multiresistant bacteria, ingested through food intake, according to a study published in February 2015 by researchers working at the *Unité d'hygiène et de lutte contre l'nfection nosocomiale* – Unit for Hygiene and Control of Nosocomial Infections – of the Bichat-Claude Bernard hospital in Paris (north of the city). One of the co-authors of the study, Jean-Christophe Lucet, indicated the mathematical model developed by the Israeli researchers does not take into account a key parameter: the persistence of antibiotic resistant bacteria in the patients' digestive tract (Lucet, 2015). J. Carlet thinks that the digestive microflora is the "resistance epicentre" (Rosier, 2015e).

In hospital conditions the risk of resistance emergence is decreased thanks to a mixing or a rotation of antibiotics prescription among patients. But none of these strategies has been found really effective: "If some progress has been observed with regard to the dissemination of some antibiotic-resistant bacteria, such as meticillin-resistant straphylococci (…), the situation is worsening in the case of multiresistant enterobacteria," summarized François Bourdillon, director of the Health Vigilance Institute, at the end of 2014 (Rosier, 2015e).

It is worth mentioning that bacteria are not the only microorganisms which develop resistance. The HIV (human immuno-deficiency virus) "becomes resistant to current treatments over a period of two weeks or two months, further to mutations," indicates Vincent Calvez, a professor at the Paris La Pitié-Salpêtrière hospital. The recommended response is to use at once powerful and well-tolerated molecules and to see the patient every three months (Rosier, 2015e). In oncology "chemotherapies select tumour cells that already have resistance mutations," underlines Marie Dutreix of the Institut Curie, Paris. "This selection of resistant cells is the main likely cause of the failure of these chemotherapies." Those treatments which target a specific enzyme are easy to counter: the tumour cell modifies that enzyme or activates another biochemical pathway. In other cases, tumour cells can increase their capacity to degrade the drug or to stimulate its outflux. As recommended in the case of antibiotic-resistant bacteria the appropriate strategy is "to use sufficiently high doses of the drug, so as to kill the cells before they find a way to escape the treatment. Another method consists of combining treatments that may induce different kinds of resistance; the limitation of this approach is the toxicity of drug combinations" (Rosier, 2015e).

### *A French biotechnology startup at the forefront for combating antibiotic resistance*

On 5 October 2015, under the aegis of the French health ministry, a new startup, named Eligo, inaugurated its laboratory within the Pasteur Institute in Paris. The French health minister, Marisol Touraine, seized that opportunity to announce the creation of a €100-million investment fund, devoted to innovation in health. She stated: "The French startups are too often and unfortunately facing the danger of the so-called financial "death valley", before reaching commercialization. "Due to the lack of sufficient capital, they have to struggle for trying to find funding, instead of devoting most of their time to research and development. They run out of steam. My ambition is to break this negative spiral through supporting the startups financially." The minister also wanted to seize that opportunity in order to announce the organization of a national day for innovation in health, to be launched on 23 January 2016 at the Paris Science and Industry City. The minister added: "Innovation in health will upheave tomorrow's medicine. That is why we must allow everybody to be fully aware of this revolution and to participate in it" (Hecketsweiler, 2015u).

Eligo was created in 2014 by two young French researchers, Xavier Duportet (27 years old) and David Bikard (30 years old). X. Duportet did his PhD at the Massachusetts Institute of Technology (MIT) which awarded him in the spring of 2015 the title of "French innovator of the year." David Bikard also worked in the United States at New York Rockfeller Institute, and he specializes in the use of the gene editing technique CRISPR-Cas 9 (see p. 143). Both researchers are developing genetically modified viruses which selectively attack bacteria. These new "antibiotics", called "eligobiotics", are weapons of massive destruction, because they do not harm the "good bacteria" living in the human body, while they kill the pathogenic ones. This is a new tool to combat the antibiotic resistance developed by an increasing number of pathogenic bacteria. While in France, as mentioned above, *ca*. 13,000 patients die annually because of this resistance to antibiotics, the health minister wanted to decrease that mortality under the threshold of 10,000 deaths per year (Hecketsweiler, 2015u).

Eligobiotics have been effective in mice, but another two or three years are needed before carrying out the first clinical trials in humans. The first patients that will benefit from this kind of treatment are suffering from Crohn's disease, an inflammatory intestine disease associated with certain bacteria. There are other options which are being determined by both French scientists. To that end Eligo is recruiting high-level scientists, including from the United States (they have received 180 applications from Stanford, Harvard universities and MIT). X. Duportet hopes to create a "dreamteam" on synthetic biology. The startup also raised €2.4 million – an important amount whereas the development of eligobiotics is just starting. Most of these funds were brought in by Seventure Partners, a subsidiary of Natixis bank, which invested €2 million in July 2015. Seventure Partners, which is supported by agrifood big corporations such as Danone (dairy products), Tereos (sugar) or Lesaffre (yeasts), has created in 2013 a fund devoted to the research on the microbiome, i.e. all bacterial populations that live on the surface of, and inside, the human body. The fund already raised more than €100 million, part of it from Bpi-france, and Eligo is its fifth investment venture (Hecketsweiler, 2015u).

## *Antibiotics market and new business models*

Big drug manufacturers scaled down investments into antibiotics R&D in recent decades to focus on higher-margin and lifelong medicines such as drugs against cancer as well as against the so-called "civilization" diseases – diabetes, hypertension and excess of blood cholesterol. The overall result has been a sharp drop in the number of new antibiotics reaching the market. In 2013 the antibiotics global market was estimated at US$40 billion (or €31.9 billion) and it was expected to remain stable during the following years; by contrast the sales of antidiabetes drugs soared to US$38 billion in 2013 and would amount to US$70 billion in 2020 (Hecketsweiler, 2014h).

Although Pfizer, one of the pioneering big pharmaceutical companies in the area of antibiotics, closed down its research centre in Connecticut in 2011, and Bristol-Myers Squibb and Eli Lilly also decided to scale back their antibiotics manufacturing units or factories, there have been signs of revival since the end of 2012, with Roche, GlaxoSmithKline and Sanofi all pledging fresh investment or forging research partnerships to develop new pathogen-fighting drugs (Ward, 2014b; Hecketsweiler, 2014h). Massachusetts-based biotechnology company Cubist is at the forefront of efforts to develop a new generation of anti-infective medicines to tackle the spread of drug-resistant pathogens. In fact Cubist aimed to deliver at least four new antibiotics by 2020 and it committed US$400 million in research and development in 2014 alone. Cubist shares surged as high as US$93.50 in after-hours trading on Friday 5 December 2014, up 26% from their US$74.36 close, after the *New York Times* reported Merck &Co. was closing in on a US$100-a-share deal as early as the week starting on 8 December 2014. For Merck &Co. a takeover of Cubist would mark its second large deal in six months after its US$3.85 billion acquisition in June 2014 of Idenix Pharmaceuticals, a hepatitis C specialist (Ward, 2014b).

Launched in October 2014, the Drive-AB consortium involves academia, physicians and industry in the design of new business models in antibiotic production. M. Harbarth, one of the participants in the consortium, mentioned that "a common fund that would be created through the contribution of States, according to their respective resources, could serve to allocate bonuses to laboratories that make a discovery. Such grant would cover part of the expenses. As a counterpart these laboratories would make the commitment to limit the sales of their molecule." "In order to conserve their efficiency, it is indeed necessary to reserve these new medicines to a limited number of patients with the shortest therapy duration," underlined Antoine Barouky who heads Cubist French subsidiary (Hecketsweiler, 2014h). Another business model, suggested by Kevin Outterson, a British economist, consisted of a commitment made by the States to buy a given quantity of antibiotics and store them "just in case." This approach would guarantee an amount of sales to the laboratories, independently from the volume of prescriptions. According to the WHO an amount of US$250 to US$500 million per antibiotic molecule would be sufficient to drive the engine again (Hecketsweiler, 2014h).

Other projects rather focus on research. In Europe, for instance, the innovative Medicines Initiative (IMI) has allocated €224 million to funding the programme called "New Drugs for Bad bugs". In the United States, since 2010, the government, who is concerned about superbugs and bioterror, has been funding several programmes, such as those of the biotechnology companies Basilea and the Medicines Company. The American government also signed a US$200-million contract with GlaxoSmithKline (GSK). This political commitment is having results. For instance, in 2013 Roche made a spectacular return in antibiotic research and development: in 2013 the global leader in anticancer medicines, pledged up to €415 million for an antibiotic developed by another Swiss pharmaceutical company Polyphor in order to control a superbug of the *Pseudomonas* group; in 2014 Roche signed partnerships with the British pharma Discuva and Spero Therapeutics, an American startup (Hecketsweiler, 2014h).

## Towards the end of tuberculosis pandemia

### *A major public-health scourge*

In 2014 9.6 million people, including 1 million children, were diagnosed with tuberculosis across the world. And 12.5% of these people were seropositive (AIDS/HIV). The death toll in 2014 amounted to 1.5 million, including 140,000 children who were seronegative with respect to HIV. The global death toll was 4,000 per day. Most of these deaths (95%) occur in low- and intermediary- income countries. "This is unacceptable," stated José-Luis Castro, executive director of the International Union against Tuberculosis and Respiratory Diseases (IUATRD). There has been nevertheless some progress in the struggle against tuberculosis: "Mortality has decreased by 47% since 1990," recalled Jane Carter, president of IUATRD. "But the problems are before us: 9.6 million patients and 1.5 million deaths a year. These figures must boost our action," she added. Tuberculosis kills more than AIDS/HIV (1.2 million deaths a year) [Rosier, 2015g].

### *The World Health Organization new objectives*

In May 2014 the general assembly of the WHO adopted three objectives for 2035: reduction by 95% of the number of deaths caused by tuberculosis, compared with the 2015 figure; reduction by 90% the incidence rate of tuberculosis (this rate has decreased by an average 1.5% since 2000); and take all the necessary measures so that not a single family would bear the catastrophic costs relating to this disease. On 3 December 2015 José-Luis Castro opened the 46$^{th}$ global conference on respiratory health in Cape Town, South Africa. *Ca*. 4,000 experts from across the world attended the conference, researchers, physicians, decision-makers, civic society representatives, humanitarian organizations, patients and human rights activists (particularly those who defend the rights of patients). They all gathered in the city where Nelson Mandela, "Madiba", had spent 27 years in jail at Robben Island, and was infected with the Koch bacillus. Madiba suffered from tuberculosis, that was aggravated by the very bad health conditions during his detention. Meeting in Cape Town was therefore a great symbol for all the participants, who were convinced that a successful control of

tuberculosis will come only if all their efforts were coordinated, thus giving rise to an effective synergy. "Everything is linked," summarized Mark Dybul, executive director of the Global Fund to Fight AIDS, Tuberculosis (TB) and Malaria (Rosier, 2015g).

## *A road map towards the eradication of tuberculosis*

In 2014 the WHO road map towards the eradication of tuberculosis included three major stages. In the first place treatments of the disease should be focused on the patients, i.e. they must be adapted. For instance children are a first vulnerable target population. Until now the parents and the health-care personnel had to break or grind the available drugs in order to help the youngest children take them. In Cape Town the Alliance against Tuberculosis and its partners announced the delivery of the first antituberculosis drugs to children. These drugs are a combination of the three most frequently used medicines. They are soluble pills, with a good taste, easy to ingest and with affordable prices. "This is an important progress," underlined Philippe Duneton, a physician of UNITAID which funded the development of these pediatric forms of antituberculosis drugs (Rosier, 2015g).

Treatments should also be simplified and shortened. In the case of the patients where the Koch bacillus (*Mycobacterium tuberculosis*) is sensitive to antibiotics, the treatment lasts six months; four antibiotics are used. But there are multiresistant forms of the pathogen. In 2014 worldwide 480,000 were suffering from tuberculosis with multiresistance to antibiotics. The regions that are mostly affected by these antibiotic resistances are Eastern Europe, Asia and some parts of Africa. The treatment recommended for these multiresistant forms of the disease should last between 18 and 24 months. "These very long treatments are not generally followed correctly," indicated Nicolas Véziris, a physician at Paris La Pitié-Salpêtrière hospital. A progress was highlighted at the Cape Town conference: the treatment of these multiresistant forms of tuberculosis can be shortened, and last only nine months, as shown by a study of a cohort in nine French-speaking African countries. According to preliminary results the treatment led to: "a cure rate of 82%, compared with 40%-50% with the current treatment lasting more than 18 months," summarized Arnaud Trébucq, the French physician who launched that study. The WHO decided to analyze these data in order to revise its recommendations in 2014, indicated Marco Raviglione, director of the global programme on tuberculosis at WHO (Rosier, 2015g).

However, according to a report by Doctors Without Borders (MSF), almost 60% of the countries reviewed still use obsolete treatments which lead to the spread of antibiotic-resistant tuberculosis. After a first therapeutic failure treatment is renewed in these countries but with inefficient drugs. "National programmes aimed at controlling tuberculosis must therefore change their policies in order to be on line with international recommendations," warned Lucica Ditiu, a physician of the NGO Partenariat Halte à la Tuberculose ("Stop Tuberculosis Partnership"), who co-authored the MSF report. In 2012-2014 two new antibiotics were commercialized: bedaquiline of Janssen and delamanid of Otsuka. They are very efficient against multiresistant forms of the disease. But "these treatments have been administered to only 2% of the

patients with multiresistant tuberculosis," stated MSF. "In the relevant countries, all the measures have not been taken in order to introduce these new antibiotics and treat the patients," indicated Christophe Perrin, also of MSF. And "there are already some forms of resistance to bedaquiline," warned N. Véziris (Rosier, 2015g).

Vulnerable populations must be targeted, including prisoners. A study presented at the Cape Town conference showed that in Brazil the rate of tuberculosis among prisoners had doubled between 2007 and 2013, from 4.1% to 8.2%. The incidence rate of tuberculosis in prisons was 28.3 times higher than in the general population. "There is a risk that Brazilian prisons become a "reservoir" for the transmission of tuberculosis through the rest of the population," remarked Paul Bourdillon of Yale University, Connecticut. In South Africa public decision-makers and politicians are seriously considering this issue: "In the prison of Pollsmoor, a regular diagnostic test of tuberculosis is performed among all the prisoners," indicated Paula Fujiwara, scientific director of the IUATRD (Rosier, 2015g).

Another vulnerable group of patients that must be targeted are those living with HIV; the risk to be infected with the Koch bacillus is 20 to 30 times higher than that among seronegative persons. Also diabetes increases the risk of tuberculosis. There is therefore a need for developing an integrated approach to dealing with these diseases. Reducing drastically smoking is another challenge. "Anything that damages lungs facilitates the occurrence of tuberculosis," summarized Mark Dybul (Rosier, 2015g). Mario Raviglione drew the attention on migrants who also run a higher risk to be infected with the Koch bacillus and who have limited access to health-care. "Persecution, conflicts, violence or violations of human rights: there are *ca*. 60 million refugees across the world," he stated. Moreover these populations are often discriminated. "To say that refugees are the vectors of communicable diseases, is inhuman. We ought to find more efficient responses to these issues," claimed Mark Dybul (Rosier, 2015g).

Another crucial challenge is to develop new diagnostic tools, faster, simpler and that need less infrastructures. A molecular-biology test, GeneX-pert, has been available since 2010. "This test applied to a sputum can lead to a result in less than two hours, compared with two to three weeks in the case of tests using cultures of the bacillus," underlined Philippe Duneton. The test also detects the resistance to rifampicine. Consequently the treatment can be adopted rapidly. In 2016 other diagnostic tools were expected: they will rely on solar energy and will be therefore more autonomous (Rosier, 2015g).

The second stage of WHO road map is to have bold policies and the support of local populations. "We must cooperate with these communities in order to identify the patients where they live and work," stated José-Luis Castro. There are *ca*. 3 million persons that have not been tested. "An important programme, TB Reach, resulted in duplicating the number of persons who were tested in many countries. This programme is focused on targeted communities, such as teenagers, in South Africa," indicated M. Dybul. "In Uganda, one of our projects, based on the involvement of physicians working in the slum areas of Kampala, has led to a 59% increase in the

number of detected cases of tuberculosis in one year," rejoiced P. Fujiwara. An important component of this second stage of the road map is the funding of the activities aimed at ending the tuberculosis pandemia. According to Lucica Ditiu "US$56 billion will be needed for the next five years (2015-2019)." "In 2016 the expected annual investment should rise from US$8 billion to US$11 billion (or from €7.3 to €11 billion). We do not know where to find this supplementary US$3 billion," reckoned Mario Raviglione (Rosier, 2015g).

### *New candidate vaccines against tuberculosis*

The third stage of the road map is to markedly increase the research-and-development and innovation efforts. "We need new diagnostic and therapeutic tools. Even in the case of non-antibiotic-resistant tuberculosis, treatments are too long. We must convince the big pharmas to invest in these developments. The market exists, due to the number of infected persons," stated J.L. Castro. In particular, the search for new vaccines is a primary objective. "This is the priority of the priorities," thought Jean-Michel Molina, a professor at Paris Saint-Louis hospital. There is an old vaccine available: the BCG (bacillus of Calmette and Guérin) that has been used since the 1920s in humans; but its efficacy is limited in adults. According to Camille Locht, research director at the French National Institute for Health and Medical Research (INSERM) and working at the Pasteur Institute in Lille (north of France), "the BCG vaccine protects children very efficiently against severe forms of tuberculosis, particularly meningitis. In countries with high endemicity of tuberculosis the WHO recommends the vaccination with BCG of infants that are seronegative (HIV). In France vaccination with the BCG has not been mandatory since 2007" (Rosier, 2015g).

Some 15 candidate vaccines against tuberculosis are being tested in humans. "It is impossible to say which are the most promising. We must carry out more basic research on the relations between the Koch bacillus and its infected hosts," explained C. Locht. For instance, why, after infection, only 5% to 10% of individuals fall sick? Which are the immunity mechanisms that distinguish the 90% to 95% who remain free of tuberculosis? Another challenging factor for the researchers is that, like HIV, the Koch bacillus finds a haven in certain cells of the human body. "This microbe is not so virulent, its installation in the body is slow and latent. But the most successful germs in terms of evolution are those with relatively weak virulence," underlined C. Locht (Rosier, 2015g).

In Cape Town, at the Desmond Tutu Foundation Emavundleni Centre, in the heart of Nyanga township, vaccination trials are being carried out with a view to evaluating the efficacy of two candidate vaccines among 12 to 17-years-old teenagers. The trials also aim at analyzing the immune response of the vaccinees. The centre's missions are to prevent and treat infections with HIV, as well as those diseases which accompany AIDS, such as tuberculosis and pneumonia. The centre is also carrying out research on these issues. It should be underlined that the incidence of tuberculosis is very high in South Africa. "With 30,000 cases of tuberculosis recorded per year, Cape Town has more infected persons than the whole of the United States," indicated Linda-Gail

Bekker of the University of Cape Town. In that city 20% of children are infected with Koch bacillus (they had their "primo infection") when they are admitted in schools. But this infection rate reaches 50% at the beginning of their sexual life. Regarding adults infected with HIV three-quarters of them are also infected with the Koch bacillus. But "only" 5% to 10% of these infected persons will have an active form of tuberculosis (Rosier, 2015g).

Recruiting volunteers for the vaccination trials has not been easy. "We had to test 600 of them before selecting 62 who were pathogen-free," explained L.G. Bekker. In order to encourage people to participate in the clinical trials, the Emanvundleni Centre relies on an in-depth social work within the communities: "We have a team who devotes its work to raise people's awareness; they carry out a daily education work, while meeting with the people," explained L.G. Bekker. "They also inform them about the research conducted at the centre," she added. Philippe Duneton, on his side, commented that "this type of centre is very useful, because it combines prevention, research and services aimed at solving daily problems. We are inspired by their action when we carry out our field activities, particularly with respect to the struggle against AIDS/HIV" (Rosier, 2015g).

Eradicating the tuberculosis pandemia by 2035 is one of the objectives for sustainable development (OSDs), that followed the Development Millennium Goals (DMGs) and were adopted by the Organization of the United Nations in 2014. The alliance against the Koch bacillus and tuberculosis – a disease of poverty and exclusion – has been promoted by the WHO and many other actors, through a road map adopted in 2014. Finding the resources and accelerating research-and-development activities worldwide can make the eradication of tuberculosis an achievable goal.

# ERADICATION OF PARASITIC DISEASES

## Introduction

Some diseases are not suitable for eradication because the organisms that cause them hang around in the environment, or have other animal hosts. Others, such as tuberculosis, can infect people "silently", without causing symptoms, so are invisible to physicians. A list of five plausible targets – measles, mumps, rubella, filariasis and pork tapeworm – has hardly changed since the early 1990s, yet measles, mumps and rubella are all subjects of intensive vaccination campaigns that could easily be converted into ones of eradication. Hepatitis C should be made a target, too. It kills half a million people a year and affects rich and poor countries alike, yet new drugs against it are 90% efficient and there are no silent carriers. Eradicating these seven diseases – the five, plus malaria and hepatitis C – would save a yearly total of 1.2 million lives (*The Economist*, 2015j).

The reason filariasis is on the "possible" list is the development of ivermectin, a drug that kills the worm which causes it. The inventors of this drug, William Campbell of the United States and Satashi Omura of Japan, won half of the 2015 Nobel Prize for Medicine or Physiology (the other half was won by Tu Youyou, the Chinese researcher who discovered the antimalarial efficacy of artemisinin, see p. 267). W. Campbell and S. Omura claim their shares for the discovery of avermectin, derived from *Streptomyces* spp. of which S. Omura grew thousands of strains during the 1970s. W. Campbell picked up on this work and found that one of S. Omura's compounds killed nematode worms of the sort that cause filariasis and river blindness or onchocercosis. Today avermectin's descendant, ivermectin, is regarded as so important that it is on the WHO list of Essential Medicines which catalogues those drugs that even the most basic medical system needs (*The Economist*, 2015j).

Even better technology is in the pipeline. In the case of mosquito-borne illnesses such as malaria and dengue, genetic engineering promises ways of making the insects resistant to the pathogens that they pass on to people, of crashing the mosquito population and even of attacking insects and pathogens with genetically modified fungi and bacteria (*Wolbachia*). Genetic engineering also promises a wide range of new vaccines (see p. 273). Another reason for seeking the eradication of parasitic diseases (and other transmissible diseases) is a change in political attitudes. The emergence of AIDS/HIV, in particular, made governments everywhere sit up and take notice. In 2014 the West African outbreak of Ebola hemorrhagic fever only reinforced the message. Political attention leads to better medical infrastructure. To deal with

AIDS/HIV new networks of clinics were created and staffed with trained personnel. These can serve as the backbone of the campaigns that would be the starting-point for many extermination programmes (*The Economist*, 2015g).

## Eradication of malaria

### *One of humankind's deadliest plagues*

Malaria has killed people since the dawn of humankind. In 1900 it was endemic in almost every country on Earth and throughout the first half of the 20th century it killed 2 million people a year. The Protozoan *Plasmodium falciparum* causes the most deadly form of malaria. Together with four cousins it infects 214 million people per year and is responsible for *ca*. 850,000 deaths a year, and the ruination of the lives of millions more people who survive the initial crisis of disease. Roughly 40 species of *Anopheles* mosquito, found all over the world, are hosts for the types of Protozoans that cause malaria in humans. After a bite from an infected mosquito, which injects a small number of parasites into its human victim's bloodstream, the parasites travel to the liver where they multiply rapidly. They then infect red blood cells (erythrocytes) and continue to proliferate. Flu-like symptoms begin when the parasites break out of the erythrocytes, one to four weeks after the bite. Other mosquitoes can then pick up the parasite when they bite an infected person and pass it on when they bite another one (*The Economist*, 2015j).

Five types of malaria cause illness in humans. *Plasmodium falciparum* is responsible for the vast majority of deaths, having killed virtually all of the 528,000 people who died from malaria in sub-Saharan Africa in 2013. *Plasmodium vivax* is the most geographically widespread species, responsible for most cases of malaria outside sub-Saharan Africa; it is less lethal than *P. falciparum* but can remain dormant in the liver and cause illness to recur when it emerges into the bloodstream; frequent relapses weaken its victims, making them more susceptible to other diseases. Up to 85% of people infected with malaria do not show symptoms and the parasite can lay dormant for months or years after an initial infection before emerging. This make it especially difficult to fight (*The Economist*, 2015j).

Eradication efforts focus on the two most virulent species of the Protozoan: *P. falciparum* and *P. vivax*. Since 2000 malaria deaths across the world have fallen by nearly half. Campaigns against the disease have brought the toll down markedly. The steepest drop has come from sub-Saharan Africa where 90% of fatalities occur. Malaria still kills *ca*. 850,000 people each year – most of them children in Africa. But the World Health Organization (WHO) estimates that better control prevented the deaths of 3.9 million African children between 2000 and 2013. The WHO believes that malaria cases and deaths could both fall by another 90% in the next 15 years. At a summit in November 2015 heads of state from East Asia endorsed a plan to make the region free of malaria by 2030. The Bill and Melinda Gates Foundation, an important source of funds for antimalaria research and control efforts, believes it can be eradicated completely by 2040. At that time this would bring the world US$2 trillion of benefits and this would rank among humankind's greatest achievements (*The Economist*, 2015j).

Optimism, however, should be tempered by the recollection that past endeavours have failed. A global eradication effort begun in 1955 dramatically decreased malaria deaths over the following decade. But because of flaws in the programme, such overreliance on too few drugs and a lack of adequately trained doctors, and because funding dried up as malaria cases fell, the disease came flying back. Laboratory equipment that can detect the parasites is not available in every country. Laboratory tests take over a week to complete, which leaves plenty of time for mosquitoes to transmit the parasite to new victims. Taking on the malaria parasite and its insect hosts has proven equally hard. Both are frustratingly skilled at developing resistance to drugs or insecticides; and resistant strains tend to spread fast. In the early 20th century several countries reduced malaria by using DDT, a powerful insecticide, against *Anopheles* mosquitoes, until the insects developed resistance. Strains of *P. falciparum* resistant to chloroquine, once a common antimalaria drug, developed independently in several countries in the 1950s and 1960s; nowadays there are found everywhere. Resistance works the other way, too. People living in endemic areas become partially immune to the parasite as a result of repeated infections. Symptoms are most severe among children under five and become less serious as immunity builds over time, almost disappearing in some adults. Where malaria is eliminated people lose immunity over time – so as recurrence of the disease can lead to an outbreak that affects everyone (*The Economist*, 2015j).

"The mosquito is the most efficient transmitter of disease in the animal kingdom," stated Grayson Brown, director of the University of Kentucky Public Health Entomology Laboratory. "They are responsible for more deaths than any other form of insect – or any other animal, period," he added. Drugs and insecticides have been used and are still being used to combat the diseases transmitted by mosquitoes, as well as to control the populations of the latter. These tools take up several years and hundreds of millions of dollars to develop, and in the meantime millions of people die. "We do not have that kind of time," stated Richard Kamwi, former health minister of Namibia, who now coordinates public-health efforts to eliminate malaria across southern Africa. "A malaria vaccine has been ten years down the line for the past 25 years. We need something now, before the tools we do have stop working." New genetic controls, R. Kamwi argued, have to be part of the solution – and by that he meant eradication. "The mosquito vectors must be eliminated to eliminate the parasites," … "I want to call on all the researchers and say that where they have been walking, they must start running" (Sifferlin, 2016).

## *Artemisinin-based combination therapies (ACTs)*

Artemisinin is derived from *Artemisia annua*, a plant used for treating malaria in China for well over 2,000 years. The earliest recipe consulted by artemisinin discoverer, Tu Youyou, who shared the 2015 Nobel Prize for Medicine or Physiology with two other drug discovers, was the *Handbook of Prescriptions for Emergencies*, written in 340 BC by Ge Hong. It gave her helpful hints on how to extract the herb's active principle. Artemisinin has played a pivotal role in the halving, since 2000, of the number of deaths inflicted by malaria. Tu Youyou took an idea she had developed originally in order to help keep North Vietnamese soldiers malaria-free during the Vietnam war, and pushed it forward to become the saviour of a field in which existing drugs were becoming less and less useful, because of the evolution of resistance to them by *Plasmodium* spp. It was nevertheless the combination of artemisinin-derived

compounds and other antimalaria drugs that reduced malaria deaths in children by more than 96%. But artemisinin-resistant Protozoans were found in five South-East Asian countries: Cambodia, Laos, Myanmar, Thailand and Vietnam. So far, most people with *P. falciparum* malaria recovered when treated with a combination of other drugs, but some strains proved resistant to almost all available antimalaria medications (*The Economist*, 2015j).

### *Resistance of mosquitoes to insecticides*

In Africa mosquitoes are rapidly developing resistance to the four insecticides used to treat nets and spray houses. Bed nets treated with insecticide are among the more effective and widespread low-cost measures. Most countries distribute them free. The share of at-risk population sleeping under one rose from 3% in 2004 to 44% in 2013. But everywhere in Africa there are mosquitoes that have become resistant to pyrethroids, the chemicals used in two-thirds of house spraying and the only type to treat bed nets. Resistance to two or more insecticides has developed in nearly two-thirds of malaria-stricken countries worldwide. If the high levels of resistance already seen in western and southern Africa spread, then the most effective interventions of the past decade could become ineffective (*The Economist*, 2015j).

### *Funding the combat against malaria*

The tenacity of malaria means that much more money will be required to wipe it out. For many years the research budget came from the military purse – particularly of Western armies that had to fight in tropical climates. Charities and governments of wealthy countries now pay for the fight against malaria. In recent years their contribution has grown dramatically, accounting for 82% of the US$2.7 billion spent on control and elimination of malaria in 2013. The largest single source of funding the combat against malaria is the Global Fund to Fight AIDS, TB and Malaria, which pools cash from developed countries, the private sector and charities. Between 2002 and 2013 the Global Fund spent US$8 billion battling malaria. The United States is a big donor to the Global Fund : US$1.35 billion in 2015 fiscal year; other aid programmes exist, including the President's Malaria Initiative, launched by George W. Bush in 2005 and now with an annual budget of US$674 million (*The Economist*, 2015j).

Even so, the Gates Foundation states that to remain on track to eradicate malaria, funding must double between now and 2025, when costs are expected to peak at US$6 billion a year. The foundation puts the total cost of eradication at US$90 billion between now and 2040. Despite the steep price tag, the pay-off is far bigger: the Gates Foundation puts the total economic benefits of eradication from productivity gains and health savings in the same time period at more than US$2 trillion (*The Economist*, 2015j).

### *Developing new weapons against malaria*

As well as paying for current schemes, some of the cash goes to developing new weapons against the disease. These weapons belong to three broad categories: new applications of existing treatments, novel treatments, and better diagnosis

and surveillance. In the first category treating everyone in a village or a region with antimalaria drugs can be effective. It tends to work best on settled populations, which may explain why its greatest success has been on a small island: Aneityum in Vanuatu. Nine rounds of mass drug treatment in 1991 helped eliminate malaria there. That lasted until 2002 when the disease was reintroduced, prompting another round of treatment that wiped it out again. Also mass treatment of villages on the Thai-Myanmar border decreased the number of *P. falciparum* cases transmitted during the rainy seasons from 290 to 46 between 2011 and 2012 – but without a better health system, mobile populations could well undo that success. More tests are under way in Asia and Africa on various drug combinations and on comparing their costs with other ways of eliminating malaria (*The Economist*, 2015j).

The second category of new weapons includes entirely new malaria treatments. Research-and-development spending on drugs, vaccines and basic research more than quadrupled between 1993 and 2013, reaching US$550 million annually. Several candidates to supplement artemisinin are in the pipeline but they will not be ready for release for many years. Nine additions to the array of insecticides are in development, but none will be on the market before at least three years (*The Economist*, 2015j).

After decades of near misses a vaccine against malaria, RTS,S, was developed by GlaxoSmithKline (GSK) and the Malaria Vaccine Initiative, an American not-for-profit organization. RTS,S works, but not very well. It cuts the number of malaria episodes among infants and toddlers, typically one to five a year, by 36% over four years. It is not known how it will perform over a long period. That is about half the efficacy needed for such a vaccine to become a potent weapon of eradication by greatly diluting the human reservoir of the parasite, stated James Beeson of the Burnet Institute, an Australian research outfit. An ideal vaccine, according to J. Beeson, would have two components: one would stop infections in humans or stop infected humans from becoming sick; and the other would target transmission by making mosquitoes unable to pass on the malaria parasite (*The Economist*, 2015j). See further down.

Rather than attacking the Protozoan, another method is to modify its hosts. Researchers at Johns Hopkins University (JHU) in Baltimore have developed a genetically modified *Anopheles* mosquito that is highly resistant to the malaria parasite. Mating in the wild would pass this trait on to offspring, though mass release remains at least five years away. With the help of the new genome-editing technology, CRISPR-Cas9, malaria-resistant genes could spread much faster through mosquito populations. It can enable a gene on one of the two chromosomes inherited by a mosquito to copy itself to the other, thus ensuring that all offspring inherit it (*The Economist*, 2015j; see also p. 147). Researchers are trying another technique in JHU large greenhouse in Zambia: feeding mosquitoes sugar laced with a bacterium that blocks their ability to pass on the Protozoan. A fungus with similar properties has also been discovered (*The Economist*, 2015j).

The third category of new weapons against malaria includes better surveillance and diagnosis of the disease. Diagnostic measures help with eradication for three reasons: people treated for malaria are less likely to develop the transmissible form of the parasite; correct diagnosis prevents overuse of antimalarials, thus slowing resistance;

and better surveillance or monitoring improves the aim of antimalaria activity by, say, giving priority to spraying and bed-net distribution in areas with plenty of confirmed cases of the disease. In countries that have reached the elimination stage, defined by the WHO as those with less than one diagnosed case of malaria per 1,000 people at risk per year, strong surveillance systems are critical to prevent outbreaks. For instance the WHO and the Cambodian government have trained a network of Village Malaria Workers – usually farmers or shop owners – to administer cheap, quick and reliable diagnostic tests to anyone suspected of having the disease. Such programmes reach people that national health systems cannot. In Swaziland, southern Africa, where someone with malaria shows up at a clinic, a nurse calls a national emergency number to report the case, which triggers an automatic-text message to the phones of malaria-control programme managers. This helps them track the investigations of foot soldiers, who find out whether it has led to more cases. Such surveillance system has brought Swaziland to the threshold of becoming the first malaria-free country in sub-Saharan Africa. If it succeeds Swaziland will join more than 100 countries that have eliminated malaria within their borders (*The Economist*, 2015j).

Analyzing information gathered on the ground in conjunction with other types of data can be used to direct interventions more accurately. Google and researchers at the University of California, San Francisco, have developed a prototype of a tool that uses weather, landscape and epidemiological-surveillance information to predict which villages are at high risk of malaria in certain months of the year – and should be first on the list of insecticide spraying and other preventive measures. Better mapping could also help to track infections among mobile populations, who carry the malaria parasite across borders. Swaziland's eradicators are busiest straight after Christmas, when the Mozambicans who work on the country's sugar-cane plantations return from visits across the border. Half Swaziland's malaria cases are now imported (*The Economist*, 2015j).

In South-East Asia malaria spreads through areas with high shares of migrant workers. Systems to track it across borders are not yet in place. Botswana, Namibia, Swaziland and South Africa, which aim to eliminate malaria by 2020, are now setting up a regional coordination system with their northern neighbours that will include a joint reporting system, routine sharing of information on patterns of transmission in border regions and screening at border crossings. The biggest challenge to eradication, however, is not mobile populations, the slow progress of vaccines or stiffening resistance, but the eradicators themselves. In the last 1960s malaria rates had been brought close to zero in India, Pakistan, Haiti, Myanmar and dozens of other poor countries. But donors, governments and health systems declared victory too soon: their attention wandered, funding dried up and, over the following two decades, malaria came back (*The Economist*, 2015j).

## *Perseverance in combating malaria*

As a growing number of countries eliminate malaria eradication costs will fall overall, but rise sharply in the remaining countries. These places, often war zones or failed states, tend to have a high prevalence of malaria and populations in remote and inaccessible areas, making eradication particularly costly. Funding needs to be maintained at sufficient levels and directed effectively to complete the task. As it

grows less prevalent, malaria moves from being a disease that can strike anyone to one that still afflicts the rural poor but that urban middle classes can ignore. Previous efforts to rid the world of malaria failed because the political will and funds dried up before the disease was conquered (*The Economist*, 2015j).

Malaria is one of the worst examples of the damage that transmissible diseases can wreak. But it is not alone. AIDS/HIV carries off fit, young adults by the millions and tuberculosis by the hundreds of thousands. Measles, whooping cough and diarrhea together kill 1 million children a year. Like malaria, since 2000, deaths from measles have fallen by 75%, to *ca*. 150,000. These successes are to be celebrated, but an even greater prize exists: to go beyond controlling infections and infestations, and instead to eradicate some of them completely by exterminating the pathogens and parasites that cause them. That was achieved a couple of times in the past, for smallpox (a human disease) and rinderpest (a cattle disease similar to measles). The end is reckoned to be close for poliomyelitis (a virus that once killed and crippled millions) and dracunculiasis (a parasitic worm). But more must follow (*The Economist*, 2015j).

That is in fact the rather long-term objective of malaria eradication : combating the insect vectors of the disease. As explained in the case of dengue progress is being made in the eradication of *Aedes aegypti* and *Aedes albopictus*, using the gene drive technique (see p. 147). Likewise the team of Andrea Crisanti is expected to launch a trial in semi-wild conditions at the genomic pole of Perugia in Italy. The objective of the trial is to be far from the standardized laboratory conditions and to introduce in large-sized cages several wild mosquito strains. "The genetic diversity of *Anopheles* is very important," warned Ken Vernick, in charge of the genetic unit of insect vectors at the Pasteur Institute in Paris (Herzberg, 2016). Once this obstacle is overcome the trial must be tested in the field, i.e. in Africa. Five sites have been selected, including those in Mali, Burkina Faso and Uganda, which are members with Imperial College (London) of the project Target Malaria – a consortium funded in particular by the Bill and Melinda Gates Foundation. The big challenge remains, that of "social-risk," underlined Kevin Esvelt, assistant professor at the Massachusetts Institute of Technology: "If a country is tired to see its children dying, can he make alone a decision that might affect its neighbourhood? And how these neighbours will react?"

Kevin Esvelt thinks that instead of being paralyzed by the possible danger or risk, there is "an extraordinary opportunity to do science differently." He went on saying that: "Innovations have always been imposed on populations. The best example is that of GMOs. They have been developed by big private companies in a hidden way, without obvious benefit, without the will to have a debate with the public, nor to inform on secondary effects. So let us do the contrary and act in full transparency, through the publication of all what we do. Let us take the time to accumulate and disseminate the knowledge of possible risks. And let us help the populations to make decisions by themselves. In doing so, both science and democracy will be fostered" (Herzberg, 2016). To sum up the promoters of gene drive techniques, aimed at eradicating mosquito populations, are convinced that these techniques could save a lot of lives in the future, even though the current weapons against malaria have been and are still effective, with the goal to reduce by 95% the occurrence of the parasitic disease by 2035.

## Malaria and the African pharmaceutical market

### *Antimalaria drugs*

The French pharmaceutical group Sanofi is at the forefront of the struggle against malaria. It developed and marketed an artemisinin-based drug, called Asaq, and sold it at a price between 15 and 45 cents of a Euro for a dose to be taken by a child. The patient is cured in three days. The manufacturer of Asaq is Winthrop, a subsidiary of Sanofi specialized in the production of generics. Burkina Faso where 6.9 million cases of malaria were recorded in 2013 (out of the 207 million cases reported worldwide by the WHO), is among the first of Sanofi clients in sub-Saharan Africa, behind Côte d'Ivoire and Senegal. Asaq is marketed in Burkina Faso according to a policy of no profit, no loss, that is to say at its production cost. Sales amounted to less than €2 million – a tiny proportion of the €33-billion Sanofi annual turnover in 2013 (Hecketsweiler, 2014a).

Sanofi policy aims, through the commercialization of antimalarial drugs, to become in Africa a "health partner". In countries where there is a lot to be done in terms of public health, to show an interest in endemic diseases like malaria would lead to build up collaborative links with health authorities, physicians and patients. According to IMS Health, a consulting firm, Africa's pharmaceutical market value was estimated at US$30 billion (or €22 billion) in 2016 and at US$45 billion in 2020. Sanofi strategy was therefore to take advantage of these prospects. Thanks to the development and commercialization of the antimalaria drug Asaq, Sanofi has been able to better design its business model and improve both its image and network (Hecketsweiler, 2014a).

### *Sanofi strategy in sub-Saharan Africa*

Still in Burkina Faso Sanofi delivered by early April 2014 teaching kits which were highly appreciated by the country's health authorities which cannot fund such kind of teaching tools. They are particularly useful in villages where Sanofi provides audio-visuals and advisory services aimed at educating people who are not always aware of malaria impact. As in other African countries the activities carried out by Sanofi in Burkina Faso aim to highlight the name of Asaq in the patient's mind when the pharmaceutical group is facing an important competitor which is traditional medicine. It is true that "tradipractitioners" are the first recourse for the patients; they offer to malaria-stricken people plant-based extracts ("infusions"), while there is a need to send the patients to local health centres where they can receive the appropriate treatment (Hecketsweiler, 2014a). Another concern of Burkina Faso's – and more generally of African countries' – health authorities is the sales of counterfeit antimalaria drugs. In their struggle against such a rife Sanofi can team up with the health authorities and offer them its expertise. Sanofi has a laboratory in Tours, centre-west of France, where counterfeit drugs can be identified; furthermore this laboratory collaborates with Interpol – the international police agency (Hecketsweiler, 2014a).

Sanofi competitive edge on the African pharmaceutical market is that 70% of its drugs which are marketed in Africa are produced in seven factories located in the continent. For instance Asaq is manufactured in Morocco and Sanofi built up a partnership with

Drugs for Neglected Diseases Initiative (DNDI) in order to develop the antimalaria drug. Asaq was marketed in 2007 with a major competitive advantage: its price was less than US$1 for adults and less than USCents50 for children. "Asaq was three to four times less expensive than Novartis Coartem, also artemisinin-based and marketed a few years earlier. François Bompart, in charge of Sanofi programmes of access to medicine, stated that thanks to Asaq Sanofi "set up a new price standard" (Hecketsweiler, 2014a). Even though Sanofi arrived late in the sector of antimalaria drugs, it was able to conquer about one-quarter of the African market. This result was also made possible thanks to the international support and assistance for the control of malaria, which grew up to US$2.3 billion in 2014 from US$100 million in 2000. Sanofi strategy regarding malaria was expected to help the French big pharma occupy an increasingly powerful position on the African pharmaceutical market; its sales reached more than €1 billion by 2014 and the company hoped to sell there its blockbuster drugs (Hecketsweiler, 2014a).

## Antimalaria vaccines

The European Medicines Agency (EMEA) Committee for Human-Use Health Products, on Friday 24 July 2015, gave a favourable scientific advice on Mosquirix, a vaccine against malaria and hepatitis B developed by GlaxoSmithKline (GSK). This candidate vaccine is the most advanced in terms of clinical trials, but its protection against the parasitic disease is still considered modest. The WHO is going to examine the whole file in order to decide whether it will recommend the use of Mosquirix (Benkimoun, 2015f). GSK does not intend to commercialize its antimalaria vaccine in Europe. The pharmaceutical group has used an approach called "article 58" that permits the evaluation by the EMEA of the quality, safety and efficacy of a drug or a vaccine, whose commercialization is not foreseen in Europe. The Mosquirix is aimed at vaccinating in endemic zones children aged between six weeks and 17 months against malaria caused by *Plasmodium falciparum*. Mosquirix has undergone a large-scale trial, called of "phase 3" and carried out in seven African countries (Burkina Faso, Gabon, Ghana, Kenya, Malawi, Mozambique and Tanzania). The results of this trial were published on line on 5 November 2015 in *The Lancet* (Greenwood and Doumbo, 2015). As summarized by the EMEA, "the data collected during the trial have shown that Mosquirix gives a modest protection against *Plasmodium falciparum* malaria among children during the 12 months following the vaccination. The vaccine was efficient in the prevention of the first or the only malaria clinical episode among 56% of children, aged between five and 17 months, and among 31% of children, aged between six and 12 months. The vaccine's efficacy decreases after a year." The EMEA concluded that the vaccine's safety profile is "acceptable" (Benkimoun, 2015f).

GSK and the humanitarian organization PATH have created a partnership in order to develop and produce this candidate vaccine. GSK invested US$365 million (or €334 million) to that end and was expected to add another US$200 to US$250 million. Thanks to the funds provided by the Bill and Melinda Gates Foundation, PATH malaria programme brought in €200 million to the whole project. These amounts are much higher than those allocated to other candidate vaccines. GSK and MVI (Malaria Vaccine Initiative) have warmly received the green light given by

the EMEA to the development of their vaccine, but other reactions have been more cautious. In an editorial disseminated on 24 July 2015 on the site of the BBC, Seth Berkley, executive director of the Global Alliance Vaccine Initiative (GAVI), and Mark Dybul, executive director of the Fund to Fight AIDS, Tuberculosis (TB) and Malaria, have raised the issue regarding the risk for the vaccine to give "a false feeling of safety" to the vaccinees. This viewpoint was shared by Marc Thellier, director of the National Malaria Reference Centre at Paris La Pitié-Salpêtrière hospital who recalled that since 2005 "the long-lasting insecticide-soaked antimosquito nets, fast diagnostic tests, as well as combinations of artemisinin-based drugs have significantly reduced the impact of the disease. The resources devoted to all these means of controlling malaria must not be diverted towards a means of partial protection and whose duration is not known." He also stated that the modest but tangible results of Mosquirix, in terms of lives saved, were obtained in very well conceived clinical trials, carried out in experienced centres; these results may not be as good in real-life conditions and on a larger scale (Benkimoun, 2015f).

The WHO agreed on the fact that the funds needed for producing the vaccine should not be diverted from those being used to support global and regional operations aimed at combating malaria. The WHO recalled that "it will take into account, in its evaluation of the large-scale utilization of the vaccine, such factors not examined by the EMEA as the feasibility of that utilization, the affordability of prices, the cost-efficacy ratio and the vaccine's interest for public health compared with other antimalaria interventions" (Benkimoun, 2015f).

## Lyme's disease

### *An anthropozoonosis transmitted by ticks*

Lyme's disease is an anthropozoonosis, i.e. it affects both animals and humans. The pathogen is transmitted by ticks to humans: it has been identified in 1982 as *Borrelia burgdorferi* (Bb) by Willy Burgdorfer, an American scientist who died on 19 November 2014 in Hamilton's hospital, Montana. Also called Lyme's borreliosis the disease has been diagnosed in the 1970s in the town of Lyme, Connecticut. The symptoms are: extreme fatigue, joint aches, facial paralysis, and even loss of movements (arms and legs) in the most severe cases of the disease (Maruchitch, 2014).

The incidence of Lyme's disease is increasing worldwide. In the United States, in 2013, the Centers for Disease Control and Prevention (CDCs) estimated at 300,000 the number of new cases annually, ten times more than former estimations. In Europe there are between 65,000 and 85,000 new cases annually with important regional variations, according to a report on Lyme's disease by the High Council for Public Health (HCSP, French acronym) published in March 2014. In France there are 35,000 new cases of Lyme's disease annually, according to the data provided in 2013 by a network of general practitioners, called Sentinelles ("People on the lookout"). But the actual number of cases could be much higher: the patients' association France Lyme estimated at 650,000 the number of chronic cases in the country (Maruchitch, 2014).

In 2012 the French Directorate-General of Health requested the HCSP to prepare an updated state-of-knowledge on Lyme's borreliosis. The report, published on 4 December 2014, makes a survey of the current situation that is rather complex, and issues recommendations, particularly with respect to improving diagnostic tools and care for patients. On the other hand, by mid-August 2014, a European resolution requested the European Commission to undertake activities on Lyme's disease. Finally in France, in October 2014, a draft law was submitted by 70 deputies to the National Assembly (parliament) with a view to carrying out an action plan between 2015 and 2020 (Maruchitch, 2014).

## *Reservoir and vectors*

Wild animals are the reservoir of the pathogen, *Borrelia burgdorferi*, e.g. birds, small rodents, mammals. The vector of the pathogen is the tick, an acarid whose development comprises three stages (larva, nymph and adult) and which feeds itself by sucking the blood of the hosts to which it clings. During its lifetime the tick will have three blood meals. It goes often unperceived because of its small size and also because it injects anesthetic substances that make its bite painless. "There are more than 800 different species of ticks worldwide," explained Muriel Vayssier-Taussat, a research director at the French National Agricultural Research Institute (INRA, French acronym). "In Europe, ticks which transmit bacteria belonging to the genus *Borrelia*, belong to the species *Ixodes ricinus*." Their specific trait is that they can feed on a wide range of animal species, wild and domestic animals, as well as on humans (Maruchitch, 2014).

It should be mentioned that all tick bites do not systematically transmit borreliosis. But it is highly recommended in infested areas to wear long and closed clothes, or even to use skin repellents (except in children under 30 months of age and in pregnant women). After being exposed, e.g. during a walk in a forest or woodland, or a picnic, the whole body should be scrutinized. If a tick is found, it must be withdrawn with a teaser, because the use of chemicals may induce a regurgitation that increases the risk of infection (Maruchitch, 2014).

## *Diagnosis*

Three clinical stages of the disease have been described. In the first one there appears a circular inflammatory zone, called migrating erythema, around the site of the bite by the tick. This is not, however, systematic and it may escape the sight of the patient, who does not always remember whether he/she has been bitten by a tick. The second stage occurs after one to six months and it comprises several kinds of alterations: joint aches, cardiac, neurological, cutaneous, ocular, muscular and liver disorders. Finally, during the third phase (after several years), the disease consists of joint aches, neurological and cutaneous disorders. Christian Perrone, head of the department of infectious diseases at Raymond Poincaré hospital in Garches, near Paris, has become a renowned specialist of Lyme's disease. He reckons that the illness symptoms are numerous and not very specific. That is probably why Lyme's borreliosis is called, like syphilis, a "great imitator" (Maruchitch, 2014).

There are, nevertheless, indirect diagnostic methods that help detecting the presence of specific antibodies induced by the disease – direct methods consisting of isolating the bacterium itself. The technique to be followed has been agreed upon and published after a consensus conference on antiinfectious therapeutics of Lyme's borreliosis in 2006. It is recommended to start with an ELISA test which is less accurate for Lyme's borreliosis than for HIV, for instance. Thus, if the ELISA test is positive or uncertain, it must be confirmed by using another test, Western Blot. Unfortunately physicians often base their conclusions mainly on serological results that are not very reliable, particularly if the disease is in its first clinical stage, according to Benoît Jaulhac, in charge of the National Reference Centre on *Borrelia* in Strasbourg. The detection of the disease should rely above all on a clinical identification and biological tests are there just to help, underlined Philippe Boullenger of Siemens Healthcare Diagnostics, who develops the ELISA tests. In fact Lyme's disease can be caused by several species of *Borrelia*: *burgdorferi*, but also *afzelii*, *garinii*, etc. And the available serological tests do not detect all the strains of the pathogen. Another species, *Borrelia miyamotoi*, has been described in Japan. It has been found in Lyme's disease outbreaks in the United States, Russia, Eastern Europe, the Netherlands, but not in France, where it is never searched in humans – while it has been found in ticks. *B. miyamotoi* causes a disease identical to Lyme's disease, or another pathology – recurrent fevers. Tests should be developed for detecting this species of *Borrelia* (Maruchitch, 2014). While patients and physicians are still puzzled by the lack of reliable tests for detecting Lyme's disease, veterinarians have access to a wide range of information on the illness which is little exploited. There should be more dialogue between physicians and veterinarians in order to reach a consensus about how to detect the disease and to design reliable tests.

### *Treatment*

Still according to the consensus conference held in 2006, in France, it is recommended during the first stage of the disease, to administer antibiotics for 21 days maximum. During the second and third stages, antibiotherapy should be pursued for 28 days. However physicians advise to still continue with antibiotherapy because the patients are not completely cured. Richard Horowitz, who published an important state-of-knowledge on the treatment of Lyme's disease, recommends both antibiotherapy and phytotherapy. According to the American physician such combined treatment was successful among 70% to 75% of his patients (Maruchitch, 2014).

In many patients the disease becomes chronical. Daniel Christmann, professor of infectious and tropical pathologies at the university hospital in Strasbourg, explained that muscular pain, memory disorders or the difficulty to focus attention that can be noted among patients who have been treated, would be somewhat residual symptoms of the disease. He underlined that "Lyme's disease, if well treated, does not become a chronic illness" and that "repeated antibiotherapy is useless." But he also mentions the existence of "co-infections, " because ticks are multi-infected and can transmit up to seven pathogens. Therefore, when the symptoms persist, one should make sure that there is not another infection, such as anaplasmosis or

bartonellosis. D. Christmann has found cases of co-infections that should be taken care of during the treatment, but he stated that their frequence is low (Maruchitch, 2014). Richard Horowitz, on his side, considers that co-infections are frequent, and, in addition to anaplasmosis, ticks can transmit several other diseases such as ehrlichiosis, brucellosis, babesiosis. According to him "more than 80% of patients are co-infected by Lyme's borreliosis and *Babesia* which causes babesiosis." "These are pathogens that physicians do not often see, while veterinarians are much more familiar with them and for many years," remarked Denis Fritz, a veterinarian in charge of a veterinary-analysis laboratory (Maruchitch, 2014).

The French High Council for Public Health (HCSP) report has drawn the following conclusion: "It seems clear that there are many patients showing chronic and invalidating symptoms which are considered as those of 'Lyme's disease', but without certainty because of negative biological tests or the persistence of antibodies (…). Some of these symptoms may be those of a Lyme's disease which cannot be diagnosed due to imperfect biological tests. But one could also think that a large number of these patients might suffer from an infection caused by other microorganisms living in ticks and transmitted by the latter when they bite humans." Muriel Vayssier-Taussat of INRA has discovered thanks to her numerous studies of ticks that among the pathogens these ticks can transmit half of the bacteria were unknown, as well as half of the parasites and 80% of the viruses. "Ticks are a world which is still largely unexplored," she concluded (Maruchitch, 2014). See also Lenglet and Perrin (2016).

# GENETIC AND RARE DISEASES : A NEW FRONTIER FOR MEDICAL BIOTECHNOLOGY AND PHARMACEUTICAL COMPANIES

The so-called rare diseases are those which affect few or very few persons within the whole population. The United States National Institutes of Health (NIH) recognize 7,000 rare diseases – defined as those which affect fewer than 20,000 people, each. Research published in 2013 in the *Journal of Rare Disorders* stated that *ca*. 8% of Americans – some 25 million people – were affected by rare diseases, and that it took an average of seven and a half years to diagnose a rare disease. In Europe and the United States *ca*. 60 million people suffer from these diseases and around 80% of the latter are genetic diseases (Cassino et al., 2013).

These genetic diseases are often detected during infancy and the mortality is generally high. In Europe there is no more than 1% treatment. In France, for instance, there are 3 million patients (2015) and 30,000 new cases of genetic diseases are diagnosed annually; one is dealing with 5,000 severe, chronic and complex diseases. At Paris Necker-Enfants Malades hospital – an old and renowned pediatric hospital –, of the annual 300,000 medical consultations, half of them concern genetic diseases, with more than 40,000 patients. In that hospital genetic diseases are considered the primary reason for medical visits; the medical areas concerned span from nephrology, cardiology, dermatology, ophtalmology, hematology and endocrinology to infectious diseases, metabolism, vascular and skeletal malformations, mental illness and immunology.

## Need for fast and appropriate diagnostic tools

In the United Kingdom, with all the resources of the country's National Health Service at a general practitioner's disposal, rare-disease diagnosis takes an average of five years and a half, compared with seven and half years in the United States. Also physicians often get it wrong. A survey of eight rare diseases in Europe found that *ca*. 40% of patients received an erroneous diagnosis at first. This is something that can lead to life-threatening complications (*The Economist*, 2015f). Current research aims to reduce the diagnosis uncertainties and to develop tools for a faster and appropriate diagnosis; to identify the disease mechanisms and understand the physiopathology; to promote therapeutic innovations and applications; to use the models of rare diseases in order to understand more frequent illnesses; and to disseminate knowledge and knowhow relating to this complex area of medical research.

Diagnostic crowdsourcing is an interesting approach to reducing the time for an appropriate diagnosis of a rare disease through a website called CrowdMed. The volunteer diagnosticians are students, retired physicians, nurses and even lawyers and women who enjoy pooling their intelligence against a good medical mystery. CrowdMed seems to work. One woman, for instance, had been burping as many as 150 times a day. Two endoscopies, a laryngoscopy and an abdominal ultrasonic scan all failed to elucidate her illness, and eliminating wine, chocolate and gluten from her diet failed to solve it. Crowdsourcing offered a diagnosis of "supragastric belching." That suggested treatment by a specialist called a speech pathologist, which cured her. In another case a 78-years-old man who was himself a doctor had had muscle pains in his face, upper body and extremities for decades. Over two months 50 medical detectives reached a diagnosis of fibromyalgia. In a review that CrowdMed carried out of several hundred cases posted on the site, *ca*. 80% of patients said the suggestions made to them were accurate. But diagnostic crowdsourcing faces competition from machine-based systems such as Watson, designed by IBM that digests large amounts of data and draws inferences from them (see p. 53). *The Economist* (2015f) stated that "it will be interesting to see whether the collective wisdom of practitioners and enthusiastic amateurs prevails over an algorithmic synthesis of the world's medical literature" (*The Economist*, 2015f).

In fact the approach to understand and to mitigate rare (genetic) diseases must rely on the close collaboration between clinicians (including general practitioners) and biologists (including geneticists). Even though the most recent machines that enable the sequencing of whole genomes at an everdecreasing cost and in a short lapse of time, certainly contribute to the diagnosis of rare (and genetic) diseases, this technology entails the necessary collaboration with bioinformaticians and physicians for the interpretation of the results and for an appropriate treatment or mitigation.

## *Downe's syndrome or trisomy 21: controversy about its discovery*

In 1959 three French researchers, Jérôme Lejeune, Marthe Gautier and Raymond Turpin, signed in this order the publication on the discovery of trisomy 21 – the cause of the genetic disease known as Downe's syndrome or mongolism. The Foundation that bears the name of J. Lejeune has always claimed that he was the discoverer of the genetic defect: an additional chromosome to the 21 pair of chromosomes (hence the name of trisomy 21). However, on 14 September 2014, the Ethics Committee of the French National Institute for Health and Medical Research (INSERM), which had been requested in 2013 by a dozen geneticists to review the issue of who was the real discoverer of trisomy 21, stated that the role of J. Lejeune is "unlikely to have been a major one." It could be surprising that an ethics committee be requested to give its opinion on the paternity of a discovery. Hervé Chneiweiss, chairperson of INSERM Ethics Committee, explained: "We have accepted to do so because what happened to Marthe Gautier raises two current ethical issues, that of gender and that of scientific honesty. That is why we recalled, along with our statement, the rules concerning the order of those who author a scientific article, the first author being the scientist who carried out the experimental work" (Chevassus-au-Louis, 2014).

In the process of preparing its statement the INSERM Ethics Committee requested the Jérôme Lejeune Foundation to provide any useful information on the history of the discovery. There was no response and the committee had to work on the basis of the statements made by the last witnesses of this story. However the Foundation broke its silence by publishing on line and as a reaction to the INSERM Ethics Committee's statement, a selection of archives (including letters, experimental copybooks), which aimed to demonstrate that Marthe Gautier had just played the role of a laboratory assistant, while J. Lejeune was the mastermind behind the discovery. This argument did not convince H. Chneiweiss who stated: "We are willing to change our view on the basis of the work of historians, but the archives published on line are not convincing enough so as to change our main message, i.e. that J. Lejeune cannot be considered as the discoverer of trisomy 21" (Chevassus-au-Louis, 2014).

In February 2014 Marthe Gautier who was 89 years old, could not receive the Prize of the French Federation of Human Genetics, as a reward to her participation in the discovery of the additional chromosome responsible for the Downe's syndrome. She was impeded by the Jérôme Lejeune Foundation who sent court bailiffs in order to record the statements of M. Gautier. The organizers of the symposium in which M. Gautier was to take the floor, had to cancel her address. The intimidation gesture of J. Lejeune Foundation had a very bad impact on its image: the journals *Nature* and *Science* widely publicized the event and gave it an international relevance. It should be mentioned that the behaviour of the foundation causes embarrassment among many researchers; the vice-chairwoman of the foundation's scientific council resigned. It is true that the foundation allocates *ca*. €2 million a year to the research on mental handicap and this makes it the main fund provider in this area. On the other hand the foundation supports many initiatives against abortion; it also played a key role in the movement against the decision made by the French education minister to promote gender equality at school (Chevassus-au-Louis, 2014).

Marthe Gautier, on her side, received the Légion d'honneur (with the grade of officer) on 16 September 2014 – a distinction she refused twice earlier on and she finally accepted it as a reaction to the "impudence of the foundation." She said so during the ceremony which took place at Paris Trousseau hospital, where the geneticist had discovered, more than 50 years before, the additional chromosome that causes Downe's syndrome (Chevassus-au-Louis, 2014).

## A lucrative market for pharmaceutical companies; orphan drugs

Pharmaceutical companies have shown an increasing interest in the development of drugs that could mitigate the effects of rare diseases; these drugs were called "orphan" drugs because they target precisely rare or very rare diseases which do not generally draw the attention of big pharmas. Thus, according to Jonathan Gardner of EvaluatePharma – a consultancy and study firm – "the sales of orphan drugs will have an annual growth of 11% from 2015 to 2020, while those of conventional drugs will have a growth rate of only 4% a year during the same period." As a result the value of this market will rise to US$178 billion from US$102 billion in 2015 (Hecketsweiler, 2015b, 2016b). These annual sales of orphan drugs will amount to 20.2% of global sales

of drugs in 2020 (excluding the sales of generics), compared with 15.5% in 2015 and 8.7% in 2006. These figures can be completed by the average cost per patient per year of these orphan drugs compared with the equivalent cost of a non-orphan drug: US$111,820 compared with US$23,331 in 2014. In 2010 the figures were: US$83,550 and US$16,448. Soliris, the drug developed by the American biotechnology company Alexion Pharmaceuticals to treat a very rare blood disease, has a cost per patient per year of US$410,000 – a record figure (see p. 286) [Hecketsweiler, 2016 b].

When the United States Congress voted the Orphan Drug Act (1983) physicians had less than 40 drugs to treat their patients. Nowadays they have many more at their disposal. According to the Orphan Drug Report 2015, published by Evaluate Pharma, of the 50 drugs approved in 2014 by the FDA, 19 were orphan drugs and 31 non-orphan medicines, the percentage being 38% of the total for orphan drugs; compared with 31% in 2010 (8 orphan drugs approved and 18 non-orphan ones). In order to obtain such result the American authorities have given several incentives to the pharmaceutical laboratories for investing in those treatments. Research on rare diseases is funded by public grants and the R&D investments made by the companies benefit from a 50% tax exemption. Also the overall costs of developing drugs were decreased further to simplified clinical trials. The latter include less stages and involve less patients: a few hundreds or dozens in the case of very rare diseases, compared with several thousands for a conventional drug. The requests for commercializing the drugs are also reviewed more rapidly. For instance the French biotechnology company Erytech, which developed a molecule that could "starve" tumours, was expecting the authorization for commercializing its drug by the end of 2016 from the United States FDA. The first indication of the drug is a very rare blood cancer, acute lymphoblastic leukemia. "We have invested €50 million in the research and development of our molecule, i.e. ten times less than for a non-orphan drug. The main difference between the two kinds of drugs is the size of the trials: in our study only 100 patients have been involved, compared with more than 1,000 usually," stated Gil Beyen, Erytech manager. "The dialogue with the health authorities is much easier. We benefit from the scientific advice of their experts and we discuss with them the protocols of clinical trials," he added (Hecketsweiler, 2016b).

And, above all, every drug has a commercial exclusivity during seven years, or even ten years in Europe, where a system comparable to that of the United States had been set up in 2000. This means that during this period of exclusivity (seven or ten years) any laboratory or company cannot launch another drug for treating the same disease (except if it is more efficient). Such kind of protection enables the laboratories or companies to ensure a return on investment before the commercialization of drugs developed by rivals. This is an outstanding advantage in the global drug market where the competition is so harsh (Hecketsweiler, 2015b). "Another key factor is the generally high price granted to the treatments of rare diseases: up to several hundred thousands euros per patient per year," recalled Nadège Penhaleux of Alcimed, a consultancy firm on innovation. For instance the drug Soliris, developed by the American biotechnology company Alexion Pharmaceuticals (see p. 286) has generated

in 2014 *ca*. US$410,000 per patient per year. Another drug that is prescribed to hemophiliacs, Advate, cost US$450,000 per patient per year. One can find on the same list and behind Soliris and Advate, the Revlimid of Celgene (US$165,000 per patient per year) and Glivec of Novartis (US$100,000), that are prescribed for the treatment of rare bone-marrow and blood cancers (Hecketsweiler, 2015b).

Specialized in hematology, Celgene has developed Revlimid, a drug prescribed for the treatment of multiple myeloma – a blood disease that affects one person out of 100,000. In 2014 Revlimid sales amounted to US$3 billion. In 2020 it might become the orphan drug that is most sold worldwide, with annual sales estimated at US$10 billion. "We invest 30% of our revenue in R&D. That is the best way to fund innovation," underlined Jérôme Garnier, medical director of Celgene in France. Celgene, valued at almost US$80 billion in the stock of exchange in 2015, not very far from the French big pharma Sanofi (US$105 billion), can build a real pharmaceutical group. With the acquisition of Abraxis and Pharmion for almost US$3 billion each, Celgene has beefed up its portfolio of "niche" anticancer drugs. In June 2015 the acquisition of Receptos for US$7 billion enabled Celgene to play a significant role in the treatment of inflammatory diseases. These three biotechnology companies had in their portfolio promising molecules for the treatment of rare diseases. "Our R&D is guided more by science than by the market," insisted J. Garnier. "A superdrug is above all a drug that brings in a significant progress in an area where there was nothing before," he added (Hecketsweiler, 2016b).

While in 2014 it was often stated that the era of blockbuster drugs was over, orphan drugs were expected to become sources of large profits for some pharmaceutical companies. The following top 10 companies present on the global market of orphan drugs show a predominance of American and Swiss pharmas and biotechnology companies :

1. Celgene (USA), with an annual turnover of US$12.7 billion in 2020.
2. Novartis (Switzerland), US$ 12.7 billion in 2020.
3. Bristol-Myers Squibb (USA), US$12.6 billion in 2020.
4. Roche (Switzerland), US$12.5 billion in 2020.
5. Shire + Baxalta (Ireland), US$9.5 billion in 2020.
6. Alexion Pharmaceuticals (USA), US$6.3 billion in 2020.
7. Pfizer (USA), US$6 billion in 2020.
8. Vertex Pharmaceuticals (USA), US$5.9 billion in 2020.
9. Merck & Co (USA), US$5.8 billion in 2020.
10. AbbVie (USA), US$5.7 billion in 2020.

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13. Sanofi (France), US$4.5 billion in 2020 (Hecketsweiler, 2016b).

Genzyme, acquired by Sanofi for more than US$20 billion in 2011, is the most active subsidiary of the French big pharma. The drugs developed by Genzyme target three pathologies associated with a lack of specific enzymes: Gaucher's, Pompe's and Fabry's diseases that affect *ca*. 10,000 persons each worldwide. The sales of these drugs amounted to *ca*. €2 billion in 2014, or 7% of the annual turnover of Sanofi pharmaceutical division. The Irish company Shire is Genzyme big competitor; in January 2016 it acquired Baxalta for US$32 billion so as to become the world's fifth-important company in the development and commercialization of orphan drugs (Hecketsweiler, 2016b).

However all orphan drugs do not reach the above-mentioned prices and sales, because most of them concern small populations. That is why the pharmaceutical companies develop other kinds of drugs that target various diseases. The only concern is that health-care insurers or social-security agencies have difficulties to cope with the increasing costs of the drugs; several laboratories had to lower their prices when agencies could not pay the price agreed on initially. This is the case of Kalydeco, a drug developed by the American company Vertex Pharmaceuticals against certain forms of cystic fibrosis, which is sold in France at the price of more than €19,000 the box of 56 pills. This drug is carefully prescribed in some countries, like Canada, because of the lack of agreement on its price. It is not always easy during the negotiations on that price to say "no", when the orphan drug can save lives. In this respect Hervé Ronin of Bryan, Garnier & Co. – an investment bank – stated that to make savings "the States had rather save one US dollar on the price of an antidiabetes drug which is prescribed to millions of patients" (Hecketsweiler, 2015b).

According to the Institute for Clinical and Economic Review – an American non-governemental organization that has evaluated in 2015 the cost/benefit ratio of new very costly treatments in California, including orphan and non-orphan drugs – the overcost for this American State was estimated at US$3 billion. And after 20 years, taking account of the savings made in treating the diseases' complications the bill would still amount to US$1.8 billion. The question raised was : to make this investment should we abandon other public-health activities, perhaps more useful? In France a decree was issued in March 2016 with a view to indicating the conditions of bearing the costs of the most expensive drugs, and this put this issue at the heart of public debate (Hecketsweiler, 2015b). See also Part One, p. 102.

## France's combat against genetic diseases

### *The Institute of Genetic Diseases (IMAGINE)*

France is at the forefront of the battle against genetic diseases. In June 2014 a new Institute of Genetic Diseases (IMAGINE, French acronym) was inaugurated in Paris; it is pegged to Necker-Enfants Malades university hospital. IMAGINE status is that of a "scientific cooperation foundation", based on the alliance between the public and private sectors and created by the ministry for higher education and research. It has six

founding members, including the National Institute for Health and Medical Research (INSERM), Paris Descartes University, the Public Assistance/Hospitals of Paris, the French Association against Myopathies and Paris City Hall. The main objective of IMAGINE is to gather in the same area patients, physicians (clinicians) and geneticists with a view to better understanding and treating genetic diseases. Of the latter, some like cystic fibrosis, hemophilia and sickle-cell anemia (drepanocytosis) have been known for a long time, while the majority of them are still an enigma and some affect a very small number of patients. An international day is devoted on 28 February to genetic diseases which are very often detected during childhood (Hecketsweiler, 2015f).

At the French IMAGINE, *ca*. 421 persons, including 50 researchers, 50 professors-and-researchers, 164 engineers and technicians, 50 post-doctoral scientists, 71 PhD students and 29 students and trainees, were working in 2015, in a building where the architecture enhances a close cooperation among the staff and with the patients. Twenty-four research groups are working at the institute, each one having a leading renowned scientist; they deal with a wide range of subjects, from human genetics of infectious diseases, molecular and physiopathological bases of cognitive disorders, to the genetics of monogenic auto-inflammatory diseases and the immunogenetics of pediatric autoimmune diseases.

Before the creation of the institute most of the current IMAGINE scientists and researchers were located in several buildings and the dialogue was far from being easy; rivalry and defiance between the research teams was often the case. The new facilities' advantages have led to good results, such as the development of an electronic chip for the diagnosis of intellectual deficiencies and autistic syndromes of genetic origin. Of the 20,000 children attending IMAGINE over a year, 5,000 are affected by these diseases. The latter are very difficult to understand and are a real headache for physicians and biologists, who cannot often determine their etiology. However 250 genes have been identified and paly a role in these deficiencies, too often erroneously diagnosed as psychiatric disorders. The teams of IMAGINE and Necker-Enfants Malades university hospital have closely collaborated to develop a chip called iDFix which will help detect these deficiencies among all young children simply and at a moderate cost (Hecketsweiler, 2015f).

Scientists and physicians working at IMAGINE have learnt to share their data, with a view to creating an in-house tool for gathering all the informations – clinical tests, biological analyses, gene sequencing – concerning the patients and cohorts followed by the various research teams. A biobank has been created in the underground floor and it contains more than 40,000 biological samples taken from patients. "Some samples are more than 30 years old and the research work on them has been made possible through the immortalization of the cells of the patients who are doomed to die," stated Anne Esling, a biological engineer, who is working at the biobank. All the written agreements of the patients are being filed and the identity of every one of them is hidden behind a bar code stuck on each test tube (Hecketsweiler, 2015f).

Also located in the underground floor of the institute are the facilities for laboratory animals used in the experiments: a total of *ca*. 25,000 lines of mice, zebra fish and flies, that are mostly genetically modified in order to test research hypotheses. "Even though the animal models cannot always predict what would happen in human beings, they are indispensable," claims Alain Fischer, IMAGINE director, renowned for leading with Claude Griscelli – a former director-general of INSERM – the team who treated in 2000 the first so-called "bubble babies". These are newborns who suffer from a very severe immune deficiency and have therefore to be maintained in sterile "bubbles"; they have been treated using a "drug gene" introduced in their defective cells (Hecketsweiler, 2015f).

The proximity between patients and clinicians/biologists, that is an important feature of IMAGINE concept, facilitates much faster clinical trials. "Software scientists are in charge of following up the patients in the different departments of Necker hospital," indicated A. Fischer who received in January 2015 the most prestigious Japanese scientific award, the Japan Prize, for his "considerable work on gene therapy." A. Fischer wants to make IMAGINE a model from both the scientific and economic viewpoint: "Our goal is also to make some money," he stated. In 2015 public funding was the main source of its €60-million budget, but the institute intends to attract private funds for an increasing number of research projects. IMAGINE is protecting its researchers' work through patents and thereby tries to draw resources from their discoveries or innovations. For instance Arnold Munnich, a renowned geneticist and pediatrician, working at both IMAGINE and Necker hospital, indicated that in the so-called "glass-bone" disease, researchers are developing a molecule that can increase bone density in the affected children. If clinical trials are successful that molecule could become a drug produced by a pharmaceutical or biotechnology company, in exchange of royalties transferred to IMAGINE. In fact the institute signed research contracts with Novartis (€400,000) or Genzyme (€135,000) [Hecketsweiler, 2015f].

It has also welcomed in 2015 the first European research centre of the American biotechnology company Alexion Pharmaceuticals, one of the pioneers of orphan drugs. This was founded in 1992 by Leonard Bell, a scientist at Yale University, Newhaven, Connecticut. In 2007 the company developed the drug Soliris, used in the treatment of patients suffering from night paroxystic hemoglobinuria, a rare disease associated with a genetic mutation that cannot be corrected and which is characterized by a severe anemia. Alexion Pharmaceuticals Soliris can reduce the symptoms markedly and decrease the number of blood transfusions that the patients must have. The drug must be taken during the whole life. The number of patients suffering from this disease was estimated at *ca*. 8,000 in Europe and *ca*. 3,000 in the United States. Soliris is one of the drugs that is best sold worldwide, with annual sales reaching US$2.2 billion (or €1.9 billion) in 2014. The figure could rise to US$5.4 billion in 2020 if other indications for the drug were approved in the meantime. The price of Soliris per patient per year was estimated at US$410,000. Alexion Pharmaceuticals invests part of its revenue in research and development of new molecules for the treatment of other orphan diseases such as hypophosphotasy which leads to a deficiency in the mineralization of bones and teeth (Hecketsweiler, 2016b).

Accommodated in the heart of IMAGINE Alexion Pharmaceuticals researchers hope to find new targets for their drugs, while the institute initiates with them a new approach to medical research, not entirely public, nor completely private (Hecketsweiler, 2015f). "Here we are creating cellular models, thanks to the samples taken from IMAGINE patients. It is a unique opportunity!" stated Jean-Philippe Annereau who heads Alexion Pharmaceuticals R&D centre. "We also benefit from the very precise knowledge of these diseases by the physicians who follow them. Their hypotheses are very valuable to us," he added (Hecketsweiler, 2016b).

### *Searching for a cure for beta-thalassemia*

Beta-thalassemia is one of the most frequent genetic diseases; its worst severe form, called major, affects *ca*. 100,000 newborns annually throughout the world, particularly in Asia, the Middle East and the Mediterranean basin. In France, the number of patients is estimated at 600. The disease is due to a defect in the synthesis of hemoglobin; this is made of four amino-acid chains, two alpha- and two beta-globin chains. In beta-thalassemias the production of beta-globin chains is deficient or even nil in homozygous persons (who have both mutant alleles of the gene that controls the synthesis of beta-chains). The overall result is a very serious anemia since early childhood (Cabut, 2014c).

Another approach to treating of this orphan disease has been the focus of the research work by two French teams led by Olivier Hermine, head of the department of hematology at Necker-Enfants Malades hospital in Paris, who published their results online in *Nature* (24 August 2014) and *Nature Medicine* (23 March 2014). Their objective is to decrease the severity of anemia, not by correcting the defect in the synthesis of beta-globin chains, but rather by trying to prevent the accumulation of alpha-globin chains – a consequence of beta-chain-synthesis inhibition – which are toxic for blood stem cells in bone marrow. A compound, called sotatercept, could be the tool for such therapy. The pharmaceutical laboratory (Celgene in partnership with Acceleron) which was developing this drug in order to treat osteoporosis, approached O. Hermine because in some patients the treatment resulted in the increase in their hemoglobin concentration. Such observation was checked in mice and the molecular mechanism of this therapeutic effect was elucidated in both laboratory animals and patients' cells. The French researchers have thus been able to establish that sotatercept inhibits GDF11, a molecule that is overproduced in beta-thalassemia and thereby contributes to anemia. O. Hermine stated that "by blocking the interaction of GDF11 with is receptor, the production of defective erythrocytes in bone marrow is reduced, and normal red cells go into the bloodstream. After publishing these results in *Nature Medicine*, the team carried out a clinical trial. About 50 patients were treated using that drug (till September 2014) and in some of them there was an increase of hemoglobin: 4g/dl. This was an unprecedented result, according to specialists, who therefore hope that subcutaneous injections of sotatercept, every three weeks, will reduce by at least 20% the frequency of blood transfusions among beta-thalassemic patients. This will have obvious benefits in terms of quality of life and perhaps of life expectancy (Cabut, 2014c). But finally another drug, luspatercept, was more efficient than sotatercept. "Every year molecules are abandoned due to disappointing clinical-trial-results," explained Jérôme Garnier, Celgene medical director in France. "One has to be humble on this issue," he added (Dussiot et al., and Hermine, 2014; Arlet et al., Hermine and Courtois, 2014; Hecketsweiler, 2016b).

Two other French physicians, belonging to the team of O. Hermine, Benoît Arlet and Geneviève Courtois, have discovered another therapeutic target for treating beta-thalassemia: the Hsp70 protein. The results of their work, carried out over a decade, were published in *Nature* (Arlet et al., Hermine and Courtois, 2014). The natural role of Hsp70 is to be a "chaperonin" of proteins whose structure has been modified by thermic shocks, or of overproduced or abnormal proteins. The presence of Hsp70 in the nucleus of red-cell precursors is indispensable to the maturation of erythrocytes in bone marrow. The French researchers demonstrated that in patients suffering from the severe form of beta-thalassemia, the Hsp70 protein is sequestrated in the cytoplasm of these red-cell precursors, by the alpha-globin chains which are overproduced. "This at least partly explains the defects in the production of erythrocytes. Drugs that could disentangle these interactions and maintain the Hsp70 protein in the nucleus could be used in new therapies," stated B. Arlet. The French team is looking for partners in order to try to isolate these molecules through screening drug libraries. If this approach is successful clinical trials can be carried out rapidly, because these may be drugs which are already commercialized and prescribed for other diseases (Cabut, 2014c).

A third approach to treating beta-thalassemia is through gene therapy that aims to introduce into blood-stem cells the gene coding for beta-globin chains (which is defective). A trial was carried out by a group of French researchers and physicians of INSERM, Paris Necker-Enfants Malades university hospital and Paris René Descartes University, who received in 2006 the authorization to carry out the trial by the then called French Agency for the Sanitary Safety of Health Products. The patient, of Vietnamese and Thai origin, used to receive a blood transfusion every month since his childhood. The initial stage of the trial consisted of taking a sample of the patient's bone marrow and of isolating hematopoietic stem cells, which give rise to all blood cells, including erythrocytes. These stem cells were modified using a viral vector – derived from HIV that has been made innocuous – carrying the correct gene for beta-globin. The viral vector was supplied by the biotechnology company Bluebird Bio, created by Philippe Leboulch of INSERM. The HIV has the advantage of penetrating into the host-cell nucleus and becoming integrated into its genome. Before the genetic transformation of the hematopoietic cells with the viral vector carrying the normal gene, a chemotherapy treatment helped to eliminate all the cells carrying the defective version of the gene encoding beta-globin (Cavazzana-Calvo et al., 2010).

The following stages, including the intravenous injection of healthy cells into the patient, the monitoring of his health status and analysis of his blood cells, were implemented by Eliane Gluckman, a physician of Paris Saint-Louis hospital, Françoise Bernaudin, a physician and professor at the Intercommunal Hospital of Créteil (south of Paris) and Frédéric Bushman (University of Pennsylvania). It was found after a few months that the patient's blood cells contained sufficient beta-globin so that no blood transfusions were needed for about two years. These good results led the AFSSAPS to authorize this gene therapy for a second patient in January 2010. In both trials the research work was partly funded by the French association against myopathies (AFM, French acronym) [Cavazzana-Calvo et al., 2010; Benkimoun, 2010; Sasson, 2011, pp. 264-265].

# CANCERS

## What is cancer?

Hopes were high when Richard Nixon declared war on cancer in 1971. But over 45 years later *ca*. 15 million people worldwide still die from the diseases that fall under the broad name of cancer. Can it therefore be said that the war on cancer has failed? No, says Paul Marks in his book *On the Cancer Frontier: One Man, One Disease, and a Medical Revolution*, co-authored with James Sterngold and published in 2014. According to the former head of Memorial Sloan-Kettering Cancer Center in New York, the goal should be containment, not victory, because the enemy is uniquely intractable. "Medical science has never faced a more inscrutable, more mutable, or more ruthless adversary," wrote P. Marks who has taken part in many of the developments that have enhanced the understanding of the disease (*The Economist*, 2014b).

Cancer is actually a term that embraces hundreds of specific ailments caused by an even larger number of genetic and epigenetic traits. Cancer is not just one disease, it is hundreds, potentially thousands. And not all cancers are caused by just one agent – for instance a virus or a bacterium that can be destroyed. Cancer is an intricate and potentially lethal collaboration of genes that do not function correctly, of cell-growth inhibitors gone missing, of hormones and epigenomes changing and cells escaping any control. "This disease is much more complex than we have been treating it, and the complexity is stunning," stated Phillip Sharp of the Massachusetts Institute of Technology (MIT), a Nobel Laureate for Medicine or Physiology (1993, for the discovery of introns), who studies the genetics of cancer (Saporito, 2013).

The old ways of characterizing cancer by the anatomical site of its initiation (e.g. kidney or prostate gland) and the histology of its cells seem increasingly out of date. Instead, thanks to genomics, researchers have unprecedented information on the nuclear change which propel it. But one should make a difference between genomics and heredity. Only 10% of cancers are hereditary, but all cancers are genetical, i.e. associated with DNA instability often caused by environmental factors (e.g. tobacco and smoking, alcohol, food, sun exposure, viruses, chemicals), and consequently not inherited from our genitors. In other words cancer(s) is (are) always a DNA disease that is generally acquired (in 90% of cases) [Alexandre, 2011].

The Human Genome Project, terminated in 2003 after 13 years of work, had resulted in the sequencing of the 3 billion nucleotide pairs of human DNA and in the identification of our 25,000 genes (including *ca*. 23,000 coding genes), starting from the analysis of

a few individuals' DNA. In ten years the cost of sequencing the whole DNA has been divided by 1 million and this has changed the analysis of the extreme complexity of the genetic, and therefore biological modifications, of tumour cells. The sequencing of one or two genetic markers that led to simplifications is followed by the sequencing of the whole genome of tumour cells that lead to clearcut conclusions such as: there is not one gene for cancer, but a large number of variants that differ from one patient to another. Each cancer is therefore a unique illness that is propelled by DNA mutations and not by one punctual modification (Alexandre, 2011).

## Cancers by the numbers

In 2012, worldwide, 14 million new cases of cancers have been recorded, or 182 cases per 100,000 persons. Also in 2012, 8 million deaths were caused by cancers, or 102 deaths per 100,000 persons. The most frequent cancers (per 100,000 persons) were: for women, breast cancer (43.3), colorectal (14.3) and lung (13.6); for men, lung (34.2), prostate (31.1) and colorectal (20.6). In 2014, according to the WHO, *ca*. 15 million people died from cancers throughout the world. This figure may be higher because in many countries a large part of the population has not access to any kind of cancer diagnosis (Rosier, 2014a).

In the United States, according to the American Association for Cancer Research, the number of new cancer diagnoses or cases was *ca*. 1.7 million in 2013, compared with 1.4 million in 2005. This figure was not including non-invasive *in situ* carcinomas as well as skin and baso-spinocellular cancers (Saporito, 2013). In 2013 the leading types of new cancer cases were: breast (235,030), prostate (233,000), lung and bronchus (224,210), colon (96,830), melanomas (81,220) and brain cancers (23,380) [*Time*, 8-15 September 2014, p. 51]. Cancer is the United States most deadly disease: an estimated 580,350 people died from the disease in 2013 according to the National Cancer Institute. It was estimated that in 2008 the costs of cancer amounted to US$77.4 billion (medical costs) and to US$124.0 billion (lost productivity) [Saporito, 2013].

The probability of developing some type of cancer over one's lifetime was estimated at 1 in 2 for men and at 1 in 3 for women, in the United States. On the other hand more Americans who have a history of cancer (including those cancer-free and in treatment) are alive today than in the past: 10.8 millions in 2004; 12.0 millions in 2008; and 13.7 millions in 2012. Five-year survival rates are climbing: 68% in 2002-2008, compared with 56% in 1987-1989 and 49% in 1975-1977 (Saporito, 2013). That is why Paul Marks claimed America was winning this particular war against cancer. The death rate from cancer has fallen, although total deaths were up because of a growing and ageing population. *Cancer is now a less lethal enemy, but P. Marks doubts it can be eliminated* (*The Economist*, 2014b).

In France, in 2012, and according to the National Cancer Institute (INCa, French acronym), 355,354 new cases of cancers were recorded, including 200,350 among men and 155,504 among women. During the same year 148,378 deaths were associated with cancers, including 85,255 men and 63,123 women. The incidence of cancer has

been increasing: 362.6 cases per 100,000 men and 252 cases per 100,000 women in 2012, while mortality has been falling: 133.6 cases per 100,000 men and 73.2 cases per 100,000 women in 2012, compared with the figures recorded in the 1980s and 1990s. The most deadly cancers were in 2012: for men, lung cancer (21,300 deaths; 28,200 new cases), colorectal cancer (9,200 deaths; 23,200 new cases) and prostate cancer (8,900 deaths; 56,800 new cases); for women, breast cancer (11,886 deaths; 48,800 new cases), lung cancer (8,700 deaths; 11,300 new cases) and colorectal cancer (8,400 deaths; 18,900 new cases) [Benkimoun and Santi, 2014].

In 2015 in France (excluding overseas departments) 385,000 new cases of cancers have been recorded (including 211,000 among men and 174,000 among women). The incidence rate of the diseases among men decreased by 1.3% per year between 2005 and 2012, while among women the rate decrease slowed down: +0.2% per year between 2005 and 2012, compared with +1.6% between 1980 and 2005, due to the lesser incidence of breast cancer. In 2015, also in France, the number of deaths due to cancers was estimated at 149,500, including 84,100 among men (where lung cancer was still the deadliest one) and 65,400 among women (with breast cancer as the deadliest one). Standardized mortality rate went down by 2.9% among men between 2005 and 2012 and by 1.4% among women (Santi and Cabut, 2016).

In France cancer remains the disease where social inequality is more pronounced than in other pathologies. Reducing the social inequalities associated with cancer is the main objective of the third Cancer Plan for the period 2014-2018, presented on 4 February 2014 by the French president François Hollande. The second Cancer Plan had also that main objective, but inequalities have not decreased. In a report published by the INCa and titled *Cancers in France in 2013*, it was shown that the risk to die from cancer between the ages of 30 and 65 years was twice higher among workers than among white collars and liberal professions. Mortality due to breast cancer was lower among female white collars, while the incidence of that cancer was higher among them. These disparities were related to the various risk factors (smoking, alcohol drinking, lack of exercise, food and environment) versus the prevention behaviours (in other words with respect to the impact of the relevant health-care messages and the capacity for these to be disseminated and followed). The disparities are therefore noted in the compliance with early or regular detection tests as well as in the access to health care. Furthermore the INCa mentions the so-called "intermediary determinants", such as living and working conditions. Consequently the persons belonging to less privileged social categories are exposed to a higher risk of suffering from cancer and to die because of it. The case of cancers associated with specific working conditions is striking: in France, of the 2.37 million employees exposed to carcinogens during their work, 70% are workers who are also exposed to the risks of smoking and alcohol drinking (Benkimoun and Santi, 2014). These inequalities do persist in 2016 according to Santi and Cabut (2016).

### *Breast and cervix cancers*

A study published in *The Lancet* on 15 September 2011 (EBCTCG, 2011) revealed that the number of new cases of breast cancer detected annually worldwide had been multiplied by three in 30 years. The new cases of cervix cancer had also increased, but to a lesser extent: +20% during the same period, worldwide. Based on an analysis

of the data on breast and cervix cancer, recorded in 187 countries between 1980 and 2010, American researchers from the University of Washington, Seattle, and Australian scientists from the University of Queensland, calculated that the incidence of breast cancers increased from 641,000 cases per year to 1,643,000 cases in 2010. The number of deaths due to breast cancer also climbed up to 425,000 in 2010, from 250,000 in 1980. This increase was less pronounced and might reflect the benefit resulting from an earlier diagnosis and more effective treatments in the industrialized countries. The increase in the number of cases and deaths due to breast cancer was the result of the interaction of different factors, according to the researchers: "the increase in the number of women during the most exposed part of their lifetime and the ageing of the population which heightens the average age in most of the regions of the world" (Benkimoun, 2011b).

Regarding cervix cancers their number reached 454,000 in 2010, compared with 378,000 in 1980; the increase in the number of new cases was higher in Asia, Latin America and Africa, while there was a decrease in the wealthier countries. The deaths due to this type of cancer increased moderately: from 174,000 in 1980 to 200,000 in 2010. Both the American and Australian researchers observed that 51% of the new cases of breast cancer and 76% of those of cervix cancer occurred in the developing countries. Among the women aged between 15 and 49 breast cancers were twice more frequent in developing countries than in the industrialized ones (Benkimoun, 2011b).

## New promising tests for cancer detection, treatment assessment and prediction: "blood biopsies"

In the usual cancer biopsy a surgeon cuts out a piece of the patient's tumour in order to make a histological analysis of the tissues. On the other hand physicians routinely monitor patients for symptoms like pain or shortness of breath, but some people do not have any. In those who do it can take time for such symptoms to wane – the tumour can disappear because of the chemotherapy or radiotherapy, but the body has to heal. Another standard method of assessing a treatment's effectiveness is to make scans to determine whether the tumours are shrinking, but it can take weeks or months before a tumour looks smaller on a scan, in part because a scan shows not just the cancer but also connective tissue, immune-system cells and scars at the site. Physicians can thus be mistaken thinking a tumour is present when, in fact, it is gone (Kolata, 2015b).

There is now hope that a simple blood test – far less onerous for patients than a conventional biopsy or a scan – will enable an oncologist to rapidly figure out whether a treatment is working and, if it is, to continue monitoring the treatment in case the cancer develops resistance. Failing treatments could be abandoned quickly, sparing patients painful side-effects and allowing doctors to try alternatives. In fact researchers in the United States are testing this potentially transformative innovation, called liquid biopsy. It consists of detecting in a simple blood sample the presence of tiny fragments of DNA belonging to cancer cells. "Every cancer has a mutation that can be followed with this method," stated David Hyman, at Memorial Sloan-Kettering Cancer Center in New York. "It is like bar coding the cancer in the blood," he added (Kolata, 2015b).

The idea for this blood test for cancer detection and evolution grew out of the discovery made about fetuses: they shed tiny fragments of DNA into the bloodstream of mothers-to-be. It turned out that all growing cells, including tumours, shed tiny DNA pieces. But finding those minuscule bits of DNA floating among a very large number of other molecules is not easy. They remain in circulation for just a couple of hours before they are metabolized. And the detection method became useful only when cancer researchers, using highly advanced methods of DNA sequencing, found hundreds of mutations that could serve as "bar codes" for cancers and developed the technology for finding a snippet of DNA (Kolata, 2015b). "This could change forever the way we follow up not only response to treatments but also the emergence of resistance and down the line could even be used for really early diagnosis," stated José Baselga, physician in chief and chief medical officer at Memorial Sloan-Kettering Cancer Center. A National Cancer Institute study published in April 2015 in *The Lancet Oncology*, involving 126 patients with untreated diffuse large B-cell lymphoma, revealed that the blood test predicted recurrences more than three months before they were noticeable on scans. The liquid biopsies also identified patients unlikely to respond to therapy (Rochewski et al., 2015).

### *Monitoring tumours through "blood biopsies"*

Researchers indeed are finding out facts about individuals' cancers that surprise them. For instance a fifth-grade teacher from Gardiner, N.Y., had lung cancer that resisted two attempts at chemotherapy and a round of radiation. Her physicians at Memorial Sloan-Kettering Cancer Center detected cancer DNA in her blood when she began taking an experimental drug in October 2014 that was her last hope. Four days later the cancer DNA fragments had disappeared, a sign, the physician hoped, that the treatment was working. Within weeks the patient began to breathe easier. Months later she had a scan, an X-ray test that uses a computer to assemble detailed images of slices of tumour tissue. It confirmed her tumours were shrinking. D. Hyman, the oncologist who was leading the study of the experimental drug the patient with lung cancer was taking, was of the opinion that every cancer (with a specific mutation) could be followed using this method. The blood tests or liquid biopsies also allow frequent monitoring of tumours as they spread and mutate or develop resistance to treatment. "I cannot do a weekly liver biopsy and see how things are going, but I can do a blood test every week," commented J. Baselga (Kolata, 2015b).

Another possible application of liquid biopsies is early diagnosis of cancer, but it is more complicated. If a blood test showed cancer DNA, what would that mean? Where is the tumour and would it help to find and treat it early? Some cancers stop growing or disappear on their own. With others the outcome is just as good if the cancer is found later. One early use for DNA blood tests may be helping physicians decide which patients with stage-2 colon cancer need chemotherapy; 80% of patients with these large tumours that have not spread outside the colon are cured by surgery alone; the rest have recurrences. Six months of intense chemotherapy reduces the risk of cancer recurrence but there is no way to predict who needs the treatment (Kolata, 2015b). Two Australian scientists, working with Bert Vogelstein of Johns Hopkins University School of Medicine in Baltimore, wondered whether a cancer DNA blood test might be

predictive. They began with a study of 250 patients, looking for cancer DNA fragments in blood after surgery. The tumours recurred in 80% of those with cancer DNA in their blood, but only 6% to 8% of those whose blood did not contain detectable cancer DNA fragments. The Australian researchers, Jeanne Tie and Peter Gibbs of the Walter and Eliza Hall Institute of Medical Research, Melbourne, are starting a study of 450 patients randomly assigned to have the blood test or not. Those who have it will receive a chemotherapy treatment if the test reveals the presence of cancer DNA fragments. Those who do not have the blood test will receive usual care, whatever their physician prescribes. The patients will be told their blood-test results, although the investigators worry how some of them would react (Kolata, 2015b).

## Cancer: a result of bad luck rather than of "bad" genes or environmental factors?

### *Stem-cell division rate, environmental factors and genetics interplay*

A paper published in the 2 January 2015 issue of *Science* by Cristian Tomasetti and Bert Vogelstein of the Sidney Kimmel Cancer Center (Johns Hopkins Bloomberg School of Public Health, Baltimore), the Ludwig Center for Cancer Genetics and Therapeutics and Howard Hughes Medical Institute suggested that two-thirds of human cancers were caused by nothing more than bad luck. The American researchers were looking at why cancers are more frequent in some parts of the body than in others. They wanted to know if the rate was related to the frequency of division of cells in these tissues. Every time a cell replicates there is a chance of mutation; more divisions mean more mutations and perhaps more cancers. C. Tomasetti and B. Vogelstein plotted the average number of times a stem cell divides during the course of a lifetime in 31 types of tissue against the lifetime risks of cancer developing in those tissues. They found that two-thirds of the variation in cancer risk among different tissues was caused by chance mutations associated with cell divisions. This is not the same as saying that two-thirds of cancer cases are caused by chance, because the results do not offer any information about the relative rates of occurrence of the cancers in question. Moreover C. Tomasetti and B. Vogelstein were unable to include two of the most common cancers – breast and prostate – in their analysis, because the relevant stem-cell data were not available for these (Tomasetti and Vogelstein, 2015).

What the study does not explain is the long-known but curious phenomenon that apparently similar parts of the body suffer different rates of cancer. Thus the risk of having a malignant tumour during a lifetime reaches 6.9% for the lung, 0.6% for the brain and only 0.00072% for the larynx cartilages. Stem-cell turnover means for instance that tumours of the small intestine are twenty times less frequent than those of the colon and rectum, while basal-cell carcinomas in the skin are commoner than melanomas (Cabut, 2015a; *The Economist*, 2015a). The American researchers have distinguished two groups of cancers: the first one includes 22 cancers (including those of the pancreas, melanomas and lung tumours among non-smokers) where the role of chance seemed prominent; the second one includes nine tumours (including colon cancers and lung cancers among smokers) where other factors play a significant role.

Even in this second group the dynamics of tissue renewal play an "essential" role; the impact of environmental factors and genetics is added to the effect of stem-cell division rate. Regarding the cancers of the first group preventive measures would not be efficient and it is therefore better to rely on early detection. Conversely prevention would be justified in the case of tumours where the role of environmental factors is prominent (Cabut, 2015a).

Fabien Calvo of the French Institut Gustave-Roussy – the biggest centre for cancer treatment in Europe, located in Villejuif in the southern suburbs of Paris – and scientific director of Cancer Core Europe, which networks six European centres for cancer research, was of the opinion that the conclusions of the American researchers were quite attractive and he found their graphs impressive. "In fact their results consolidate the classical theory according to which cancers are a disease of ageing, the risks being proportional to the total number of stem-cell divisions," he stated. Fabrice Denis, an oncologist and associated researcher at the University of Rouen, north-west of Paris, agreed with Fabien Calvo, when he stated that "for a few years much emphasis has been laid on the exogenous causes of cancer, such as pollution and food diet, but the work carried out by the American researchers confirm that cancers are above all diseases of old persons; they also give some credit to the idea that, when life expectancy increases rapidly – which is the case for human beings whose life expectancy has trebled over two centuries – biology does not follow suit" (Cabut, 2015a).

None of this, though, is reason for fatalism. Cristian Tomasetti did make a clarification about the interpretation of the results of their work: "We have not showed that two-thirds of cancer cases are about bad luck. Cancer is in general a combination of bad luck, bad environment and bad inherited genes." Copying errors during cell division are by no means the only source of cancer-causing mutations. Chemicals that damage DNA, ultraviolet light, ionizing radiation and viral infections are all culprits too – and causes that can often be avoided by thoughtful behaviour (*The Economist*, 2015a).

### *Impact of exogenous factors, within an individual's control*

Overall, according to research carried out in the United Kingdom by Cancer Research UK, a charity, 42% of cancer cases are tied to factors within an individual's control. These include smoking (which, through the carcinogenic chemicals it creates, causes 86% of lung cancer, 65% of oesophageal cancer, 37% of bladder cancer and 29% of pancreatic cancer), poor diet (51% of stomach cancer and 56% of head and neck cancer), overexposure to sunlight (86% of malignant melanomas) and infection with papilloma virus (almost 100% of cervical cancer). Obesity, alcohol and lack of exercise play also a role (*The Economist*, 2015a). That was also the opinion of Annie Thébaud-Mony, a sociologist, honorary research director at INSERM and researcher associated with the *Groupement d'intérêt scientifique sur les cancers professionnels* (GISCOP93, scientific association for professional cancers) of the University of Paris-XIII. She reacted strongly to the paper authored by C. Tomasetti and B. Vogelstein in an article published in the French daily newspaper *Le Monde* on Wednesday 7 January 2015 (Thébaud-Mony, 2015).

She underlined that the American researchers omitted to mention that a stem cell does not spontaneously evolve into a cancer cell. This occurs further to mutations that are themselves caused by exogenous carcinogenic factors, e.g. asbestos, ionizing radiation, diesel fumes, pesticides and other toxic compounds whose carcinogenic effect has been known for a long time. The second comment made by A. Thébaud-Mony dealt with the social disparities within the French population (and also most likely in the American population) regarding the occurrence of cancers in the various social categories, e.g. a worker runs a much higher risk (10 times) of dying from cancer (and before the age of 65) than a highly qualified civil servant or engineer or scientist. According to an inquiry carried out by the French labour ministry, named Sumer 2010, workers are ten times more exposed to carcinogenic factors in their working conditions than highly qualified civil servants (Thébaud-Mony, 2015).

Finally, A. Thébaud-Tony highlights the fact that cancer which affects an individual is the result of the repeated exposure to toxic agents during his/her personal life, behaviour and lifestyle, and of the body's defence reactions which are extremely variable among individuals. The more frequent is the presence of toxic compounds during daily life, the more numerous are the mutagenic or carcinogenic effects of each one of these compounds, as well as the synergy between them and the ways processes interfere with the body's defence mechanisms. A. Thébaud-Mony's main conclusion is that the statistical work carried out by the American researchers, once again, tends to underestimate the lethal effects of exogenic factors associated with industrial risks. She even added that this kind of published scientific work and its impact on public opinion support the contaminating industries' cause. In her opinion cancers can be avoided to a very large extent if carcinogenic compounds are completely eliminated in the working place, the environment and from a wide range of products (e.g. foodstuffs, cosmetics, genetically modified crops) [Thébaud-Mony, 2015].

Regarding the role of genes that make persons susceptible to cancer, it should be underlined that they are involved in 5% to 10% of cancers and their inventory is far from being terminated. Human genomics discoveries will therefore shed new light on the occurrence of cancers and tumour cells. Without underestimating the role and impact of genomic factors, many oncologists agree that the impact of some exogenous factors such as tobacco remains a major one: for instance, in the case of lung, the "spontaneous" risk is multiplied by 70. Tobacco which plays a significant role in 17 types of cancer causes more than 20% of deaths due to these diseases globally (Cabut, 2015a).

## Sequencing tumour-cell genomes

### *Genetic identity of cancers*

On 20-22 March 2012 was held in Cannes, south-east of France, the sixth scientific conference of the International Cancer Genome Consortium (ICGC) which started in 2008: 14 countries, including France, have launched a vast programme of sequencing the genomes of the main types of cancers. Initially 47 projects were to be carried out in order to determine the genetic basis or identity of various cancers. To that

end the analysis of tens of thousands of individual tumours will be necessary. Around 25,000 were proposed in the initial project, but there will be more by the end of the project because of the very fast progress in sequencing techniques and of their plummeting cost. It was estimated that in 2016 the sequencing of the genome of a type of cancer cell would be carried in two hours at a cost of less than US$1,000. It should be emphasized that the whole sequencing of a tumour cell genome generates a very huge number of data, the analysis of which relies on supercomputers (Cabut, 2012).

With the current classification of tumours, a type of cancer sometimes includes tens of distinct diseases, prognoses and health-care approaches. For instance lung tumours are subdivided into epidermoid carcinomas, adenocarcinomas, small-cell tumours, etc. According to Fabien Calvo, "the genetic approach will enable the physicians to improve their diagnosis and to optimize therapeutic strategies, thanks to targeted therapies. In the medium or long term, biomolecular analysis will replace the histological test which is presently used to define tumours" (Cabut, 2012). At the time of the ICGC conference in Cannes (March 2012) more than 22,000 tumour samples had been sequenced. The sequencing work included not only the tumour genes, but also their epigenome and their transcriptome (messenger RNAs from the expressed genes). The existing colossal database is available to the scientific community. As underlined by F. Calvo, "a cancer can involve about 1,000 mutations, which are of a diverse nature: nucleotide change, deletion, etc. But one has to distinguish the anomalies that are important from those which just express genetic instability." In the early 2000s, herceptine, a monoclonal antibody, has been used in targeted therapies in 20% of breast cancers with a mutation on the HER2 gene. Since then 15 other molecules had been authorized in targeted cancer therapies and in 2012 about one hundred of similar anticancer drugs were being developed (Cabut, 2012).

British researchers presented at the ICGC conference their findings concerning mutations – like those of the Pi3kinase – that are specific to some breast tumours with a good prognosis. The French research team of Jessica Zucman-Rossi, on the other hand, has been studying 24 types of liver cancer and has identified a genotoxic signature of liver tumours associated with excessive uptake of alcohol or with overeating. According to F. Calvo "such strategy enables the researchers to trace back the origin of cancer development and to identify different mechanisms depending on the causal factors; that will be particularly useful in the case of environmental toxic compounds, where the epidemiological analysis is not sufficient to make a clearcut judgement (Cabut, 2012).

### *The Cancer Genome Atlas*

The Cancer Genome Atlas, a project launched by the United States National Institutes of Health (NIH), part of the ICGC, has assembled genetic data on thousands of tumours and had made them available to anyone who wants to analyze them. Two papers, published online ou 26 September 2013 in *Nature Genetics*, are early fruits of that endeavour. One study carried out by Rameen Beroukhim et al. of the Broad Institute in Cambridge, Massachusetts, looked at cancers with an unusual number of copies of certain sections of their DNA. R. Beroukhim examined 4,934 specimens from

11 traditionally defined types of cancer and found 140 regions of DNA that were sometimes either multiplied repeatedly or deleted altogether. Only 35 of these regions contained either genes known to suppress tumours or those, known as oncogenes, which when mutated trigger cancer development (Zack et al., and Beroukhim, 2013).

The other study, carried out by Chris Sander et al. of Memorial Sloan-Kettering Cancer Center, in New York, proposed a way of categorizing tumours by the genetic and epigenetic changes in their cells, rather than by anatomy and histology (Weinstein et al., and Sander, 2013). C. Sander developed an algorithm to examine 3,299 tumours from 12 traditionally defined types of cancer. He created two overarching groups: those with somatic genetic mutations (i.e. mutations that have occurred in a person's body cells during his/her lifetime, and which are not inherited) and those, like R. Beroukhim's, which had an unusual number of copies of some sections of their DNA. Tumour cells with many somatic mutations rarely had many unusual copy numbers. But less surprising, was that within C. Sander's two main classes of cancer he recognized 31 subclasses. As cancer biologists have long suspected, tumours from the same type of tissue often had different genetic traits, while those from different tissues were frequently similar genetically. For instance a type of lung cancer shared characteristics with a type of head and neck cancer. This has practical implications. Both diseases might be good candidates for a specific combination of drugs that are targeted to their specific combination of mutations. Increasingly clinical trials of drugs are likely to span conventional categories of cancer by pooling those in different parts of the body that have similar genetic characteristics, and to test more than one medicine at a time (*The Economist*, 2013).

The work of R. Beroukhim and C. Sander has been made possible thanks to advances in genomics and computational biology. These studies are just the start of better understanding cancer. Scientists at the United States National Cancer Institute published the most complete genomes yet of the more than 20,000 genes in a set of 60 tumour-cell lines that include breast, prostate, lung and colon cancers (2013). Previous sequencing projects had focused on mapping only specific genes or discrete sets of genes that induce tumours. Scientists have used 60 cell lines to test anticancer drugs since the 1980s; 16,000 compounds have been screened and *ca*. 300 approved by the FDA. With the added gene-sequencing data some of the failed candidates could be retested against previously unidentified mutations. *To take full advantage of that possibility researchers will have to sequence thousands more tumour-cell genomes; such enhanced databases could produce a new generation of precisely targeted drugs* (Park, 2013).

## Genetic testing for targeted therapies

No two cancers are alike; even within an individual patient tumours may change over time. And physicians are learning that a melanoma growth might have more in common with a lung cancer or brain cancer than another melanoma. "We are moving away from the concept that all lung cancers are the same and all breast cancers are the same and all colon cancers are the same," stated David Solit, director of the Kravis

Center for Molecular Oncology at Memorial Sloan-Kettering Cancer Center (MSKCC, New York City). "Now we are going to know if you have EGFR mutant lung cancer or an ALK fusion lung cancer or a BRAF mutant brain cancer. And we are going to know better ways to treat those cancers based on those mutations" (Park, 2015a). That led to a new consensus that to truly fight cancer, physicians need to understand it from the inside out, which means decoding its DNA and exposing the ways it co-opts the body's healthy cells. Once that is known, the task becomes to develop drugs that can thwart the way a given cancer wrecks the body. Fast-moving developments in genetics and molecular biology are of a great help. "This type of testing is not standard of care yet, but everyone agrees it will be at some point," said D. Solit. The mantra for the precision medicine approach is to learn from every single patient (Park, 2015a).

### *Basket clinical trials*

Park (2015a) reported on the case of a patient suffering from glioblastoma, a brain cancer with the worst prognosis among cancers. Within a year or two of diagnosis 75% of patients are dead. The treatment that was chosen is standard for most cancers: surgery, radiation, chemotherapy. But glioblastomas infiltrate brain tissue with tiny fingers of malignant cells, making the tumours hard to treat through the conventional way. That is why, even after treatments, they almost inevitably come back. But there seems to be a better way of attacking glioblastomas. First, the patient's physicians at MSKCC sequenced her tumour DNA. If it contained any of the few hundred mutations they know can prompt healthy cells to become cancer cells and grow uncontrollably, the physicians could then check her mutations against databases of ongoing clinical trials to see whether she would be eligible for experimental drugs that might improve the prognosis. David Hyman, acting director in 2015 of developmental therapeutics at MSKCC, met the patient who underwent brain surgery earlier on: 75% of her tumour had been removed, but the rest was too enmeshed in her brain to take away safely. D. Hyman ordered a genetic test of her tumour after surgery. And on the basis of the results he thought she should join a trial he was running (Park, 2015a).

His study which is called a basket trial brings together people with 20 different types of cancer – including glioblastomas, lung and colon cancers – whose tumours all share the same genetic mutation. MSKCC genetic test scans for known aberrations in 410 genes that have been linked to cancer. In the case of the patient with glioblastoma her brain cancer is driven by a mutation called BRAF. While common in melanoma the mutation is rarer in glioblastoma. Basket trials are an efficient way of checking whether different cancers with the same mutation respond in the same way to a drug that is designed to mitigate it. That is precisely what MSKCC researchers were testing on the patient with glioblastoma and others with a drug called vemurafenib, approved in 2011 for the treatment of melanoma (Park, 2015a).

Of course, there is no guaranteed success. Even if they share the same mutation cancers that start in the skin, where cells divide and die more rapidly than almost anywhere in the human body, are almost certainly a little different from cells in the brain, which are more protected and conserved. The side-effects of such drugs are largely unknown. It is also very difficult to guess how many of the people in the basket

will be alive a year, two years and five years after taking the drug. In the case of the patient with glioblastoma D. Hyman, who knew how effective vemurafenib is on BRAF melanomas, had good reasons to hope for similar results with the treatment of the glioblastoma. In fact he turned out to be right. So far (until March 2015) the tumour had shrunk an additional 55%. Choosing to try an experimental drug the patient was eligible for hardly felt like a choice. But it was still more of a choice than many people with glioblastoma were expected to make (Park, 2015a).

### *Access to drugs and genetic testing*

Many factors, indeed, determine which drug trial a patient has access to. There is geographical location, since most Americans are treated at the hospital closest to home, and most hospitals do not have a lot of clinical trials under way at any given time. There is also the cost: both a patient's financial situation and that of their cancer center can influence what is available to them. Finally there is the human temperament. Physicians know that vemurafenib works on tumours with BRAF mutations, for instance, but simply hearing about the trial at MSKCC may be enough to prompt patients who had not genetic testing, to plead and bargain for access to the drug – and for physicians who might not have anything else to offer them to prescribe it. Any drug approved by the FDA can be prescribed for any purpose, as long as the physician has reason to. Covering the cost of the treatment is another matter: insurers use FDA approval as a criterion for deciding which drugs to reimburse. So when it comes to off-label prescribing there is no guarantee that insurance will cover the cost, which in the case of vemurafenib is exorbitant – up to US$65,000 for the recommended six-month treatment period, by mid-2015, (Park, 2015a).

In 2015 less than 5% of the 1.6 million people diagnosed with cancer each year in the United States can take advantage of genetic testing, which could cost between US$3,000 and US$8,000, depending on how many genes are analyzed. At most hospitals this kind of testing is limited. And even at centres like MSKCC *ca*. 70% of patient's genetic testing is not covered by insurance, so the programme operates at a loss. For its part the University of Texas MD Anderson Cancer Centre in Houston funds its testing almost entirely with donations. "It is clearly not a long-term sustainable model," stated Funda Meric-Bernstam, medical director of the Institute for Personalized Cancer Therapy at MD Anderson Cancer Center (Park, 2015a).

In 2015 researchers knew of a few hundred mutations linked to cancer but there were targeted therapies for only *ca*. 20 to 40 of them. The physicians' objective in the United States is clearly to give their patients more than what they have nowadays – to offer them tailored and scientifically tested therapies. There are, however, issues such as "Who will guide physicians to make clever decisions?" or "How will they decide when to test and when not to test?" Regulatory agencies like the FDA will also have to play a key role and review the drug approval process. "Doctors and patients are way ahead of where the FDA and insurance companies are in using different medication," stated Julie Vose, president of the American Society of Clinical Oncology (ASCO). While by its nature cancer is unpredictable and untameable, treating one patient and one mutation at a time, may provide the best chance yet of finally putting cancer under control (Park, 2015a).

In fact hundreds of tumour-cell lines are cultivated *in vitro* with a view to testing new therapeutic molecules. Kelloff and Sigman (2012) described of how their genetic profile and several biomarkers could be used in order to predict anticancer-drug efficiency. Thus 24 active molecules were tested on 500 tumour-cell lines whereas on the other hand 130 molecules were tested on 600 types of cancer cells. The data obtained were made available in the public domain. *All these new tools aim at better targeting the candidate anticancer drugs to be tested in clinical trials, thanks to the knowledge of the genetic characteristics of the patient's tumour cells. At a later stage this could lead to a more personalized health care and medicine* (Cabut, 2012).

## Specifically tailored anticancer drugs

Knowing which genes are going wrong (mutated) it would be possible to develop specifically tailored anticancer drugs. In August 2011 the FDA approved a medicine called Xalkori (generically crizotinib) for patients who have a particular type of non-small-cell lung cancer, the most common form of that disease. Xalkori blocks the growth of tumours caused by a mutant form of the gene encoding a signalling molecule known as anaplastic lymphoma kinase. This mutation occurs in 3%-5% of lung-cancer patients and in trials Xalkori caused a dramatic shrinkage of the tumour in around half of those treated. However the respite does not last. Typically someone will respond for about a year, but after that the tumour will grow again and the disease continues on its course. This is a pattern seen very often with the new generation of drugs that genomics helped to create: they slow the disease, but only for a few months. The presumption is that further mutations are arising in a tumour all the time, and that eventually one of them makes a molecular change that nullifies the effects of the drug (*The Economist*, 2011).

One way of restoring the sensitivity of tumour cells to Xalkori was presented by René Bernards of the Netherlands Cancer Institute at a meeting of the American Association for Cancer Research, held in San Francisco on 18 September 2011. The solution discovered by R. Bernards could be applied to many other cases in which an anticancer drug is having its effectiveness hampered by the development of resistance. It must be recalled that the mutations which cause cancer are often hidden in a large number of others that have no direct bearing on the disease. Normal DNA sequencing cannot distinguish which mutations are important for tracking cancer and which are not. But R. Bernards considers he can, by using molecules called short hairpin RNAs. The assumption was to make a hairpin RNA with an appropriate gene sequence so that it will combine strongly with the messenger RNA of a specific gene to form a double-stranded RNA molecule (double-stranded RNA does not exist in mammalian cells, but mostly in viruses; if a cell's defence mechanisms detect double-stranded RNA they destroy it, to protect against infection). R. Bernards synthesized hairpin RNAs that combined with the messengers of 20,000 genes, to see which, if any, are involved in the development of resistance to Xalkori (*The Economist*, 2011).

He found three. *Mediator-12* (*MED12*) which helps to transcribe genes from DNA into messenger RNAs. The other two genes help maintain the structure of chromosomes. R. Bernards and his colleagues looked for hairpin RNAs that restored sensitivity to Xalkori in cells whose *MED12* messengers were being blocked and they found one.

Disabling the messenger of the gene that encodes a protein called TGF beta-R2, found on cell surfaces, caused cells that had once been resistant to Xalkori to shrivel in its presence. Moreover treating these same Xalkori-resistant cells with an experimental drug designed to block TGF beta-receptors restored sensitivity to Xalkori; though it had no effect on cancer cell growth on its own. Subsequent studies by members of R. Bernards' group revealed that interfering with *MED12* messenger RNAs causes resistance to numerous other drugs, including Iressa and Tarceva, which are prescribed for lung cancer, Zelboraf, effective against melanoma, and Nexavar, which is used for kidney and liver cancers. If these laboratory-based results were confirmed in people *then TGF beta-receptor inhibitors may prove a way of extending the useful lives of many medicines* (*The Economist*, 2011).

Indeed at least three other research teams are using short hairpin RNAs to study cancer in the same way as R. Bernards' group. One of them, led by William Hahn of the Dana-Farber Cancer Institute in Boston, found what may be an important molecular link in the development of ovarian tumours. Turning these sorts of laboratory discoveries into treatments is a long process that often fails. However, *the work of R. Bernards and his colleagues on short hairpin RNAs is just the vanguard and, like for the Human Genome Project, it opens the way for understanding the functioning of genes involved in the generation of cancer* (*The Economist*, 2011).

## Breast cancer: better-targeted chemotherapies

### *Avoiding unnecessary chemotherapies*

Several genomic tests that have been developed since 2000 and that consist of analyzing the activity of several dozens of genes in the patients' tumoural tissues, aim at trying to identify those women suffering from breast cancer who may not need chemotherapy. This strategy is part of the "therapeutic de-escalation or decline", i.e. avoiding a treatment that has severe secondary effects. These tests are not recommended in France and are not reimbursed by the social security. By contrast, in the United States, Germany, Netherlands, United Kingdom and Italy, they are used in the case of some patients. In Switzerland, since the beginning of 2015, their costs have been borne by the mandatory health insurance (Rosier, 2015f).

In France, every year, *ca*. 55,000 new cases of breast cancer are recorded. "*Ca*. 40% of these women are treated with chemotherapy. Using these genomic tests, that proportion could be decreased to 30%," stated Roman Rouzier, medical director of the Senology Pole at the Institut Curie in Paris. "*Ca*. 12,000 women per year could be tested, but because the cost of the tests is not reimbursed by the social security, only 1,500 women are using them," underlined Suzette Delaloge, head of the committee on breast pathology at the Institut Gustave-Roussy (Rosier, 2015f). Chemotherapy is systematically applied to patients with a high risk of recurrence. Its intensity depends on the size and grade of the tumour, the presence of "hormonal receptors", the existence of ganglia invasion and the age of the patient. When the tumour is considered a "low-risk" one, chemotherapy is not recommended, and such secondary effects as "nausea, complete loss of hair on the scalp, fatigue and temporary cognitive troubles," are avoided; not to mention "professional sick leaves and psychosocial effects," recalled S. Delaloge (Rosier, 2015f).

To make the things simply there are three main breast cancers: hormonodependent cancers – the most frequent (70% of cases), and associated with estrogens; HER2 cancers which are most often aggressive – their multiplication is the result of the overproduction of a harmful protein; and cancers called "triple negative," which do not belong to any of the two previous categories and which are often aggressive. Regarding the usual treatment of hormonodependent cancers, when the tumour is small (less or equal to 3 cm of diameter), it is taken out surgically. Thereafter, the patient undertakes radiotherapy treatment during five to six weeks, focusing on the area of the tumour. Then an antihormone treatment is prescribed during at least five years. In the case of aggressive hormonodependent cancers, a four to five-month chemotherapy is also prescribed. There are good results with patients suffering from a non-aggressive hormonodependent cancer, with a 10% to 20% rate of women being again affected. Among those treated for a more aggressive hormonodependent cancer (even without affected ganglia), the average rate of being sick again is 20%.

A targeted therapy was developed at the Institut Gustave-Roussy, south of Paris, by Fabrice André, a professor of oncology in that institute, using a molecule called palbociclib. The trial carried out for that purpose included 100 patients with breast tumours of different kinds: 75 received the drug orally during 15 days, while the others had no treatment between cancer diagnosis and surgery. In the group of 61 patients whose cancer was hormonodependent, 43 were diagnosed with no proliferation at all of malignant cells. This result was considered by F. André a major progress in anticancer treatment. Furthermore the side-effects such as diarrhea for two patients and the decrease in blood leucocytes for two others were reversible. A new national trial with palbociclib is being carried out by the Institut Gustave-Roussy under the supervision of Suzette Delaloge and Paul Cottu and in which participate 40 hospitals. The objective of this national trial is to compare the new targeted therapy with conventional chemotherapy treatments implemented in the case of hormonodependent cancers. The results of the trial are expected in 2018. To sum up, Fabrice André highlighted that: the new targeted therapy could reduce the risk of being again affected by breast cancer, as well as the toxicity of current treatments; it would increase the likelihood of cure; and it might be efficient in the treatment of other cancers, such as certain lymphomas and lung cancers, which is being studied.

### *Genomic tests as an approach to better targeted chemotherapies*

But what to do for "intermediary-risk" cancers. In order to predict the likelihood of recurrence and evaluate the need for a chemotherapy, four kinds of genomic tests have been commercialized: Oncotype DX (American biotechnology company Genomic Health), EndoPredict (German company Sividon, commercialized by the American company Myriad), MammaPrint (Dutch company Agendia) and PAM50-Prosigna (American company NanoString). In 2015 the cost of an Oncotype DX test was €3,180; that of PAM50-Prosigna test was €2,300. "If the Oncotype DX test were reimbursed in France, €44 million or even more would have been saved through avoiding useless chemotherapies. Taking account of the overcost due to the use of that test the savings would have amounted to €12 million," has estimated Roman Rouzier. And S. Delaloge added: "A chemotherapy costs only €8,500 in France, compared with €18,000 in the United States (Rosier, 2015f).

At the French INCa the "very aggressive lobbying" of the laboratories or companies that commercialize these tests have been criticized, and the need to study and compare these tests has been underlined. Another problem is the monopolistic situation imposed by some companies. "If the health authorities decide, after evaluation, to authorize the use of Oncotype DX test, the latter will be carried out only by Genomic Health, and not by the hospital platforms (laboratories) of cancer molecular genetics, set up by the INCa," explained the Committee on Ethics and Cancer in a statement issued on 15 January 2013. At least five studies have evaluated the interest of using the Oncotype DX test which is the oldest one. "The results of these evaluations indicate that this test has a prognosis value with respect to a recurrence risk. It is also predictive with regard to the response to chemotherapy and hormonotherapy," signals the Committee on Ethics and Cancer (Rosier, 2015f).

But because the results of "prospective and randomized" studies (where patients are randomly chosen in order to make the test or not) are not available, the INCa position is a cautious one: "These tests are potentially very important. They may serve to avoid useless chemotherapies for certain women," stated Agnès Buzyn, the INCa president in 2015. "However the INCa remains cautious, because we still need data on the long-term benefit of these tests. We are not sure that women who are not treated today with chemotherapy, on the basis of criteria that are in part related with these tests, will not have a cancer recurrence in ten or fifteen years …," she added. "Our second concern has to do with the unconsistent results of these tests in 20% to 30% of the cases. Which one to choose? We are awaiting the results of prospective randomized studies," A. Buzyn commented. In fact several large-scale prospective studies were being carried out and their results were expected by the end of 2016. Suzette Delaloge concluded: "I hope that before I retire, I shall not have to prescribe an adjuvant (auxiliary) chemotherapy; because a large number of targeted therapies are being developed to combat breast cancers. These therapies which also have secondary effects, are nevertheless better tolerated than standard chemotherapy" (Rosier, 2015f). It should be mentioned in this regard that recent studies suggest that 3-D mammography is a highly accurate breast cancer screening tool. Not all hospitals and physicians offer it in the United States, but all signs point to its becoming much more common after 2015. Hologic, one of the two American companies selling 3-D mammogram machines, stated there was at least one device in all 50 States in 2014-2015. This screening tool, when applied on a large scale, will help detecting breast cancer at a very early stage and making the most appropriate treatments.

## A team-based, cross-disciplinary approach to winning the war on cancer

### *Bringing science and medicine together*

"You no longer do science and medicine differently," stated Lynda Chin, director of the Institute for Applied Cancer Science at MD Anderson Cancer Center, in 2013. "It brings science and medicine together." In 2008 Stand Upto Cancer (SU2C) was founded: an organization started by entertainment-industry figures unhappy with the progress being made against cancer in the United States. SU2C raises money through foundations and corporate, organizational and private donors, and then grants it to teams in the form of unusually large amounts (up to US$18 million, vs about

US$500,000 for a typical grant from the National Institutes of Health, NIH) to produce results in a short time, initially three years. All the chosen projects are monitored by the American Association for Cancer Research. An SU2C scientific committee, headed by Phillip Sharp and other renowned scientists, reviews each team semiannually. *The team model is also disrupting the normal course of business across the medical research communit*y. For investigators it means changes in the way careers are developed, the way data – and especially credit for achievement – are shared. For institutions team research means changes in contracts, compensation, titles and the path of intellectual property. For pharmaceutical companies it means restructuring the way experimental drugs are allocated and clinical trials are conducted (Saporito, 2013).

This model is being adopted by the NIH itself. Francis Collins, NIH director, stated that under his watch the 27 institutes he oversees will be less independent organizations pursuing their own goals, and more trustworthy collaborators can be teamed up to answer common and complex biomedical issues. And for patients, Ronald DePinho, president of MD Anderson Cancer Center, is adopting a similar collaborative approach and around what the world-renowned institute calls its Moon Shots programme, assembling six multidisciplinary groups to mount comprehensive attacks on eight cancers : lung, prostate, melanoma, breast, ovarian and three types of leukaemia. R. DePinho is planning to receive US$300 million annually over the next decade, thanks to reallocating existing research funds and soliciting new donations. *As in the SU2C effort, teams will be judged by patient outcomes, not by the number of research papers publishe*d. He stated: "It is about integration across the entire cancer continuum and it is about execution. People will be judged by whether they have reduced mortality in cancer" (Saporito, 2013).

This kind of institutional transformation is not easy, but it is the only way to take advantage of the fast scientific and technological advances that have occurred in just three years (2010-2012) – advances in bioengineering, nanotechnology, drug compounds and data gathering, including protein data, splicing data and mutation data. R. DePinho's use of the moon-shot analogy is not a marketing gimmick. In 1961, when President John F. Kennedy announced that the United States was going to the moon, the idea was no longer science fiction. The physics were understood. What remained was a giant engineering project: use and apply enough funds and aerospace engineers in order to enable Neil Armstrong to put his feet on the moon. When President Richard Nixon announced the war on cancer in 1971, victory was not remotely possible. "It was as if someone had announced a moon shot in 1820," said Lewis Cantley, head of the Cancer Center at Weill Cornell Medical College and New York Presbyterian Hospital in New York City (Saporito, 2013). See also p. 321.

*Although some teams of researchers have been able to reduce to two years the time from the discovery of a specific mutation to a drug to treat it*," *cancer does not wait two years*," stated Daniel Haber, director of the Massachusetts General Hospital Cancer Center. D. Haber, an oncologist, has partnered with Massachusetts General Hospital biomedical engineer Mehmet Toner to lead an SU2C-backed team that has designed and built a smart chip device to trap circulating tumour cells (CTCs) in a blood sample. Many tumours release cells into the bloodstream; if a CTC starts to grow in another organ, that is metastasis. The breakaway cells are not easy to spot; there are a billion

blood cells for everyone of them but detecting their presence is critical to stopping their spread. The device used by the research team uses antibodies to bind to certain cell proteins to isolate and capture the CTCs. It is possible the device will change the standard of care for treating several cancers, beginning with metastatic prostate cancer. *The CTC chip's role as a trapper is also being applied to lung cancer, where mutations can help direct powerful new therapies, to see how CTCs change and evolve during the course of treatment* (Saporito, 2013).

L. Cantley is a co-leader of a team backed by the SU2C that targets a mutation known as Pi3K, for phosphoinositide 3-kinase, existing in three women's cancers: ovarian, endometrial and especially breast, which involves the Pi3K mutation in 30% of cases. L. Cantley stated: "It is the most frequently mutated oncogene in cancer." Drug companies have long been targeting mutations like this one to develop compounds, that will interfere with the defective biochemical pathways. There are hundreds of drugs that may have some effect against some of the mutations, but there is a 95% failure rate for new products and half of phase-3 trials – the last before approval – are not successful."If I have 100 different drugs I can use in combination, then 100 times 100 is 10,000. You cannot do 10,000 trials," stated MIT Phillip Sharp. But which one can you do and should you do and on which patients? Since Pi3K mutations are the most common type, those seemed like a perfect place to start for L. Cantley's team, which is co-led by Gordon Mills of MD Anderson, along with women's cancer specialists from Massachusetts General Hospital, Dana Farber (Harvard), Vanderbilt, Columbia University, Beth Israel Deaconess and Memorial Sloan-Kettering Cancer Center. In one of the best examples of the new model L. Cantley, Gerburg Wulf and José Baselga, proposed combining a Pi3K inhibitor with a PARP inhibitor to combat a particularly pernicious mutation in the *BRCA1* gene that results in high risk for developing ovarian cancer and a severe type of breast cancer known as triple-negative. PARP is the abbreviation for a group of enzymes that repair damaged DNA strands. *Working on mouse models the team obtained cures for BRCA1 mutant and triple-negative breast cancers when they combined a Pi3K inhibitor and PARP inhibitor, which had never happened with other therapies* (Saporito, 2013). To move on to a human trial, the team needed a Pi3K inhibitor from Novartis and a PARP inhibitor from AstraZeneca. Neither drug was approved for cancer treatment and it is unusual to conduct a trial in which two unapproved drugs are combined. Because of concerns about intellectual property and other issues companies are wary of collaboration. The success of the Cantley-Mills team had drug firms lining up and the result was almost without precedent : *a human trial at five institutions with two unapproved drugs from two companies within about a year of discovery* (Saporito, 2013).

## *Joining forces in the struggle against cancers*

In the case of pancreatic cancer – whose less than 25% patients make it to one year – Daniel Von Hoff who leads a SU2C pancreatic team with Craig Thompson, CEO of Memorial Sloan-Kettering Cancer Center, has tried to improve survival rates. The focus of the 28-person team, scattered across five institutions, is to better understand the metabolic changes that characterize pancreatic cancers. It is a collaborative exercise that starts when surgeon Jeffrey Drebin at the University of Pennsylvania removes a

tumour from a diseased pancreas. He carries it from the operating room to a laboratory where it is flash-frozen for preservation. A specimen is sent to the Salk Institute Gene Expression Laboratory, where researcher Geoffrey Wahl and colleagues analyze the state of stellate, or star-sharped cells, that are usually involved in tissue repair but may play a role in cancer as well. Another sample is sent to Princeton, to the laboratory of Joshua Rabinowitz, who analyzes amino-acids, sugar and up to 300 metabolites. Team members at Johns Hopkins and Translational Genomics analyze the genome sequence (Saporito, 2013).

One of the theories emerging from this team is that pancreatic cancer cells communicate with stellate cells that also show up around the tumour and ward off immune responses and build resistance to chemotherapies. The tumour cells seem to uptake glutamine and other amino-acids from the rest of the body to feed the tumour – one reason people with pancreatic cancer lose so much weight. Preventing the uptake of glutamine and other amino-acids may starve the tumour. The team also discovered that vitamin D can help stop the scarring around the cancer, giving the immune system or chemotherapies better access to cancer cells. Within two years they have modeled, evaluated and tested an albumin-containing drug that shows promise in increasing the efficacy of treatments. They enrolled 861 patients in a phase-3 clinical trial of a treatment for advanced pancreatic cancer that adds the chemotherapy drug Abraxane, and the results have been encouraging: the combination stabilized the disease in 84% of the patients, doubling the two-year survival rate to 9%, indicating how it remains difficult to combat pancreatic cancer. Remarkably yet, a few patients had a complete remission (Saporito, 2013).

Something similar was happening at MD Anderson Cancer Center, where physicians and researchers were joining forces in the struggle against breast and ovarian cancers because genetic markers have shown that these cancers are related. Altogether they are accumulating data and expertise, so that they will be able to profile all the relevant mutations. For instance work is progressing on triple-negative breast cancer – so named because the receptors for estrogen, progesterone and a growth factor known as HER-2/neu are missing. This makes treatment difficult since those are targets for hormone and drug therapies (Saporito, 2013).

More hope for other patients is coming from the new platforms MD Anderson Cancer Center had added around prevention and early detection. If a patient is genetically predisposed to breast cancer, what about other women in the family? If they are offered testing for the same biomarkers the medical doctors could avoid big trouble by detecting any cancer earlier. Likewise there are 94 million ex-smokers in the United States (2012), meaning they have elevated cancer risk. Subjecting each of them to an annual CT scan would catch early-stage lung cancers and reduce mortality from the disease by perhaps 20%. Given that there are 175,000 new lung cancers diagnosed every year, that is a lot of lives. But submitting all those people to a CT machine is neither practical nor even possible. *Instead, MD Anderson is developing a simple blood test for a protein marker that could, when used in combination with diagnostic imaging and risk models, detect lung cancer earlier than it is typically found* (Saporito, 2013).

Working in teams is not necessarily appropriate for every aspect of cancer research. Nor it is issue-free. For instance how long should a team be together? SU2C initial funding is generally for three years, although some teams have secured money for additional years. The pancreatic cancer team, for instance, received two grants of US$2 million each from the Lustgarten Foundation and SU2C for another two years. At MD Anderson Cancer Center R. De Pinho is committed to the team concept, but he is also willing to defund or change the leadership of teams that do not perform. On the other hand, the researcher, sitting alone or with a couple of postdocs in a laboratory, will always have a niche in this new approach to combating cancers (Saporito, 2013).

## Immunotherapy: a promising approach to mitigating cancers

### *The immune system and its role in the evolution of living beings*

Philippe Kourilsky, professor emeritus at the Collège de France in Paris, former director-general of the Pasteur Institute (2000-2005) and a renowned immunologist, published in 2014 a book titled *Le jeu du hasard et de la complexité* (Odile Jacob ed., Paris, 336 pp.) that can be translated as "The game of Chance and Complexity" and is devoted to the "science of natural defences." In an interview with Florence Rosier of the French daily newspaper *Le Monde*, published on 24 December 2014, P. Kourilsky explained that "any living being is a 'machine' engineered by the evolutionary process in order to survive very complex haphazard events, such as its encounters with infectious pathogens, but also the dysfunctions of its own cells, errors or mutations that lead to cancer or to ageing. Henceforth the development of defence mechanisms – also very complex – against external and internal enemies." These defence mechanisms embedded in the human body's immune system make up 15% to 20% of the body's volume and between 5% to 10% of the human genes are devoted to them (Rosier, 2014e).

Philippe Kourilsky's book is a synthesis of 14 years of lecturing at the Collège de France, a renowned centre for advanced knowledge and research. The author decided to lay emphasis on evolution in connection with the immune system. The living beings that are the result of natural selection are those which most effectively resist the "dangers" of life. The engineers, before the biologists, have been aware of this process and have created a science of the complexity that takes into account the robustness, i.e. the capacity of a mechanical system (e.g. an aircraft or a computer) to withstand such events as a storm or an electric cable that is broken by wear and tear or by a fire. In the case of the bacterium *Escherichia coli*, its genome has 4,000 genes, and, among them, 1,000 are necessary to the cell metabolism, while the others' function is to enable the bacterium to survive in hazardous conditions. Henceforth, according to P. Kourilsky, a major component of evolution is the progress of robustness in living organisms, even more than the acquisition of new functions – except for breakthrough innovations that are not frequent (Rosier, 2014e).

Before the appearance of fishes with jaws, all living beings had a defence system based on "innate immunity" – a very rapid defence system, but offering partial protection. A few hundreds or thousands of molecules are involved. Those organisms which have acquired an "adaptive immunity" benefit from a universal protection. That is a real breakthrough. Antibodies are the weapons of this adaptive immunity: each antibody

sticks to a specific molecular structure, that is recognized as foreign or abnormal – the antigen – before neutralizing it. All the antibodies produced by a shark, a mouse or a human being, can recognize all potential antigens: those existing in natural conditions or those engineered by humans. Our body contains very few cells that are able to produce antibodies. For the "antibody response" to be efficient, these cells must proliferate: this clonal expansion needs about a week. Henceforth the importance to be immediately protected by the innate immunity: even in those organisms that have acquired the adaptive immunity, innate immunity is still indispensable (Rosier, 2014e).

How this adaptive immunity was developed? According to P. Kourilsky three cooperative systems had to be set up : B-lymphocytes that produce antibodies; T-lymphocytes which recognize antigen fragments; and the molecules of the major histocompatibility complex (called HLA system in humans). This complex plays a key role in the "self" recognition by the organism. It is highly unlikely that the three systems have appeared simultaneously. Lamprey that is a fish without jaws is one of the oldest Vertebrates. Equivalents of antibodies and T-lymphocytes have been found in lamprey, but not the major histocompatibility complex. With the exception of lamprey all species have acquired an adaptive immunity based on a molecular pattern, that of immunoglobulins. P. Kourilsky explains that an immune cell is a "communication minisatellite" that has several hundreds of "receptors" – molecules that respond to the immune system chemical messengers. New subtypes of immune cells are increasingly identified; they have new combinations of cell-surface receptors. Every year a few dozens of these combinations are discovered, but the functions of the cells containing them remain to be precisely determined (Rosier, 2014e).

## *Cancer immunotherapy : antimelanoma drugs*

At the congress of the American Society of Clinical Oncology (ASCO), held in Chicago in June 2013, with 35,000 specialists in attendance, immunotherapy was the focus of the meeting. Immunotherapy consists of stimulating the body immune defences in order to destroy cancer cells. *It has shown promising results in the treatment of advanced melanomas and lung cancers*. Melanoma is a malignant skin cancer whose prevalence has been increasing during the last decades and which is often cured through surgery. However clinicians have been unable to successfully treat advanced forms of melanomas, where surgery was not possible or which are metastatic. *Ca*. 76,000 Americans were expected to suffer from melanoma in 2014 and 9,700 would die from it, according to the Food and Drug Administration (FDA) experts. The latter have underlined that a major cause of the disease was exposure to sunlight (Cabut, 2013; Pollack, 2014a).

Melanoma was known in the past to be susceptible to being subdued by the immune system. It was not therefore surprising that immunotherapy drugs had first been approved for that disease (Pollack, 2014a). *In 2011 a new era for immunotherapy started with the commercialization of a monoclonal antibody, ipilimumab (Yervoy, produced by Bristol-Myers Squibb) that can improve the survival of patients with an advanced melanoma*. This monoclonal antibody inhibits a receptor of T-lymphocytes, called CTLA-4, which decreases the immune response. The results of a clinical trial were revealed at the ASCO congress, regarding the association of Yervoy with GM-CSF – a growth factor of leucocytes (Leukine, produced by Genzyme), that reduces the

potential important toxicity of the monoclonal antibody. The trial included 245 patients with metastatic melanoma, half of them were treated with both molecules, and the other half received only Yervoy. The survival rate within a year was 68.9% for the first group, 52.9% for the second group; *there was therefore a significant improvement in the reduction of the death rate* (Cabut, 2013).

Another immunotherapy, based on the use of monoclonal antibodies called anti-PD-1, is also promising in the treatment of several cancers, including melanomas. The receptors PD-1 (programmed death receptor 1), located on the surface of T-lymphocytes, reduce the immune response to tumours because of the decrease in the activation of T cells. Anti-PD-1 monoclonal antibodies, such as the lambrolizumab, developed by Merck, can neutralize the PD-1 receptors, as revealed by some presentations at the ASCO. When tested at different dosages in 135 patients with an advanced melanoma, lambrolizumab induced a response among 38% of them, and this proportion reached 52% when the monoclonal antibody was used at a high dosage. These effects need to be confirmed in controlled trials carried out with a larger number of patients (Cabut, 2013).

*On Thursday 4 September 2014 the FDA approved the commercialization of Keytruda, developed by Merck &Co. for patients with advanced melanoma who have exhausted other therapies*. "This is really opening up a whole new avenue of effective therapies previously not available …" and "it allows us to see a time when we can treat many dreaded cancers without resorting to cytotoxic chemotherapy," stated Louis M. Weiner, director of the Georgetown Lumbardi Comprehensive Cancer Center in Washington, D.C., and spokesperson for the American Association for Cancer Research (Pollack, 2014a).

Keytruda, a monoclonal antibody known generally as pembrolizumab, that inhibits the action of PD-1, is the sixth new antimelanoma drug approved since 2011, transforming care of a disease that, once it had spread, usually meant a quick death. One of those new drugs, Bristol-Myers Squibb's Yervoy or ipilimumab, was actually the first immunotherapy approved for melanoma. Keytruda was given accelerated approval by the FDA, allowing to reach the market without going through the three typical phases of clinical trials needed to show the efficacy of a drug. This has allowed Merck &Co. to win a race to market in the United States against Bristol-Myers Squibb (BMS), Roche and AstraZeneca, which were in advanced stages of testing drugs that also block the action of PD-1. BMS drug, nivalumab (monoclonal antibody), being developed with Ono-Pharmaceuticals was approved in July 2014 in Japan as a treatment for advanced melanoma (Pollack, 2014a).

Keytruda was approved on what was essentially an extra-large phase-1 trial involving 173 participants who all received the drug, with no control group. Tumours shrank in about 24% of patients, the FDA stated, with the therapeutic effect lasting at least 1.4 to 8.5 months and continuing beyond this period in most patients. "Even the very preliminary results on a handful of patients, 20 or so, indicated a high degree of activity," stated Richard Pazdur, who oversees cancer drugs at the FDA, in an interview on 4 September 2014. Merck was now expected to conduct two controlled clinical trials to verify that the drug can extend lives and delay the progression of the disease (Pollack, 2014a).

Keytruda which is administrated by infusion every three weeks is approved for now only for patients who have first tried Yervoy. Patients also have to first try pills known as BRAF inhibitors if they are eligible for uptaking these drugs. Antoni Ribas, a melanoma specialist at the University of California, Los Angeles, stated that patients who failed to respond to both Yervoy and BRAF inhibitors would probably survive only a few months. But in a clinical trial of Keytruda that he helped conduct, 69% of patients were alive after one year including 65% of those who had tried Yervoy (Pollack, 2014a). Inhibitors of PD-1, researchers indicated, activate an immune response more specific to the tumour than Yervoy does, which reduces the risk of side-effects. Keytruda labeling warns that the drug can cause immune-system reactions that could damage the lungs, colon, liver, kidney and other organs. However that warning is not inside a black box, the strongest level of caution (Pollack, 2014a).

*Keytruda action on the immune system may contribute to solving a century-old mystery of how cancerous cells manage to evade the body immune system*. And it may work for many types of cancer, although so far the main successes in clinical trials have come against melanoma. In fact researchers have found that the anti PD-1 drugs show signs of working for some patients with lung cancer. There are also signs of effectiveness against bladder, gastric and more other types of cancer. Roger M. Perlmutter, head of research and development at Merck, stated the company was testing Keytruda in about 6,000 patients with 30 different tumour types (Pollack, 2014a).

The treatment with Keytruda is expensive: on 4 September 2014, Merck said it would cost *ca*. US$12,500 a month or *ca*. US$150,000 a year. The pharmaceutical company stated nevertheless that the price was in line with that of other anticancer drugs, albeit it seemed to be higher than some. *Many physicians who treat cancer have complained about the rapidly escalating costs of anticancer drugs, which they said could put treatments out of reach for a rather large number of patients*. On the other hand some Wall Street analysts indicated that collectively cancer-immunotherapy drugs could achieve annual sales of tens of billions of dollars (Pollack, 2014a). See also Part One, p. 103.

### *Search for a test to predict the efficacy of cancer immunotherapy-drugs*

Cancer immunotherapy-drugs cost *ca*. US$150,000 a year per patient in 2015 – even more for higher doses used in some cases – and the United States health system is eventually expected to spend billions or even tens of billions of dollars on these drugs each year. On the other hand, as stated by David R. Gandara, a professor and lung-cancer specialist at the University of California, Davis, "We do not want to give these drugs to 100% of patients if only 59% or 20% will benefit." Consequently, being able to test for a *biomarker* that could predict the drugs efficacy "would make this new class of drugs easier on the wallet, the national health wallet," he said (Pollack, 2015d).

But "We do not want to have an imperfect biomarker," stated Jedd D. Wolchok, chief of the melanoma and immunotherapeutics service at the Memorial Sloan-Kettering Cancer Center in New York. At the annual meeting of the American Society of Clinical Oncology (ASCO), held in Chicago at the beginning of June 2015, J.D. Wolchok presented a study on the need for such biomarkers, that was published in the *New*

*England Journal of Medicine – NEJM* (Larkin et al., 2015). The 945-patient study showed that the combination of two immune-boosting drugs from Bristol-Myers Squibb (BMS) – Opdivo and Yervoy – is more effective than either drug alone in treating advanced melanoma. Patients treated with both drugs went a median of 11.5 months before their disease worsened, a longer reprieve than the 6.9 months for those who receive only Opdivo and 2.9 months for those who took only Yervoy (Pollack, 2015d). But the combination of both drugs also caused serious side-effects such as diarrhea and colitis in 55% of patients, compared with 16.3% for Opdivo alone and 27.3% for Yervoy alone. Antoni Ribas, a melanoma specialist at the University of California, Los Angeles, who was not involved in the study, stated Opdivo alone might be just as good as the combination for many patients, with far fewer side-effects, but that a biomarker test was needed. "The combination is outstanding, but we have to figure out who needs the combination as opposed to the single agent," he said (Pollack, 2015d).

The main test being explored is for PD-L1, a protein produced by cancer cells, that in effect induces the immune system to stand down and not to attack them. The Merck drug Keytruda, Opdivo and similar treatments work by keeping this "stand down" signal from being received by the T-lymphocytes. So it makes sense that the drugs work best against tumours that are issuing such a signal and that they may not work at all against tumours that are not issuing the signal. Studies by BMS, Merck and Roche, which is also developing such kind of drug, have shown that there was a much greater success rate using the drugs to treat tumours that were positive for PD-L1 (Pollack, 2015d). Still, at least a small number of patients whose tumours do not produce meaningful amounts of PD-L1 also seem to benefit from these drugs. So some physicians consider it is wrong to withhold the drugs from patients whose tumours test negative for PD-L1. In the melanoma study patients whose tumours were positive for PD-L1 did as well on Opdivo alone as with the combination Opdivo+Yervoy, as measured by the delay before their cancer worsened. One implication might be that those patients should receive only Opdivo, while others should be treated with both drugs. But Michael B. Atkins, deputy-director of the Georgetown Lombardi Comprehensive Cancer Center in Washington, D.C., stated that even for PD-L1-positive tumours, the combination was better at shrinking the abnormalities. "The biomarker is not good enough to make any decisions on it," said M.B. Atkins, who was not involved in the study (Pollack, 2015d).

PD-L1 is not the only possible biomarker. Merck is working with a diagnostic company, NanoString Technologies, to develop a test that measures activity levels in genes associated with immune response. A downside for drug companies, however, is that a test can narrow the market for a drug. For instance shares of BMS fell nearly 7% on Friday 29 May 2015 based on what would seem to be positive clinical trial results showing that Opdivo could prolong the lives of patients with the most common form of lung cancer. But there was a big survival difference in patients with PD-L1 positive tumours and patients without the protein. For those with PD-L1-negative tumours, there was no real difference between Opdivo and the generic chemotherapy drug docetaxel. This information dashed investors' hopes that Opdivo might be used by all patients with that form of lung cancer. Opdivo did cause fewer side-effects than docetaxel, but insurers might not be willing to pay so much more for that reason

alone. Docetaxel costs US$6,000 for six cycles of treatment; Opdivo used for the same length of time costs *ca*. US$60,000, stated Patrick W. Cobb, an oncologist in Billings, Montana. "The cost of treating these patients will be far higher than in the past," P.W. Cobb commented on webinar sponsored by Kantar Health, a consulting firm. "We really need a way of determining which patients are likely to benefit from these agents" (Pollack, 2015d).

## *Public-private partnerships in immuno-oncology*

### National Immunotherapy Coalition

Several leading pharmaceutical companies have decided to join forces in an effort to speed the testing of anticancer immunotherapy drugs. The cooperative effort, announced on Monday 11 January 2016, will include Amgen, Celgene, GSK, Pfizer, Merck of Germany and some smaller companies. The effort, known as the National Immunotherapy Coalition, will aim at rapidly testing various combinations of such drugs. "The challenge of cancer is far too great for any of us to tackle alone," stated Mikael Dolsten, head of research and development at Pfizer (Pollack, 2016b).

Three drugs that were approved in the last few years – Keytruda from Merck, and Opdivo and Yervoy from Bristol-Myers Squibb (BMS) – had produced significant and long-lasting improvements in some patients. But many other patients do not benefit at all from the drugs. Researchers believe that combinations of two or more drugs that engage different parts of the immune system might be effective for more patients than a single drug. The drugs from Merck and BMS are "looking at only one tiny aspect," stated Patrick Soon-Shiong, a billionaire pharmaceutical entrepreneur who is the driving force behind the coalition. "What we want to do is capture all these different molecules in the immunotherapy system," he added. And Mark C. Poznansky, director of the vaccine and immunotherapy centre at Massachusetts General Hospital, commented: "It is a sort of bringing all the parties involved around a single mission" (Pollack, 2016b).

Merck and BMS are already testing their drugs in combinations with dozens of other drugs, many from other companies. But Roche and AstraZeneca, considered leaders in this field, are not in the new coalition. There are a dizzying number of possible combinations and arranging such trials one-by-one can be time consuming. The Coalition stated it would have access to 60 drugs and would seek to enrol 20,000 patients by 2020. It would run early-stage trials of various combinations of drugs for up to 20 types of cancers, including breast, lung and prostate. The manufacturers of the drugs would then obtain valuable information on which combination work best for which types of cancer, information they can use to plan larger studies aimed at having the drugs approved by the FDA, P. Soon-Shiong said in an interview (Pollack, 2016b).

Academic medical centres, community oncologists and the National Cancer Institute will be involved, and patients in the trials will undergo sequencing of their DNA and profiling of their immune systems to help choose which drugs to test. P. Soon-Shiong, who is based in Los Angeles and is a part-owner of the Los Angeles Lakers, is

best known for developing the anticancer drug Abraxane. He sold the company that owned that drug to Celgene for US$2.9 billion in 2010. Forbes estimated his wealth at US$12.4 billion, making him the richest American pharmaceutical executive. The coalition is the basis for what P. Soon-Shiong calls Cancer MoonShot 2020. Vice-President Joseph R. Biden Jr., whose son Beau died of cancer in 2015, also talked about devoting the rest of his term in office and his years after that to a "moonshot" against cancer (Pollack, 2016b). See p. 321.

The announcement of the National Immunotherapy Coalition came on the first day of the huge J.P. Morgan Healthcare Conference in San Francisco, when numerous companies make announcements. In addition to the coalition announcement on Monday 11 January 2016, one company stated on Sunday 10 January 2016 that it was aiming for what it called the "holy grail of oncology" – a blood test to detect all cancers at the early, most treatable stage (Pollack, 2016b).

Chimeric antigen receptor T-lymphocytes (CART-cells)

Several studies have shown outstanding clinical results when applying a cell therapy combined with a gene therapy to the treatment of acute lymphoblastic leukemias or non-Hodgkin malignant lymphomas at an advanced stage. The patients received their own T-lymphocytes (autologous) that had been genetically modified in the laboratory before being administered. After this genetic modification the T-lymphocytes express a chimeric antigen receptor, or CAR, that can recognize a protein expressed on the surface of tumour cells. The genetically modified CART-cells behave like searching "missiles" that recognize the protein target expressed on cancer cells and thereafter destroy them (Chabannon et al., 2015). Some of the patients thus treated show severe side-effects including fever, variation of blood pressure as well as neurologic signs, which imply the need for a sophisticated care in specialized hospital units, including intensive care units. The side-effects are due, to some extent, to the production of pro-inflammatory molecules. But the overall clinical results confirm the development of immunotherapies based on drugs, monoclonal antibodies or cell therapies (Chabannon et al., 2015).

These new tools that have been initially developed by public research, also are an indication of the come-back of pharmaceutical companies to the field of cell and gene therapies. Thus, Novartis, GSK, Pfizer, Janssen, Amgen or Celgene invest big amounts of money in clinical trials of CART-cells, and more than 20 biotechnology companies, such as Juno, Kite, Pharma, Bluebird Bio or Cellectis (a French biotech, see pp. 36-37) seize the opportunity of the very good clinical results and their popularization by the media, in order to highlight the benefit of their genetic-engineering technologies. The stock value of these new industrial actors is growing rapidly, which means that they have for the first time hope to cash a significant revenue from these cell therapies. These prospects seem to be even more promising when it appears that these new therapeutic approaches can be applied not only to blood cancers – that are relatively rare – but also to more frequent cancers such as prostate or lung cancers (Chabannon et al., 2015).

These advances are mainly the result of partnerships between the private sector and academic research centres, mainly in the United States, within renowned institutions with advanced research in immunotherapy, such as the University of Pennsylvania, Philadelphia, the Memorial Sloan-Kettering Cancer Center in New York, the Fred Hutchinson Cancer Research Center in Seattle, or the National Cancer Institute in Bethesda, Maryland. The use of CART-cells raises regulatory, logistical and financial issues: how these "innovative therapy drugs" will be labeled or qualified by health agencies, and how they will be produced on a large scale when they obtain an authorization for commercialization from health authorities in Europe? Specialized industrial sites are being developed and they will have to devise their interaction with the health centres that will take care of the patients receiving those treatments. Autologous T-cells will have to be isolated in health-care centres (hospitals) and thereafter they should be dispatched to the industrial site, sometimes over long distances, before being carried back to the hospitals; that will entail a close collaboration between industrial and academic stakeholders in order to achieve a safe precision logistics. New private-public partnerships should be developed in order to ensure the long-term existence of this unprecedented model. Such partnerships or collaborative ties are absolutely needed for ensuring the safety and efficacy of these cell therapies, from the patient to the patient (Chabannon et al., 2015).

A startup success story

In this fast-growing area of immuno-oncology a French biotechnology start-up, Innate Pharma, is particularly successful. This Marseille-based firm (south-east of France) has signed an exclusive contract with the British pharmaceutical group AstraZeneca, regarding the commercialization of one of its promising antibodies, called IPH 2201. The contract was signed on 30 June 2015 and AstraZeneca granted US$250 million (or €228 million) to Innate Pharma; other grants were expected to reach US$1.025 billion, as well as royalties on the sales of the antibody. This was considered a record in current medical biotechnology (Hecketsweiler, 2015o).

On 30 July 2015 AstraZeneca announced that its three-month turnover amounted to US$6.31 billion, a 2% decrease compared with the same period in 2014. AstraZeneca was therefore seeking new partnerships with a view to increasing its innovative drug portfolio and competitiveness. According to Antoine Yver, in charge of leading the development of new drugs in oncological treatments at AstraZeneca, the lucrative partnership with Innate Pharma precisely aimed to boost the pharmaceutical group's capacity in immunotherapy. The challenge seems to be colossal: according to the *Morningstar* analysts, the global sales of these antibodies – e.g. antiNKG2A, antiCTLA-4, antiPD-1 or antiPD-L1 – could reach US$33 billion in 2022 (Hecketsweiler, 2015o).

As mentioned above, the first immunotherapeutic compound was commercialized in 2011: this was Bristol-Myers Squibb Yervoy (ipilimumab), the sales of which amounted to US$1.4 billion in 2014. Two other molecules were approved by the end of 2014 in the United States and at the beginning of 2015 in Europe: Opdivo (nivolumab), also produced by Bristol-Myers Squibb (BMS), and Keytruda (pembrolizumab) by Merck

&Co. These drugs were expected to become blockbusters. They were initially used in the treatment of advanced melanomas, but they were also exceptionally effective in the treatment of some lung cancers, much more common than melanomas. The market of these drugs, estimated at US$33 billion by 2022, is much coveted. BMS has won the first round thanks to the approval, in the spring of 2015, of the commercialization of Opdivo and its use against lung cancers. But the competition is just starting: the Swiss pharmaceutical group Roche, the world leader in the development and sales of anticancer drugs, as well as AstraZeneca are among the most important actors. The French pharmaceutical group Sanofi announced on 28 July 2015 the signing of a US$2.17-billion (or €1.98-billion) contract with the American biotechnology firm Regeneron in order to develop new immuno-oncological drugs (Hecketsweiler, 2015o).

Immunotherapy has become a must for all big pharmas. "Before, we were talking in the desert and all of a sudden we were caught in a tsunami," recalled Hélène Sicard, in charge of leading the research on the development of a drug for Innate Pharma. BMS researchers were indeed the first to look at the discoveries of Innate Pharma. An agreement was signed in July 2011 and €35 million were cashed by the French biotechnology startup in order to develop a compound tested against various cancers, in combination with Opdivo. Researchers had observed that only 25% to 30% of patients reacted to the already commercialized antibodies and that the promising approach was to use a combination of several molecules. That was precisely the purpose of the deal between BMS and Innate Pharma. The deal also opened up the American market to Innate Pharma. "Before, nobody was aware of our existence and American investors were not so much interested in French biotechnology firms, which were too small, with stock-exchange valutations lower than €100 million," recalled Catherine Moukheibir, financial director of Innate Pharma. In 2011 the French startup initiated its first road show in the United States and this was a real success: in November 2011 it was able to raise in one day €24 million. In 2015 40% of the company equity belonged to American investors, including Orbimed – one of the most renowned funds in the health-care sector. Innate Pharma value was estimated at *ca*. €800 million in 2015 (Hecketsweiler, 2015o).

Before the French biotechnology startup was supported by Bpi-france, which in 2015 owned 8% of the firm equity. The large venture fund of Bpi-france had invested a total of €18 million before selling part of that share. That was considered one of the best business deals of Bpi-france. The agreement between Innate Pharma and AstraZeneca was the most important ever concluded by a French biotechnology company, and one of the biggest at the global level at this stage of development. Martial Descoutures, a health-care analyst at Invest Securities, observed that "immunotherapeutic treatments are expected to become the cornerstone of cancer treatment, and consequently companies such as BMS and AstraZeneca are seeking to find out and secure the most effective compounds and combinations of the latter." The analyst forecast that Innate Pharma share value could rise 35% during the second half of 2015. Innate Pharma researchers are indeed working very hard in order to discover new drugs. Their method consists of "injecting a human antigen to a mouse – the "marker" which corresponds to a "receptor" identified on the surface of a human cell – so as to provoke an immune

reaction. The antibodies produced by the laboratory animal are thereafter collected, isolated and tested in *in-vitro* models, eventually on patients' cells, in order to evaluate their biological properties," explained Hélène Sicard (Hecketsweiler, 2015o).

## Anticancer drugs from plants

In the 1960s the United States National Cancer Institute (NCI) had systematically listed the natural substances with pharmacological properties. Among them was narciclasin. But "the NCI did not carry out further *in-vitro* tests on this compound. We bought kilograms of bulbs of this kind of daffodil in order to extract and study it," recalled Florence Lefranc of the department of neurosurgery at the Erasmus hospital, Free University of Brussels (ULB, French acronym), and co-author with Robert Kiss, a toxicologist at the ULB Pharmaceutical Institute, of the research on narciclasin. They hade been testing this anticancer compound on glioblastoma – a deadly brain cancer – for several years (Benkimoun, 2011a).

The team of F. Lefranc and R. Kiss had also been working on another natural substance extracted from *Calotropis procera*, a sub-Saharan plant species. In the early 2000s R. Kiss visited with Pierre Guissou, a researcher at the ULB and working in Burkina Faso. He met women with advanced breast cancer lesions who were supposed to die from their illness. But one year later they were still alive; they had been treated with infusions of *C. procera*. The substance isolated from the plant slowed down "both the proliferation and the migration of cancer cells, and particularly those of glioblastoma," explained F. Lefranc. Preliminary clinical trials had been carried out in Belgium and the Netherlands. "The molecule is the property of the private company Unibioscreen, a startup created in 1999 and incubated at the ULB," added F. Lefranc. As the work had been carried out within the framework of a collaboration between the ULB and the University of Ouagadougou, each institution was to receive 5% of any profit made as a result of the commercial use of the natural anticancer substance. This concern about not to plunder the country of origin of the natural substance was also reflected in the price of the drug. "The bioactive principle is so powerful that a treatment based on it would cost €15,000 per patient per year. In fact the cost is €7 and eight countries of the Economic Community of West African States (ECOWAS or CEDEAO, French acronym) can have access to it," added R. Kiss (Benkimoun, 2011a).

This kind of discovery of antitumour substances extracted from plants is not extraordinary. More than 60% of traditional pharmacopea is derived from plants. Madagascar periwinkle is an emblematic example. In 1952, Robert Laing Noble of the University of Western Ontario, Canada, received a mail from his brother, Clark Noble, a physician, with 25 leaves of a plant, *Vinca rosea*. A patient gave him those leaves and indicated that in Jamaica they were used to prepare an infusion that had antidiabetic properties. Robert Laing Noble did not observe any particular efficacy on blood glucose concentration, but he realized that all the mice on which the substance had been tested died from a wide-ranging infection. They had no leucocytes to defend themselves. Hence the idea to use *Vinca rosea* compounds against cancers, starting with leukemias where abnormal leucocytes proliferate (Benkimoun, 2011a).

"Gordon Svoboda, a chemist working at Eli Lilly, isolated from *Vinca rosea* two anticancer molecules: vincablastin and vincristine. The former was commercialized in 1963 and the latter twenty years afterwards. These were the first commercialized anticancer drugs of natural origin," recalled François Tillequin of the Paris René-Descartes University and the French National Scientific Research Centre (CNRS, French acronym). The content of the bioactive compound in the plant is very low: "5 to 50 g of bioactive compound can be extracted from one ton of dried leaves, or ten tons of fresh leaves. Henceforth a very high price. By the end of 1968 vinblastin cost 850 French Francs, bearing in mind that at that time the minimum salary was 3FF per hour," indicated F. Tillequin (Benkimoun, 2011a). In order to increase the yield of plant-derived bioactive compounds one could rely on partial chemical synthesis. Pierre Potier and his team of the Institute of Chemistry of Natural Substances (CNRS), at Gif-Sur-Yvette, south of Paris, designed a chemical method of partial synthesis, with the help of François Guérite. In 1976 they could synthesize a new compound, vinorelbin, which is made of vinblastine and a synthetic molecule; its cost was much lower than that of vinblastin (Benkimoun, 2011a).

The same story occurred with paclitaxel. At the beginning of the 1970s two researchers of the NCI, Monroe Wall and Mansukh Wani, identified a bioactive compound in the bark of the Pacific yew tree, *Taxus brevifolia*. The compound, paclitaxel, was tested successfully in breast cancer. But there was again a problem of yield : to treat a cancer, one tree or half of it was needed for the extraction of the necessary amount of the drug – and the yew tree takes decades to reach its full size. "Pierre Potier decided to use the tree needles and not the bark; he isolated a compound close to the molecules discovered by M. Wall and M. Wani, and transformed it into a partially synthetic analogue, named docetaxel, which became a very successful drug developed by the French chemical group Rhône-Poulenc," recalled F. Tillequin. A second generation of these drugs, called toxoids, is replacing the initial compounds and they are more efficient. But these developments can take a very long time: in the early 1970s M. Wall and M. Wani had also isolated an interesting substance, camptothecin, from a tree of Chinese origin, *Camptotheca acuminata*. It had been tested on patients whose cancer resisted all available treatments. Susan Horwitz, an American pharmacologist, found the target of camptothecin, and this had been followed by the development of semisynthetic compounds which were less toxic, irinotecan and topotecan, and were commercialized between the mid-1990s and the early 2000s, 30 years after the pioneering work of M. Wall and M. Wani (Benkimoun, 2011a).

## *Reluctance from the pharmaceutical industry*

Relying on natural substances in order to produce drugs is fraught with difficulties that hinder the possible interest of pharmaceutical companies. One of these difficulties is linked to the Rio Convention on Biological Diversity (CBD). "There have been cases of bribery concerning the local civil servants in charge of delivering the authorizations to use or exploit biological diversity, particularly when biologically diverse countries were told or brought to think that they were sitting on a gold mine. That is why pharmaceutical companies do not believe in natural substances as potential drugs. They want to avoid court trials as well as problems of sustainable supply. They do prefer synthetic molecules," commented F. Tillequin (Benkimoun, 2011a).

There are nevertheless those who are convinced that a large number of natural substances could be isolated and exploited, because they have shown their efficacy over long periods of time through the very selective filter of evolution. "Plants which cannot flee from their predators, have produced a wide range of chemical substances for defending themselves, because these compounds are toxic," concluded Robert Kiss (Benkimoun, 2011a). For the time being and with respect to the war on cancer compounds extracted from plants play a minor role, except perhaps at the level of traditional pharmacopea. But it is almost impossible to quantify (evaluate) their contribution to cancer treatment. See also Sasson (2011).

## Some conclusions

According to Harold Varmus, director of the United States National Cancer Institute (NCI) in 2014, Nobel Laureate (1989, for Medicine or Physiology), *the identification of tumours will still rely on tissue and molecular diagnosis. Both types of tests will coexist*. To identify a mutation associated with a tumour does not imply that treatments targeting such anomaly will be effective. On the contrary, surgery, radiotherapy and conventional drugs are curing one cancer out of two nowadays. Fabien Calvo emphasized the huge progress made in cancer genomics since 1976 when the first oncogenes had been discovered. Two wide-ranging programmes contributed to this leap in our knowledge: *the International Cancer Genome Consortium (ICGC) and the Cancer Genome Atlas (CGA). It has been possible to establish the genetic identity card of more than 50 distinct cancers and the numerous genetic anomalies associated with them. Many of them are found in cancers affecting various organs. Also, an increasing number of epimutations associated with cancers and affecting genes that interfere with the expression of many other genes, are discovered. This new area of research is expected to have a therapeutic impact quite soon* (Rosier, 2014a). H. Varmus was of the opinion that we must be pragmatic and focus our research on the most frequent genetic modifications – or that enable the prediction of a cancer. In the United States there is a programme devoted to the *Ras* genes that are mutated in one-third of lung and colon cancers, and in 95% of pancreatic cancers (for which there are no targeted therapies) [Rosier, 2014a].

In France and since 2006, 28 "molecular genetics cancer platforms" had been created under the National Cancer Institute (INCa). That was a pioneering work, and "*in 2013 some 70,000 patients had genetic tests made thanks to these platforms. France is the only country in the world that offers these tests to those who need them*," stated Agnès Buzyn, president of the INCa. Alexander Eggermont, director-general of the Institut Gustave-Roussy, had emphasized that "with 28 platforms devoted to tumour genetics, the INCa had set up an organization that the whole world praises." "Nowadays they identify only a few anomalies, targeted by a few drugs. But in the near future the whole sequencing of tumour cell genomes using high-throughput techniques will have a considerable impact on cancer therapy." The third Cancer Plan (2014-2018), launched on 4 February 2014 with a budget of €363 million over five years – this funding had been safeguarded, compared with that of the second Cancer Plan (2009-2013) – has the following objective: in 2014, a few platforms for

high-throughput sequencing were to be set up; the whole tumour-cell sequencing will be carried out in 3,000 patients in 2015, 10,000 in 2017 and 50,000 in 2019, according to A. Buzyn (Rosier, 2014a).

Those 28 platforms are using the new-generation sequencing techniques that target, not just one gene, but a panel of genes (they are called NGS) and thus aim at identifying through one single analysis the molecular anomalies of several genes in the patient's tumour. "The main shortcoming of this novel approach is the rather long time for receiving the results and delivering them to the patient; if the average time lapse is 18 days, much longer periods have been noted for a minority of patients," in the centres that are not in the vicinity of the molecular genetics platforms, commented the INCa. But, as asserted by Catherine Simonin, president of the Anticancer League committee in the department of Tarn-et-Garonne (south-west of France), "everybody should have access to innovative treatments, wherever he/she is treated, if we want to maintain this pillar of the health-care system in France" (Santi and Cabut, 2016).

Those achievements and prospects would propel France as the most advanced country in the world in personalized medicine and large-scale clinical trials in oncology. In 2014, with 9.2% of all high-level international publications in oncology, France came fourth, behind the United States, the United Kingdom and Germany. Furthermore clinical research on cancer remained another priority of the third Cancer Plan which aimed to: firstly, duplicate the number of patients participating in therapeutic trials in oncology, so as to reach the number of 50,000 in 2019; secondly, develop early trials, with special attention to children (two centres for early trials in pediatrics will be created) [Rosier, 2014a]. According to F. Calvo, *it has been decided that in France all patients having the same molecular anomaly, will have access to targeted therapies, whatever the organ affected by the cancer and even beyond the indications recommended for these therapies* (when they received their authorization for commercialization). That was the objective of the programme called AcSé (Rosier, 2014a).

*Which are the foreseeable progresses in cancer therapy*? According to H. Varmus, to treat every tumour more specifically needs the development of more targeted therapies, that could destroy tumour cells more effectively and overcome the resistance developed by these cells to those treatments. Another challenge deals with the improvement of anticancer immunotherapies. We also must understand why only some patients are sensitive to them. Finally, *we should reduce the costs of these treatments so as to extend them to the largest number of patients*. F. Calvo indicated that in 2014 there were 20 drugs available to target molecular anomalies associated with some cancers. But more than 900 targeted therapies were being assessed in clinical trials. With an annual development of five to six new anticancer drugs the expectation was to have some 50 targeted therapies by 2019. To whom should these drugs be prescribed and after which tests? To that end the profiling of tumours using the new techniques of high-throughput sequencing must be developed. On the other hand, genomics could help understand why certain patients are cured, while others are not, after a conventional chemotherapy treatment, radiotherapy or surgery (Rosier, 2014a).

H. Varmus recalled that an efficient detection system of cancers reduced the incidence of the illness by 20% (average). *We therefore need to continue to develop new diagnosis tools for the early detection of a cancer in order to be able to determine the probability of occurrence of the disease and its risks for life*. The war on cancer entails the research on such basic issues as: which are the factors that lead a cancer cell to leave its primitive site to form a new tumour elsewhere in the body (metastasis)? Which are the tumour antigens that trigger the anticancer immune response and the biotic factors that regulate that response? (Rosier, 2014a). See also Beaugerie et al. (2014); *The Economist* (2014a).

### *The "moonshot" approach to curing cancers: an unrealistic goal*

By early January 2016 a group of 15 American cancer researchers attended a meeting at the United States FDA, invited by Vice-President Joseph R. Biden Jr. to discuss his "moonshot" approach to curing cancer. It turned out the vice-president was out of town the day of the meeting, but his staff wanted to know: What could J.R. Biden Jr. do in his remaining year in office, and over the long term, to advance cancer research and ultimately cure the disease? The cancer researchers cut short the meeting because they felt that the chances of reaching a moment of victory as the analogy "moonshot" suggests seem unrealistic. "This is not about getting to one point in a certain period of time," stated Harold Varmus (Kolata and Harris, 2016).

Unlike in 1971 when President Richard Nixon launched his "war on cancer", researchers now understand that cancer is not one disease, but essentially hundreds. Thus the very notion of a single cure – or as President Barack Obama put it, making "America the country that cures cancer once and for all" – is misleading and outdated. "Cancer is way more complex than anyone had imagined in 1970," stated José Baselga, the president of the American Association for Cancer Research and chief medical officer at Memorial Sloan-Kettering Cancer Center in New York City (Kolata and Harris, 2016).

Although cancer death rates are dropping, cancer remains a leading killer of Americans – there were an estimated 589,460 cancer deaths in 2015. But cancer specialists are optimistic, stating they have entered a new era with the ability to rapidly determine the sequences of genes in tumour cells, searching for mutations that may be driving the cancer growth. Researchers are developing more targeted drugs and immune therapies, and say that in the future they expect to hit cancers with several such treatments at once, much the way AIDS/HIV was treated when researchers developed drugs to strike the virus at its vulnerable sites. "We are in a situation now where we can really make an impact," said J. Baselga. "But at this point, funding matters," he added (Kolata and Harris, 2016).

While J.R. Biden Jr.'s focus has already made some meaningful difference – he negotiated with Republican congressional leaders a US$264-million increase in funding for the National Cancer Institute, the largest in a decade for an agency that has been squeezed by static budgets in recent years – his "moonshot" initiative consisted

of listening in the first stage. He visited the Abramson Cancer Center at the University of Pennsylvania Perelman School of Medicine to talk to physicians and researchers. He also discussed cancer research with international experts at the World Economic Forum in Davos, Switzerland, at the end of January 2016. He was to convene the first of several meetings with cabinet secretaries and agency heads to improve federal investment and support for cancer research and treatment. The researchers offered a number of ideas on how the vice-president of the United States could be helpful, not simply for new research but for making sure what is being discovered at such a rapid pace today is not squandered, that patients who could benefit were actually helped (Kolata and Harris, 2016).

# CARDIOVASCULAR DISEASES

Heart attacks are the leadings killer in the United States and *ca*. 720,000 Americans a year have them (Kolata, 2014a). Cardiovascular diseases are also a major threat to the health of people in industrialized countries (along with cancers) and also increasingly in developing ones. Reducing blood cholesterol, triglycerides, as well as blood pressure (hypertension) can help prevent heart disease. Surgery of coronary arteries is also a means to save lives. And changing lifestyle, i.e. healthier eating habits and daily exercise, also contributes to reducing the incidence of cardiovascular diseases.

## What is the optimal blood-pressure goal?

For years physicians have been uncertain what the optimal goal should be for patients with high blood pressure. The aim, of course, is to lower it, but how far and how aggressively remained a matter of uncertainty and eventually of argument. There are trade-offs – risks and side-effects from drugs – and there were lingering questions about whether elderly patients needed somewhat higher blood pressure to push blood to the brain. On 11 September 2015 the United States federal health officials declared that there were ending a major study more than a year early because it has already conclusively answered the question: How low should blood pressure go? This study, called SPRINT, randomly assigned more than 9,3000 men and women aged 50 and over, who were at high risk of heart disease or had kidney disease, to one of two systolic blood pressure targets : less than 120, which is lower than any guideline ever suggested, or less than 140 (systolic pressure is the higher of the two blood pressure numbers and represents pressure on blood vessels when the heart contracts). The study was expected to conclude in 2017, but considering the results of great importance to public health, the National Heart, Lung and Blood Institute announced them on 11 September 2015, adding that a paper with the data would be published within a few months (National Institutes of Health, 2016).

The study found that patients who were assigned to reach a systolic blood pressure goal below 120 – far lower than current guidelines of 140 and 150 for people over 60 – had their risk of heart attacks, heart failure and strokes reduced by a third and their risk of death reduced by nearly a quarter. Gary H. Gibbons, director of the National Heart, Lung and Blood Institute, stated : “This study provides potentially lifesaving information.” *Ca*. 79 million adults in the United States – one out of three – have high blood pressure, and half of those being treated for it still have systolic pressures over 140. “This study will shake things up,” predicted J. Michael Gaziano, a professor of medicine at Harvard Medical School, who was not involved in the study.

He anticipated that it would have the same effect on people's thinking about blood pressure as studies of cholesterol-lowering did when they showed that, contrary to what many had thought, the lower the better (Kolata, 2015f; see p. 325).

Mark Creager, president of the American Heart Association and director of the Heart and Vascular Center at Dartmouth-Hitchcock Medical Center, who was not involved in the study, said: "It is outstanding news. It will serve as a road map and will save a significant amount of lives." Since cardiovascular disease is still the leading cause of death in the United States, a change in blood pressure goals could also reduce the nation's overall mortality rate, commented Jackson T. Wright Jr, a blood-pressure expert at Case Western Reserve University and a study investigator (Kolata, 2015f).

A systolic pressure that is naturally 120 might be good, but it is quite another matter to artificially lower pressure down to this figure with drugs. Reaching a target that low would mean giving people more and more medications, and the side-effects could cancel any benefit. Older people might be especially vulnerable to ill effects of a much lower blood pressure since many already take an array of drugs for chronic conditions which might interact. A very low blood-pressure could lead to dizziness and falls. Twenty-eight percent of the subjects in the SPRINT study were over age 75. It was not always easy for people in the study to reach their blood pressure goals. Those assigned to have their systolic pressure below 140 took, on average, two drugs. Those assigned to below 120 took an average of three drugs. Cost is usually not an issue for high blood-pressure patients because 90% of antihypertension drugs are available as generics (National Institutes of Health, 2016).

The study also tried to find out whether a lower blood pressure would help people with kidney disease and whether people would think more clearly and have less dementia. That was one hypothesis, but it was also possible that a lower pressure would mean less blood reaches to the brain and kidney, with detrimental effects. The results of a lower blood pressure on the kidneys and the brain are still being analyzed, according to the National Heart, Lung and Blood Institute. As with all large clinical trials the SPRINT accumulating data was periodically examined by a safety and monitoring committee. Such committees keep study results to themselves unless they became so clearly positive or negative that the only ethical behaviour would be to end the trial. In August 2015 the committee told administrators at the National Heart, Lung and Blood Institute that the trial should be stopped. Administrators and researchers looked at the data and agreed. The next step, before any public announcements, was to notify the study participants. They were told the study was ended but to stay on their current medications until their next clinic visit or until they saw their health-care provider, stated David Reboussin, professor of biostatistics at Wake Forest Baptist Medical Center and principal investigator for the study coordinating center. "I was very surprised not only by how large the effect was but that it occurred at such a relatively early stage of the study," stated William C. Cushman, chief of preventive medicine at the V.A. Medical Center in Memphis, Tennessee, and a member of the trial leadership committee (Kolata, 2015f).

## Lowering blood cholesterol

### *Efficacy of statins*

Statins are the world's leading cholesterol-lowering drugs, firmly established as safe and very effective at reducing the risk of heart attacks, strokes and deaths from atherosclerotic heart disease for most people. Statins work by inhibiting an enzyme called HMG-CoA reductase that controls the production of cholesterol in the liver. More than a quarter of Americans older than 40 are taking a statin, a number that could rise to 46% of people aged 40 to 75 under the newest prescription guidelines, especially now that almost all statins are available as inexpensive generics. A study shows that prescribing statins under the new guidelines could cut in half the number of people who develop clinical evidence of cardiovascular disease (Brody, 2015).

Because statins are cheap, as well as efficient and safe for most people, physicians often prescribe them for otherwise healthy patients with elevated blood cholesterol (LDL level ≥ 171 mg per deciliter), even though they have no other cardiovascular risk factors, stated Philip Greenland, a cardiologist at the Northwestern University Feinberg School of Medicine. Yet many who could benefit – including people with established heart disease and serious risk factors like smoking, diabetes and high blood pressure – are not on a statin for reasons that include reluctance to take daily medication, concern about possible or actual side-effects and "denial that they are at risk of premature cardiovascular disease and death." Even those for whom statins are prescribed often take them inconsistently, undermining their potential benefits (Brody, 2015).

While best known for their ability to lower serum cholesterol, statins also reduce artery-damaging inflammation that can result in a life-threatening blood clot. By lowering cholesterol statins also appear to stabilize plaque, artery deposits that can break loose and cause heart attack or stroke. And they may cleanse arteries of plaque that has not yet become calcified. The new guidelines, put forth by the American College of Cardiology and American Heart Association, focus on four main groups who could be helped by statins:

- people with cardiovascular disease, including those who have had a heart attack, stroke, peripheral artery disease, transient ischemic attack or surgery to open or replace coronary arteries;
- people with very high levels of LDL cholesterol – 190 mg per deciliter or above;
- people with an LDL level from 70 to 189 mg per deciliter who also have diabetes, a serious cardiovascular risk;
- people with an LDL level above 100 mg per deciliter who, based on other risks like smoking, being overweight or high blood pressure, face a 7.5% or higher risk of having a heart attack within ten years (Brody, 2015).

Not everyone responds well to statins. About 5% of people who use them have distressing muscle aches and some experience an unhealthy rise in blood glucose. Furthermore Stephen L. Kopecky, a preventive cardiologist at the Mayo Clinic in

Rochester, Minnesota, stated that *ca*. 15% to 20% of people were "hyperresponders" – their LDL level is only minimally reduced or actually goes up on a statin. They may be good candidates for one of three other newer drugs that lower cholesterol by different mechanisms. "Statins are the most studied drugs in the world," stated S. L. Kopecky. "We know from studies in tens of thousands of people what one statin will do in numerous patients, but we do not know what numerous statins will do in one patient." Thus, physicians are encouraged to try at least three statins before moving on to another option (Brody, 2015).

Best studied among the newer statin alternatives is ezetimibe, sold as Zetia, which acts in the digestive tract to block absorption of dietary cholesterol. Zetia, taken by itself, has not been shown to reduce the risk of a heart attack or stroke, but it can enhance the cholesterol-lowering effect of a moderate dose of a statin in high-risk patients who cannot tolerate a high-dose statin. Ezetimibe does not reach the bloodstream and has not been linked to any serious adverse effects, stated Donald Lloyd-Jones, director of the Northwestern University Clinical and Translational Sciences Institute (Brody, 2015).

### *New anticholesterol drugs (PC-SK9 inhibitors) mimicking gene mutation*

An expert group recommended that the FDA approve two new drugs that protect against heart attacks. Joshua W. Knowles, a Stanford cardiologist, called the medicines "a triumph of the modern genetic revolution." Patients who have taken them have seen their LDL (low-density lipoproteins, also called "bad cholesterol") levels plummet to remarkably low values. But the definitive evidence of the drugs efficacy in reducing heart attacks and deaths will come only after large clinical trials are efficacy completed in 2017 (Kolata, 2015c).

Sanofi and Regeneron Pharmaceuticals drug Praluent (alirocumab) and Amgen drug Repatha (evolocumab) are injected once every two weeks or once a month, depending on the formulation. The companies and independent cardiologist stated they had reason to believe the drugs will perform as expected. These drugs were specifically designed to mimic mutations in a gene, PC-SK9, that protects people from having heart disease (coronary disease), even if they smoke or have high blood pressure. Sanofi estimated that 11 million Americans might qualify for these drugs. Amgen put the number at 8 million out of the 73 million who suffer from an excess of cholesterol (Kolata, 2015c; Hecketsweiler, 2015n). It was already shown that LDL levels plunge by *ca*. 40% to 65%, even if the starting level was achieved with a statin. LDL levels could be even more lowered by increasing the dose of the new drugs. Safety studies so far have shown that the drugs seem to have no more side-effects than a placebo. Both companies are conducting multiple trials looking at effectiveness, including a 27,500-patient study by Amgen and an 18,000-patient study by Sanofi (Kolata, 2015c).

Pharmaceutical companies see the prospect of multibillion-dollar blockbuster drugs in their future and are therefore trying to commercialize them as rapidly as possible. Sanofi paid the FDA US$67,5 million for an expedited review and was granted the authorization for commercializing its drug on 24 July 2015. Amgen received its authorization by the FDA on 27 August 2015. Pfizer also had a drug in this new class,

but it was further behind in development. The companies were requested to use their drugs for three groups of patients: those with high levels of LDL who cannot lower them enough with statins; people at very high risk because they already had a heart attack or who have diabetes and cannot lower their LDL levels with statins; and people with high levels of LDL who cannot tolerate statins. Steven Nissen, a Cleveland Clinic cardiologist, stated: "Imagine starting with an LDL level of 2.20 g/l," referring to a dangerously high level. "With the maximum dose of the most powerful statin you decrease it down to 1.20. Now you add a PC-SK9 inhibitor and lower it to 0.60 g/l. For those people, it will be really helpful." S. Nissen is leading an Amgen study of its drug, evolocumab, to see if it can reverse coronary disease and is on the steering committee for a Pfizer clinical trial, although he reported taking no fees from the companies (Kolata, 2015c,e).

The powerful effects of the gene, PC-SK9, whose expression is blocked only by the drugs, were discovered when researchers found people who were born with one copy of the gene mutated, so it did not function. They seemed to be protected against heart disease. Then they found two young women with both copies knocked out, and both had very low LDL levels: one had a level of 0.14 g/l and the other's was 0.15 g/l. These findings indicated that it might be safe to block the action of the gene with a drug and consequently drive LDL levels very low. With cholesterol-lowering drugs most people start treatment in middle age, after heart disease is established. Would it be better to start much earlier in the case of patients whose LDL levels are high? And how low should LDL level go? The evidence so far is that lower is better and physicians often tell people at high risk for heart attacks to aim for LDL levels of 0.70 g/l. But Harlan M. Krumholz, a Yale cardiologist, was worried by the likelihood that the patient population who takes the drug would expand beyond those who really need the drugs. "These drugs should be reserved for people who have no choice," he stated (Kolata, 2015c).

### *Cost and strategic issues*

The new drugs, like many new anticancer drugs, are monoclonal antibodies, produced from living cells at great expense. The companies have not indicated how much these drugs would cost, but William Shrank, chief scientist officer at CVS Health, an important pharmacy benefit manager of the United States, estimated they would cost *ca*. US$14,000 a year. If the drugs were restricted to those with high cholesterol levels who cannot lower enough their LDL levels with statins, the total cost would be US$16 billion, he estimated. If people, who are intolerant to statins, are included, that would add US$20 billion. If people with a history of heart disease are included, the bill would rise to US$150 billion for the American health-care system (Kolata, 2015c).

Impact on the health-insurance system; selected prescriptions

By the end of July 2015 Steve Miller, medical director of Express Scripts – the most powerful pharmacy benefit manager of the United States – warned that "the annual cost for the taxpayers and the patients consuming the new anticholesterol drugs would amount to more than US$100 billion; this would increase by 30% the country's drug-

expense bill which rose to US$374 billion in 2014" (Smith, 2015). Express Scripts mission is to help insurance companies in the selection of those drugs whose expenses they will bear and to negotiate drug prices with the pharmaceutical companies. This pharmacy benefit manager is very powerful and influential because it can kill the commercialization of a drug, if the latter is not on the list it establishes and that serves as a reference for the reimbursement by the insurance firms of the expenses made by the patients. Both Express Scripts and its rival, CVS Health, wanted to send a clearcut warning to the big pharmas: not to prescribe Repatha or Praluent to any kind of patient, as this has been the case in the past with other drugs (Hecketsweiler, 2015p).

The FDA made it clear that these drugs should be prescribed to those patients who already take the maximal dose of statins (or who do not tolerate them) and when there is a high risk for heart disease and vascular accident. The FDA however did not give a precise criterion for the evaluation of that risk. "Pharmaceutical companies had rather to well inform physicians because if the prescriptions are not well made, the pharmacy benefit managers will not hesitate to negotiate important retail prices while promoting the competition between the two drugs approved by the FDA," commented Sébastien Malafosse, an analyst at Oddo (Hecketsweiler, 2015p).

The pharmacy benefit managers might also decide to reimburse the costs of these drugs if their efficacy is demonstrated "in real life", and they would therefore impose their own evaluation criteria. S. Malafosse indicated that "because of the soaring prices of drugs the pharmacy benefit managers demand more evidence of their efficacy." In this conflict the advantage is not on the side of the big pharmas. Express Scripts and CVS Health already control the prescriptions of one American patient out of two and they tend therefore to impose their conditions. For instance regarding Sanofi Praluent its reimbursement in the United States was made on a case-by-case approach, by the end of August 2015; in other worlds the negotiations were still being carried out between the pharmacy benefit managers and the pharmaceutical company. In this regard the PC-SK9 inhibitors are not the only ones to be scrutinized by the pharmacy benefit managers. Other drugs have been declared "persona non grata": in order to limit the high increase in drug prices Express Scripts eliminated from its lists or forms 80 molecules and played on the competition between pharmas. CVS Health, on its side, eliminated from its lists *ca*. 245 speciality drugs by the end of 2015 (Hecketsweiler, 2015p).

Implications for the pharmaceutical companies and their competitivity

The commercialization of Sanofi alirocumab or Praluent (commercial name) after its approval by the FDA on 24 July 2015 has resulted in the availability of the drug to American patients since August 2015. Also the European Medicines Agency (EMEA) Committee for Human-Use Medicines has given its approval for the commercialization of Praluent. The European Commission was expected to deliver its authorization by September 2015. This was very good news for Sanofi whose CEO, Oliver Brandicourt, was appointed in April 2015. "The last great event in commercializing a new drug happened ten years ago with the anticancer medicine Eloxatine. Praluent is the first of a series of new drugs that will enable Sanofi to change its stock exchange status,"

estimated Philippe Lanone of Natixis – a French investment bank. If one adds to Praluent a drug against severe asthma or dupilumab, to be commercialized in 2017, and sarilumab – a drug against rheumatoid polyarthritis, expected in 2016 –, the resulting turnover would amount to *ca*. US$12 billion for Sanofi. All these compounds have been developed by the American biotechnology company Regeneron Pharmaceuticals of which Sanofi owned 22% of equity (Hecketsweiler, 2015i).

The commercialization of Praluent opened up a race among a few companies – Amgen, Pfizer, in addition to Sanofi – in the development and marketing of these new anticholesterol drugs. The latter are supposed to become blockbuster drugs for these companies, while the statins have lost patent protection, after making the fortune of such companies as Pfizer whose Lipitor sales reached up to US$13 billion (or €11.8 billion) – a record in the history of the pharmaceutical industry – and AstraZeneca whose Crestor annual sales amounted to US$7 billion. The annual cost of statins per patient was estimated at US$600 (average) or €536 in the United States. In the case of Sanofi the partnership with Regeneron Pharmaceuticals set up in 2007, helped the big pharma to fill the gap, due to the lack of homemade new drugs that were expected to replace the former blockbusters. In 2014, of the total annual turnover of the group (€33 billion), more than €6 billion were provided by the sales of its insulin Lantus, the patent of which expired, and €3.5 billion from the sales of two anticoagulants, Lovenox and Plavix, which are now being produced as generics (Hecketsweiler, 2015i).

According to Natixis the sales of Praluent in the United States could reach US$4 billion per year. This will depend on the labeling of the drug by the FDA (i.e. to whom the drug is reserved) and on the success of its rival, Amgen Repatha (evolocumab). As mentioned above the FDA recommended that Praluent be reserved, in the first place, to patients suffering from family hypercholesterolemia – a rare disease – and to those with a high risk of heart attacks. That would also be the case of Repatha. In order to target the patients who do not respond well to statins – a much larger population – both Sanofi and Amgen are carrying out studies aimed at showing that the uptake of their drugs is followed by a decrease in mortality among the patients treated. The publication of these studies was expected in 2017-2018. The economic stake is considerable: in the United States *ca*. 73 million people have a high level of "bad" cholesterol (LDL) and among them 15 million do not respond well to a statin-based treatment (Hecketsweiler, 2015n).

Sanofi and Amgen are doing everything possible to cash the profits of their new PC-SK9 inhibitor drugs, before Pfizer similar drug reaches the market by 2017, and its impact study on mortality is completed in 2018. This competition has to take into account important factors such as the labeling of the drugs and their price. PC-SK9 inhibitor drugs must be injected, while statins are taken orally; that is not a competitive advantage. With respect to cost it was estimated that the wholesale price of Praluent in the United States will be *ca*. US$40 per day, or *ca*. US$14,000 per year, i.e. 23 times higher than that of statins. In a press release Sanofi stated that "the real costs for the patients, insurances and health-care systems would be lower, because the wholesale price of Praluent does not take into account the discounts that could be granted." The

French pharmaceutical group, in order to successfully compete with Amgen, intends to carry out a programme for helping the patients without insurance or poorly insured, and also for distributing the drug free of charge to the patients who really need it (Hecketsweiler, 2015n).

In order to please the powerful pharmacy benefit managers Sanofi is marketing Praluent in the United States with a dosage of 75 mg and two injections per month. Amgen Repatha dosage was expected to be higher, with one single injection per month. The competition between Sanofi and Amgen may be plagued with disputes on intellectual property rights: in October 2014 Amgen filed a complaint against Sanofi and Regeneron Pharmaceuticals, because of infringement of three of its patents. This seems to be a rather frequent procedure in the United States. The court trial was expected to take place in March 2016 if a gentleman's agreement is not reached before (Hecketsweiler, 2015n).

## Lowering blood triglycerides

Many users of statins still have high concentrations of blood triglycerides and go on to have heart attacks. Daniel J. Rader, director of the Preventive Cardiovascular Medicine and Lipid Clinic at the University of Pennsylvania, stated: "We have been looking for something beyond statins." Experts indeed differ in their estimates of how many Americans might be candidates for a triglyceride-lowering drug. If the eligible group included all adults with triglyceride levels of 2.00 g/l or more – the normal level is 1.50 g/l or less – that would mean *ca*.20% of adult Americans. If it were just those with the highest levels, above 5.00, then 2% to 3% of adults would qualify (Kolata, 2014a).

In 2008 researchers from the University of Maryland medical school found that one in 20 Amish people had a mutation that destroys a gene involved in triglyceride metabolism, compared with one in 150 Americans generally. The scientists were intrigued but they did not have enough data to elucidate the role of that gene in heart attacks. On Wednesday 18 June 2014, findings of studies carried out by the members of the Triglycerides (TG) and High-Density Lipoprotein (HDL) Working Group of the Exome Sequencing Project and published in the *New England Journal of Medicine* (*NEJM*), and funded by the National Heart, Lung and Blood Institute (NIH) and the European Union, provided "a very, very strong type of evidence," that triglycerides are in fact a cause of heart attacks (Crosby et al., and Kathiresan, 2014). This research work began when scientists at the Broad Institute of Harvard University and Massachusetts Institute of Technology (MIT) started searching through an enormous data set – drawn from 70 studies involving 200,000 people – to see if there were tiny genetic changes near or in genes that seemed to lead to very high or very low amounts of triglycerides in the blood. They also asked whether people who happened to have a higher or low triglyceride concentration also had a higher or lower incidence of heart attacks (Kolata, 2014a).

The researchers discovered that people with a genetic predisposition to higher triglyceride levels had more heart attacks and those with genetically lower triglyceride levels had fewer. Their study, published in 2013 in *Nature Genetics*, did not isolate

individual genes, though. It just pointed to signposts on the long stretch of 30 million DNA nucleotide pairs that were near the genes (Do, Willer et al., and Kathiresan, 2013). Later on Crosby et al., and Kathiresan (2014) sequenced the protein-coding regious of 18,666 genes in each of 3,734 participants of European or African ancestry in the Exome Sequencing Project. They found that an aggregate of rare mutations in the gene encoding apolipoprotein C3(APoC3) was associated with lower levels of plasma triglycerides and APoC3. These results were reported in the *NEJM* on 18 June 2014. The scientists found four mutations that destroyed the function of APoC3 gene. The Amish study had shown that people with such a mutation could drink a big, rich milkshake, loaded with fat, and their triglyceride blood concentration did not increase markedly. For everyone else it spiked. "Those who have the gene mutations have a 40% reduction in triglyceride levels and a 40% lower risk of heart disease," stated Sekar Kathiresan of Massachusetts General Hospital and the Broad Institute. He is the lead researcher in the gene project. He added: "Now there is a route to heart attacks that is independent of LDL" (Kolata, 2014a).

The other study, led by Anne Tybjaerg-Hansen of Copenhagen University Hospital, used data from 75,725 subjects to learn whether low triglyceride levels were linked to a reduced heart-attack risk. They were. The researchers also asked whether people who had mutations deleting the APOC3 gene had fewer attacks. They did. Those with such mutations had a 44% reduction in triglycerides and a 36% lower heart-attack risk (Kolata, 2014a). A small Californian biotechnology company, Isis, also hit upon the gene when it was looking for ways to make triglyceride levels decrease in the small group of people with disorders leading to triglyceride concentrations so high they can be fatal. They made a drug that counteracts the function of the gene and began testing it. It decreased triglyceride levels by 71%. "It is the most important drug in our pipeline," stated Stanley Crooke, the chief executive of Isis. The company nevertheless had no plans to test whether its drug prevents heart attacks in the general population. That sort of huge study, lasting years, would require the resources of a much bigger company. But cardiologists see Isis drug as at least a proof that it is possible to come up with a new class of pharmaceuticals to protect against heart disease (Kolata, 2014a).

## Coronary disease: can exercise and drugs replace surgery?

### *Advances in coronary surgery*

Opening blocked coronary arteries dates back to the 1970s and 1980s as the means to save lives. In those decades the only treatment was bypass surgery, a major operation in which the ribs are split open and a patient is put on a heart-lung machine while the heart is stopped. The surgeon bypasses the blockage with a blood vessel taken from elsewhere in the body. Studies at that time found that surgery was better for patients with severe blockage of major coronary arteries than not having surgery (Kolata, 2015d).

Stents were introduced in the 1990s: the typical surgery act is to thread a narrow catheter up from a blood vessel in the groin to the heart, squirt in a dye that allows the surgeon to see blockages in coronary arteries on X-ray radiographies and then insert a stent in the blocked areas. A stent is a small wire cage that maintains the artery open,

so as to avoid thrombosis, i.e. an occlusion of the vessel by a blood clot, which then will provoke a heart attack or infarctus. Inserting a stent has become the preferred treatment of stable coronary disease. It has also become, in France for instance, the way to handle an emergency situation when a patient is having a heart attack (Rosier, 2014d; Kolata, 2015d).

Because they relieved pain and were far less invasive than bypass surgery, stents become the treatment of choice. Millions of people have had stents inserted in their coronary arteries. In France, for instance, 135,000 people had this kind of treatment in 2013 (Rosier, 2014d). But while stents unquestionably save lives of patients in the throes of a heart attack or a threatening heart attack, there is no convincing evidence that stents reduce heart attack risk for people suffering from the chest pains known as stable angina. These are people who feel tightness or discomfort walking up a hill, for instance, because a partly blocked coronary artery is depriving their heart of sufficient blood. But the pain or discomfort fades out if they stop and rest or just stay still. And there is a reasonable argument that drugs – cholesterol-lowering statins in particular – might be just as good at reducing pain (Kolata, 2015d).

### *Follow-up treatment to stent insertion*

Even after the insertion of a stent, the risk of thrombosis still exists, particularly at the level of the stent itself (this is called stent thrombosis). That is why the patients receive a preventive treatment with medicines, such as aspirin, that inhibit the aggregation of plaquettes or platelets – blood cells that play a key role in blood clotting. But the secondary effect of these treatments is a higher risk of hemorrhage. Most recommended current treatments consist of aspirin uptake associated with another antiplaquette drug. Such dual therapy should be followed by a lifelong uptake of small doses of aspirin. But is there a real benefit from such lengthy treatments? (Rosier, 2014d).

To answer this question the FDA has demanded to a consortium of eight device and drug manufacturers and others to fund the study called DAPT Clinical Trials, the results of which have been published on line on 16 November 2014 in the *NEJM*, and presented the same day to the annual congress of the American Heart Association (AHA) in Chicago. The results of the study have been reviewed by an academic structure at Harvard. This international study has been conducted on 9,961 patients; all of them had a stent inserted in their coronary arteries in order to treat a stable coronary disease (or stable angina) or an infarctus. They had a treatment consisting of aspirin associated with an antiplatelet drug during one year. Then, they were sorted out: half of them continued to receive this dual or bitherapy for 18 months, while the other half had only aspirin. It was found that prolonged bitherapy reduced by 71% the risk of stent thrombosis, and also decreased by 53% the rate of occurrence of "major cardiovascular accidents" (Mauri et al., 2014). The "benefits of prolonged bitherapy are obvious and homogeneous," stated Gabriel Steg, professor of cardiology at Bichat-Claude Bernard hospital in Paris, and one of the co-authors of the study (Rosier, 2014d).

Also, as expected, prolonged bitherapy increased by 61% the risk of bleeding (moderate to severe). But surprisingly it slightly raised the mortality rate (all causes): 2.0% versus 1.5%. According to G. Steg, "this effect is probably linked to hazard.

Overall mortality was not the main criterion of the study: its statistical value is less important." This seems to be verified by another study the results of which were published on line in *The Lancet* on 16 November 2014 (Elmariah, Mauri et al., and Steg, 2014). In fact these results aggregate the outcome of all the large trials of antiplatelet-bitherapy, whose effect on mortality is neutral. However, the debate is not over. Victor Aboyans, professor at the university hospital of Limoges (centre-west of France), who did not participate in the study demanded by the FDA, but who attended the Chicago conference of the American Heart Association, made the following comments: "These trials select the patients without complications during the first months of bitherapy. The duration of the treatment is not therefore decided on the day when the stent is inserted. It depends on what is considered as the greatest concern for each patient: the bleeding or hemorrhagic risk or the infarctus risk" (Rosier, 2014d).

### *Stents or medical treatment?*

Coming back to the issue whether stents do in fact prevent heart attacks, the National Heart, Lung and Blood Institute is trying to find out through a clinical trial known as Ischemia. In this trial, as well as in a previous large federal study, called Courage, that was published in 2007, participants were given stents and intensive drug therapy – a statin, blood pressure drugs and aspirin – or just the medicines. The criticism, though, was that physicians may have cherry-picked patients for the study, excluding the sickest. Because angiograms revealed blockages in arteries before patients were invited to enrol in the trial, doctors who believed stents were lifesaving may never have asked patients with the most severe disease to join the study. The result, skeptics said, was that most patients in the study were at such low risk that it did not matter which treatment they received. They were certain to do well, so the study proved nothing about whether stents worked. Because of the doubts about that study and ingrained habits, medical practice was largely unchanged by its findings. A study which analyzed recorded conversations between cardiologists and patients with stable angina, found that 75% of the cardiologists recommended stents and, when they did, their patients almost always complied. And also the study found, on the rare occasions when the cardiologists presented both stents and medical treatment as options, none of the patients chose stenting. Stents are safe, but expensive. In the United States Medicare payments vary depending on what kind of stent is used and how many, but are generally above US$10,000 and can be more than US$17,000 (Kolata, 2015d).

The International Study of Comparative Health Effectiveness with Medical and Invasive Approaches (ISCHEMIA), funded by the National Heart, Lung and Blood Institute, aimed to avoid the methodological flaw in the 2007 Courage study. Patients who agree to participate are not given angiograms before being assigned a treatment. Instead they are accepted into the trial on the basis of non-invasive tests indicating blocked arteries and high risk of a heart attack. Their physicians know only that an artery is blocked – not which one or how much – so they are not able to select patients they believe need stents and prevent them from entering the trial (Kolata, 2015d).

Underlying the debate about the utility of stents is an uncertainty about how and why heart attacks occur. For years the common notion was they were caused by a plaque that partly blocked a coronary artery and grew until no blood could get through, and

a stent was therefore needed to open an artery before it closed completely. But a leading hypothesis assumes there is no predicting where a heart attack will originate. It could start anywhere there is plaque, even if the plaque is not obstructing the flow of blood in an artery. Unpredictably a piece of plaque can burst open. Blood starts to clot on the injured area and soon the blood clot clogs the artery; the result is a heart attack. It is known that certain plaques, with thin walls and bursting with fat-filled white blood cells, are prone to rupture. A study published in 2011 found that only a third of heart attacks originated in plaques that were blocking at least half of an artery, as seen on an angiogram. The rest began with the rupture of plaques that appeared to be causing no problems (Kolata, 2015d).

According to this view of how and why heart attacks occur, stenting would not be protective because people with atherosclerosis have arteries studded with plaques. The partly blocked area visible on an angiogram is no more likely to be the site of a heart attack than any other with plaque. But statins could work because they change the nature of plaques, making them less likely to rupture. Although stents tend to relieve chest pain, today's medical therapy can, too, although it may take weeks or months (Kolata, 2015d).

### *Stenting : a paradigm tough to overcome*

But proving whether stents make a difference is turning out to be harder than expected. Many physicians and patients have such strong opinions about the value of stenting that recruitment for ISCHEMIA had been difficult. Stents have become part of the heart-disease care. In 2013 and 2014 researchers randomized nearly 2,000 patients for the trial at the 300 participating medical centres. The plan is to randomize 8,000 patients over four years. "Half of the people over 65 have blockages; if you have some degree of atherosclerosis, you have blockages," stated Gregg W. Stone, an interventional cardiologist at Columbia University. And once a stress test or an angiogram reveals a blockage, it can be hard to ignore a partly blocked artery, hard to avoid thinking a stent has to help. "People believe that if they have a blockage, they have to fix it mechanically," stated Judith S. Hochman, the study chairwoman for the ISCHEMIA trial and director of the Cardiovascular Clinical Research Center at the New York University School of Medicine Langone (Mecklai et al., 2014). "It seems logical, but in medicine, many things that seem logical are not true," she added. "Not only do cardiologists find it hard to fight their own feelings that stenting makes sense, they also find it difficult to persuade patients to try medical therapy," commented Brahmajee Nallamothu, an interventional cardiologist at the University of Michigan. The concept that stenting helps, he said, "is a paradigm so deeply set on the part of the public and a lot of doctors that it is tough to overcome" (Kolata, 2015d).

# DIABETES

## Diabetes by the numbers

Which country has the highest number of diabetics? The answer is China, with 96.3 millions, according to the estimates from the International Diabetes Federation (IDF) published on 14 November 2014. Next up is India's 66.8 millions, followed by the United States 25.8 millions. Of course these numbers are in large part simply a function of size. But they clearly show that diabetes is not a mainly western phenomenon. More than three-quarters of the 387 million diabetics worldwide live in low and middle income countries, according to the IDF's *2014 Diabetes Atlas* (Ward, 2014a).

All continents are concerned: *ca*.10% of adult population suffers from diabetes in the United States, *ca*. 12% in China. This proportion rises to *ca*. 20% in the Gulf States, such as Saudi Arabia, Kuwait or Qatar. Type-1 diabetes (autoimmune disease where the body's immune system destroys the beta-cells of the Langerhans islets that produce insulin) prevails in 10% of the total number of diabetics worldwide, and its prevalence is increasing by 3% to 4% annually since the mid-1990s. Type-2 diabetes makes up 90% of diabetics worldwide: it has a genetic background, but is also closely related to lifestyle (food habits and consumption, and exercise) [Santi, 2014b].

In France *ca*. 3 million people are medically treated because they suffer from type-2 diabetes; this represented in 2014 *ca*. 4.6% of the country's population. Another 1 million persons would be affected, ignoring they have the disease. Qualified as the "silent epidemic of the 21st century," diabetes prevalence in France increases by 2.5% annually, according to the data published in the *Bulletin épidémiologique hebdomadaire* (BEH, French acronym) of the National Institute for Health Vigilance. The French Diabetics Federation is advocating that diabetes control becomes a "great national cause." The BEH has drawn the attention to the existing disparities in the disease distribution: the overseas departments (DOM), the North-Pas-de-Calais, Picardie or Saint-Denis regions or departments are the mostly affected. Diabetes has cost €15 billion in 2013 to the social security (health insurance) which has set up a service in charge of coaching the patients (more than 500,000 beneficiaries) [Santi, 2014b].

## What is diabetes?

As mentioned above type-1 diabetes is an autoimmune disease that results in a lower production of insulin by the pancreatic Langerhans islets or even in a lack of the hormone. Consequently the concentration of glucose in the bloodstream

(glycemia) rises. The end result is a diabetic coma and death. The increase in glucose concentration "make blood much less fluid, viscous; it therefore flows more slowly and this can cause vascular lesions that heighten the risk of atherosclerosis (atheroma plaque on the walls of arteries), heart attack and stroke," explained Patrick Collombat, in charge of the diabetes genetics team of the French National Institute for Health and Medical Research (INSERM) and University of Nice Sophia-Antipolis, south-east of France. P. Collombat's team works on the regeneration of insulin-producing cells; he received the Silver Medal of the European Prize Morgagni which rewards research on metabolism (Santi, 2014b).

Type-1 diabetes also has a detrimental impact on kidney function, it can alterate the nerves of inferior limbs and even lead to the amputation of foot or leg. Vision is also affected because of alterations of the retina. The risk of heart attack is multiplied by three to five. "Despite the current therapies using injections of various kinds of human recombinant insulin, diabetics' life expectancy is reduced by five to eight years and their quality of life is very bad," stated P. Collombat (Santi, 2014b).

Regarding type-2 diabetes or diabetes mellitus, which is much more widespread than type-1, the patients also need a supply of insulin because their tissues are resistant to it and do not use it efficiently. Even though *ca*. 60 genes may be involved in this pathology, overweight (obesity) and the lack of physical exercise play a key role in this illness: "We work on the way a change in food uptake and exercise could influence metabolic homeostasis in the human body. One of the great challenges is to understand the physiopathology of diabetes through working on systems biology, i.e. how the organs, tissues, cells or proteins communicate between themselves," explained Bart Staels of the European Institute for Diabetes Genomics (University of Lille-II, north of France; INSERM and National Scientific Research Centre, CNRS), and also co-founder of the laboratory Genfit (Santi, 2014b).

Glycemia "is just one aspect of diabetes, because, in addition to insulin, other hormones are disturbed by the pathology," commented Philippe Froguel, an endocrinologist of the Pasteur Institute in Lille, north of France, and at Imperial College, London. "We are interested nowadays in what could be lethal for diabetics, such as lipidic disturbances causing arterial diseases, as well as their relationships with disorders in glucose uptake and metabolism," he added. Another research focus is to detect and identify liver pathologies, called "fatty livers", which make the individual susceptible to non-alcoholic hepatic steatosis; and to study the role of liver in diabetes and cardiovascular diseases. *Ca*. 10% of affected persons will have serious hepatic diseases, such as cirrhosis and fibrosis. According to P. Froguel 25% of the population would be affected by these liver diseases (Santi, 2014b).

## Diabetes and lifestyles

The proportion of diabetics in the global population will rise as the developing world increasingly adopts the food culture and more sedentary lifestyles that have fuelled the diabetes epidemic in the United States and Europe. Lars Sorensen, chief executive of Novo Nordisk, the world's biggest manufacturer of insulin, stated one of the main culprits is the global trend towards urbanization: "When people move to cities … we

see a tremendous rise in diabetes because of long commutes, access to junk food, stressful lives [and] little possibility for exercise … So it is a huge problem for emerging markets" (Ward, 2014a).

Between now and 2035 China is forecast by the IDF to see its number of diabetics rise by almost half to 142.6 millions while India's will climb by nearly two-thirds to 109 millions. But the biggest increase is expected in Africa where cases are projected to rise by 92% to 41.5 millions. For developing world governments this threatens to impose a heavy burden on still weak public-health systems. At an estimated US$612 billion in 2014 spending on diabetes-related treatments already accounted for 11% of total *global* health-care costs, according to the IDF. Yet more than 80% of this money is used to treat 17% of diabetes sufferers – those in the wealthiest countries (Ward, 2014a).

A study published in October 2014 by the CDCs suggested that after more than doubling between 1990 and 2008, the growth of diabetes in the United States may be starting to slow. This could signal that rising public awareness of the health risks from obesity and high sugar intake is starting to change behaviour. The rate of obesity in the United States reached a plateau at about a third of adults for a decade. But even if lifestyles do become healthier, the incidence of diabetes will keep rising in the United States and Europe because people are living longer and old age is itself a risk factor. The IDF projects a rise of *ca*. 30% in both regions between now and 2035. Globally, the number of diabetics is forecast to rise by just over half during that period to 592 millions. Petra Wilson, IDF chief executive, wants the G20 group of leading economies to help design a coordinated global response. "It is not just a problem of health ministers," she said. "It is for finance ministers too. If we are going to meet this challenge, it will take a comprehensive effort across all areas of government and policy" (Ward, 2014a).

Many health advocates believe public education is not enough. They point to Mexico which in 2013 introduced a "fat tax" on fast food and sugary beverages. In November 2014 Berkeley became the first United States community to impose a 1-cent-announce levy on soft drinks. High sugar consumption has been strongly linked with type-2 diabetes. David Cavan, IDF policy director, stated the top priority must be prevention. "Science has come a long way in developing treatments to control the disease. Where we have not moved as far forward is in changing lifestyles and getting the political and public-health communities to take this challenge seriously enough. On the other hand drug companies are increasingly recognizing they have interest in helping societies keep the cost of diabetes sustainable. Novo Nordisk, for instance, has launched a partnership with Mexico city's government to develop policies to combat diabetes and is extending the scheme to other big cities around the world (Ward, 2014a).

## Diabetes and diets

### *Mexico*

A report of the Food and Agriculture Organization of the United Nations (FAO), published in July 2013, revealed that the obesity rate among Mexicans (32.8%) was higher than that prevailing in the United States (31.8%); in the same report, higher

obesity rates were mentioned, e.g. 42.8% in Kuwait and 35.2% in Saudi Arabia. "But if one adds to the obese people those who are overweight, Mexico is undoubtedly the global leader," warned Abelardo Ávila, a researcher at the Mexican Health and Nutrition Institute (Saliba, 2013).

When it comes to diabetes, Mexico is a ticking time bomb. Seven out of ten adults and a third of children are obese or overweight – a principal cause of type-2 diabetes which has risen sharply in Latin America's second-biggest economy – and 9.2% of Mexicans have been given a preliminary diagnosis of diabetes. But the Mexican Diabetes Federation warns that the number of sufferers could in fact be twice as high (Webber, 2014). According to Abelardo Ávila, 7.5 million Mexicans are diabetics and diabetes has become the country's first cause of mortality. But he also agreed that the real number of diabetics could be much higher because "many are not diagnosed" and A. Ávila also underlines the "outburst of hypertension ailments and cardiovascular diseases." While all these pathologies, including those related with obesity or overweight, threaten to break down the public health-care system, A. Ávila warned that "if this trend continues, the life expectancy of Mexicans could be ten-years shorter from now to 2030" (Saliba, 2013).

"Diabetes used to be something we only saw in adults, but now we are starting to see it in children too. It is very worrying. These children are getting this pathology 30 years early," stated Valeria Szymanski, a nutritionist at the Mexican Association of Diabetes. "Right now, not everyone who is overweight has diabetes, but they very probably will develop it. It is a huge problem – the health system could collapse," she added. According to A. Ávila, one-third of children are overweight and children obesity has trebled in ten years (Saliba, 2013; Ward, 2014a; Webber, 2014).

Abelardo Ávila thinks that "diabetes epidemics in Mexico is caused by the market offensive of food industries, with Coca-Cola at the forefront." In March 2013 Olivier de Schutter, who was at that time special rapporteur of the United Nations on the right to food, denounced the "Coca-colisation" of Mexico. According to him, the North American Free Trade Agreement (NAFTA), signed in 1994 between Canada, Mexico and the United States, has boosted the imports of products containing high amounts of cholesterol, saturated fats and sugar. Mexicans guzzled some 163 litres of fizzy drinks per head a year before the approval of a tax on these drinks and junk food in 2013 (Saliba, 2013; Webber, 2014).

"Drinking Coca-Cola confers some kind of prestige to low-income social categories which want to become part of the consumer society, following the American model," deplored Alejandro Calvillo, director of the NGO *El Poder del consumidor* (The power of the consumer). Present everywhere, in the cities as well as in the rural areas, Coca-Cola's advertisements are adapted to the ethnic composition of the country. On 9 August 2013 in Mexico City, Maureen Birmingham, representative of the Pan American Health Organization (PAHO), made an appeal in order to support a draft law on a special tax on sugary drinks. Fernando Zárate, a leftist legislator who has been pushing in vain for the soda tax to be doubled, stated a study by the National Institute of Public Health showed that consumption of taxed sugary beverages had fallen

10% while consumption of untaxed ones such as water and milk had risen 7%. F. Zárate indicated that a 20% tax on fizzy drinks would have saved 13 billion pesos in the medical cost of treating conditions directly related to being overweight or suffering from diabetes in the next decade, and helped prevent as many as 1.3 million cases of diabetes by 2030. At the current 10% soda tax rate, the number of cases prevented was 400,000 to 630,000, he estimated (Webber, 2014). Alejandro Calvillo stated "political lobbying was blocking the new legislation," while in 2010 Mexico had signed a national agreement with the food and beverage industries on nutritional health that foresaw the withdrawal from schools of too fatty and sugary foodstuffs (Saliba, 2013).

### *United States*

In the United States processed-food makers are making efforts in order to produce healthier food and improve their image. After decades of rising sales and high popularity, makers and sellers of processed food are under pressure from policymakers, campaigners for "real" food and an increasingly sceptical public. In April 2015 Kraft stated the company would remove the artificial colouring that gives its Macaroni and Cheese – one of its biggest sellers – its neon-orange glow. On 28 April 2015 it announced flat sales and a 16% fall in net profits, year on year, in the first quarter. McDonald's, having replaced its boss in March 2015 because of poor sales, stated on 22 April 2015 that they were still falling (*The Economist*, 2015d).

Just as the restaurants promising more "natural" ingredients have been winning customers from McDonald's in recent years, Kraft and other processed-food manufacturers have lost out to smaller food firms peddling healthier fare. At the same time consumers who are less choosy about ingredients have become more concerned about price, switching to supermarkets' own-label foods. In 2014 supermarkets' own labels represented 20% of the American market share of food and beverage producers, compared with 45% for the top 25 processed-food makers and 35% for the middle tier. Some big food firms are cutting back: in January 2015 General Mills, maker of Pillsbury chocolate-chip cookies and Totino's frozen pizza, among other things, announced factory closure and job cuts. PepsiCo has finished a three-year process to cut up to 8,700 jobs, or 3% of its global workforce (*The Economist*, 2015d).

American consumers' growing interest in healthier, simpler fare is providing opportunities for all sorts of businesses. For instance family farms had been going out of business for decades, but now new ones are being founded, promising organic-locally grown produce. Kind, which makes fruit and nut snacks, went from nothing to annual sales of more than US$100 million in ten years. Even more impressively, Chobani, a manufacturer of Greek-style yoghurt, reached sales of US$1.3 billion over the same period (*The Economist*, 2015d).

One way in which the big processed-food manufacturers are reacting is by reformulating their products to answer worriers about synthetic ingredients. Just as Kraft is promising with its macaroni and cheese, so Nestlé's – the world's biggest food firm – is pledging to remove all artificial flavours and colours in more than 250 types of chocolate sold in the United States. PepsiCo also stated that it would remove aspartame, an artificial sweetener, from Diet Pepsi sold in America. Its rival Coca-Cola is promoting Coc Life, a

fizzy drink that contains stevia, a natural (extracted from the leaves of the plant *Stevia rebaudiana*) no-calorie sugar substitute. But this strategy may have its shortcomings. For instance stevia has a slightly bitter aftertaste that may put off some consumers. And in 2010 when Campbell's reduced the salt in its soup, customers forced the company to add some back. Its soup's market share in the United States continued to fall (*The Economist*, 2015d).

An increasingly popular, although expensive, alternative for big processed-food manufacturers has been to buy small but fast-growing healthy-food brands. For instance, in 2012, Campbell's bought Bolthouse Farms which makes organic juices; a year later it took over Plum Organics, a maker of baby food. In 2013 Coca-Cola bought Innocent, a maker of fruit smoothies. In 2014 General Mills bought Annie's, an organic food firm. If the decline in processed-food's popularity continues, two further strategies – consolidation and cost-cutting – will become more prevalent. Since they had bought Heinz for US$28 billion in 2013, Warren Buffet's Berkshire Hathaway and 3G Capital, an investment firm with Brazilian roots (see p. 59), have cut costs drastically, at its head office and factories. In 2014 although Heinz sales fell by nearly 5%, its earnings before interest, taxes, depreciation and amortization (EBITDA) rose by almost 25%. In March 2015 W. Buffet and 3G Capital announced that they were buying Kraft for US$50 billion to merge it with Heinz. There will most probably be a drive to apply the same "zero-based budgeting" approach to cost-cutting at Kraft that Heinz has undergone. Mondelez, a snacks-maker spun off from Kraft, has also gone for zero-based budgeting: by early May 2015 it announced improved operating-profit margins, year-on-year, in its first quarter, despite a 10% fall in revenues (*The Economist*, 2015d).

W. Buffet and 3G Capital are unlikely to be satisfied with just Heinz and Kraft, reckoned Robert Moskow – a food-industry analyst at Credit Suisse: "I think they will keep consolidating the industry." Heinz and 3G Capital stated they were planning to cut the debt on their balance-sheet to three times EBITDA in two years. At that point they will be ready for another acquisition. They are not the only ones showing how much scope there is for trimming financial fat as well as the culinary sort from the processed-food business. In 2013 Dean Metropoulos and Andy Jhawar, two entrepreneurs, bought Hostess, the collapsed maker of Twinkies cakes. They got rid of inefficient factories and made investments into automated production and an improved distribution system (*The Economist*, 2015d).

Hostility from regulators, campaigners and the media; declining popularity; consolidation and cost-cutting. Processed food sounds like it has much to learn from tobacco, an industry that has been shrinking for more than 50 years. "It would not be as bad as tobacco, but a bumpy road lies ahead for Big Food," predicted Alexia Howard of Sanford C. Bernstein, a research firm. Processed-food manufacturers have to contribute to the struggle against obesity and overweight worldwide, and at the same time slow down a declining popularity and consolidate their lucrative business.

Bringing whole wheat to more American plates

Industrial agriculture has so thoroughly expelled genuine whole-wheat flour and foodstuffs from our diet that most of us do not even notice its absence. It does however

make sense, from a healthy diet viewpoint, to reintroduce whole-wheat flour and bread into our daily food intake. The initiative of Stephen Jones, director of the Bread Lab, at the Washington State University campus in Mount Vernon, Wash., shows how this could be achieved in the United States, e.g. in the Pacific Northwest (Jabr, 2015).

A vast majority of America's 56 million acres of wheat grows in a belt stretching more than 1,000 miles from the Canadian border to Central Texas. *Ca*. 50% of the harvest is exported and most of what remains is funnelled to feedlots for cattle or to giant mills and bread factories. This industrial system forces plant breeders to first select wheat kernels of highly specific sizes, colours and hardness. Commodity wheats are defined in three ways: hard (high in protein, which is good for bread) or soft (better for pastries); red (dark colour and strong flavour) or white (pale and more delicate-tasting); and winter or spring, depending on when they are planted. "Hard red spring" for instance is often used for bread. A grain of wheat has three main components: a nutrient-rich outer coating called the bran; the flavourful germ, a living embryo that eventually develops into the adult plant; and a pouch of starch known as the endosperm. When those three parts are mashed together, flour is not the inert white powder most of us are familiar with, it is pungent, golden and speckled, because of fragrant oils released from the germ and bits of hardy bran. If freshly ground flour is not used within a few weeks, however, the oils turn it rancid (Jabr, 2015).

The giant band of wheat that stripes the centre of America is a byproduct of the industrial era. From the 18$^{th}$ to the early 19$^{th}$ century wheat was grown mainly near the coasts. During this time immigrants and American emissaries introduced numerous varieties, which breeders tinkered with, adapting them to various soils. All that preindustrial wheat had a wide range of flavours: vanilla, honeysuckle, black pepper. When the Erie Canal and transcontinental railroads opened up the vast expanse of the Great Plains to wheat farming, by the late 1800s, this crop had largely shifted to Midwestern States. At the same time the Industrial Revolution altered the process of transforming grain into flour. First appearing in Budapest, in 1839, the steel roller mill was a radical departure from previous techniques, e.g. stone mills, because it sheared the wheat kernel apart. Roller-mill spinning cylinders denuded the endosperm and discarded the germ and bran, producing unspoilable white flour composed entirely of endosperm, i.e. starch. This was a boon for the growing flour industry: mills could transport wheat across the United States without worrying about shelf-life. That newfound durability came at a huge cost, however, sacrificing much of the grain's flavour and nutrition. Nowadays whole-wheat flour accounts for only 6% of all flour produced in the United States. And most whole-wheat products sold in supermarkets are made from roller-milled flour *with the germ and bran added back in* (Jabr, 2015).

Stephen Jones is both a pioneer in conventional wheat breeding and a strong advocate of promoting whole-wheat flours and products. While studying agronomy at Chico State University in the late 1970s, S. Jones grew five acres of wheat on a campus farm. A few years after college he apprenticed with an Idaho wheat breeder named D.W. Sunderman who taught him the craft of breeding: selectively cross-pollinating plants in order to create new varieties. In 1991 S. Jones completed his doctorate in genetics at the University of California, Davis, and the United States Department of Agriculture hired him to study wheat genome at Washington State University main

campus in Pullman. Three years later S. Jones landed a job as one of the Washington State University's chief wheat breeders. He was tasked with improving the yield and disease-resistance of wheat cultivars that had been designed for industrial milling. When he tried breeding wheat with higher concentrations of nutritious minerals, like iron, he was told those traits were unimportant (Jabr, 2015).

By 2007 S. Jones had spent more than a decade breeding wheats for the commodities market. As tensions mounted between him and the university he made a difficult decision: in order to escape the commodities system he would give up wheat altogether. In 2008 he moved to Washington State university's western campus to become director of the W.S.U. – Mount Vernon Research Center, which helps small and mid-scale farmers in the surrounding Skagit Valley, halfway between Seattle and Vancouver, British Columbia, grow *ca*. 80 different kinds of fruits, vegetables and flowers. Driving around the area he was startled to discover one wheat field after another. Farmers told him it was crucial for crop rotations. They harvested and sold the grains, but only to lose less money. What would happened, S. Jones wondered, if he developed unique varieties of wheat adapted to Skagit Valley's cool, wet climate? What if he could draw the interest of local millers and bakers in dealing primarily with Washington State's wheat? What if wheat, like wine, had terroir? (Jabr, 2015).

S. Jones created the Bread Lab which serves as a headquarters for Jones' project and mission to make regional grain farming viable once more by creating entirely new kinds of wheat that have both the taste and wholesomeness of their ancestors with the robustness of their modern counterparts. S. Jones' lab, founded in 2012, has already earned the respect of the country's most celebrated bakers, like Chad Robertson of Tartine. Bread Lab breads have even made their way to the kitchens of the White House. Earlier on, in 2010, after digging through seed banks, collecting both heirloom wheats and modern varieties, crossbreeding them and adapting them to the Skagit Valley's weather, he had some of his favourite varieties stone-milled and then brought several bags of flour to the Seattle-based baker George De Pasquale for an expert evaluation. De Pasquale baked a baguette, a batard and a miche (loaf) with each flour. One of them, Bauermeister bread, came out of the oven dark brown. He sliced into it and breathed in: there was a noticeable aroma of chocolate, and alcoholic twang and hints of cinnamon and nutmeg (Jabr, 2015).

S. Jones therefore ramped up his efforts to breed for "flavour, nutrition, funkiness." Each year S. Jones and his senior scientific assistant, Steve Lyon, along with three graduate students, grow between 5,000 and 10,000 kinds of wheat. So far the Mount Vernon's breeders have produced wheat with higher than typical concentrations of micronutrients; grains that are strikingly blue, purple and black; and wheats that imbue bread with maltiness, spice and caramel. In the fall of 2015 S. Jones planned to publicly release two lines: a West Coast-adapted version of a French wheat, named Renan, and a hard red winter wheat called Skagit 09 (Jabr, 2015).

In 2013 S. Jones decided to hire a resident bater. With him the Bread Lab's fame grew even faster. By the spring of 2016 Stephen Jones and his colleagues moved to a 12,000-square-foot building that they were renting from the Port of Skagit.

Encouraged by the Bread Lab's success, a group of investors was planning to build a mill nearby, with a capacity to produce thousands of tons of flour annually. Although the new mill may produce some single-variety stone-ground flours, it focuses on roller-milling wheat and blending different types to achieve uniformity. But is it really possible to scale up without becoming the very type of system S. Jones fled? (Jabr, 2015).

Such concerns have been worsened by the Bread Lab's new high-profile partner, Chipotle, the fast-casual food Mexican chain which wanted to use of the lab's regional wheats in its tortillas (flat bread). Chipotle served 800,000 tortillas across the United States every day in 2015. Steve Ells, the company's founder, learned of S. Jones around 2012 and at that time he was determined to meet his promise of "food with integrity," of working with sustainably grown, locally produced whole ingredients when possible. Chipotle's tortillas were made with commodity flour. A visit to the Bread Lab convinced S. Ells that S. Jones and his team could replace Chipotle's tortillas with a more healthful and tastier whole-wheat version. A viable recipe was devised using five ingredients: water, oil, salt, whole-wheat Edison flour and a sour dough starter – a living mixture of flour, wheat and microorganisms. The starter is not standard procedure for a tortilla, but it was crucial for S. Jones' and his bakers's version: it magnifies flavour and extends the tortilla shelf-life. The techniques were taught to Ruben Berber and Tom Hoffert, who work in research and development at Don Pancho Authentic Mexican Foods, which is based in Salem, Oregon, and is Chipotle's primary tortilla supplier in the Northwest. So Don Pancho has been cutting Edison with white flour and the tortillas they have produced so far are dense, golden-tinged and slightly tangy. S. Ells planned to test the semi-whole-wheat tortillas in the Pacific Northwest. If things go well, he stated, he wanted to pair regional sources of wheat with tortilla-manufacturers across the country (Jabr, 2015). "There are definitely issues of scale," S. Jones stated. "If you have Chipotle come in, how big does it get, and how quickly? Do we end up with a commodity by any other name?" If the partnership with Chipotle succeeds it would bring real whole wheat to more American plates than any other Bread Lab collaboration so far. If it succeeds it will also contribute to a healthier diet for many Americans (Jabr, 2015).

### *Advice on what to eat and to drink*

The relationship between illness and diet is hard to pin down. One reason is the lack of robust long-term experiments, as well as the complexity of diets and chronic illnesses. However nutritionists and scientists agree on one thing when it comes to food and diabetes: overeating leads to weight gain and overweight people are more likely to develop type-2 diabetes than lean people. Furthermore some people have a genetic disposition that leads them to develop diabetes without necessarily being overweight (Daneshkhu, 2014). See also Sasson (2011).

When one consumes enough real sugar, his/her brain receives the message and a sense of satiety – or fullness – takes over. "Your body is used to knowing that a sweet taste means you are ingesting energy" – or kilocalories – "and that if you do not burn them off, it is going to convert to fat," explains Helen Hazuda, professor of medicine at the University of Texas Health Science Center at San Antonio. But the most popular artificial sweetener in diet drinks, for instance, is *ca*. 200 times sweeter than sugar (sucrose) without triggering a feeling of satiety. When rats ate yogurt mixed

with an artificial sweetener they consumed fewer calories and gained more weight than rats that ate sugar-sweetened yogurt, suggesting that the no-calorie sweeteners interfere with a natural ability to regulate incoming calories. This – no surprise – can lead to sugar cravings and weight gain. A recent study found that artificial sweeteners changed the colonies of gut bacteria in mice in ways that made the animals vulnerable to insulin resistance and glucose intolerance, which are metabolic disorders that can lead to weight gain and increase the risk of type-2 diabetes (Oaklander, 2016).

In a study based on dietary questionnaires of 9,500 people, those who replied they drank one can of diet soda a day had a 34% higher risk of metabolic syndrome – a cluster of risk factors that can lead to heart disease and type-2 diabetes – than those who did not drink diet soda. The study stopped short of drawing a cause-and-effect link, but the association surprised the authors, who called for more research. But evidence is mounting that low and no-calorie sweeteners may not be great choices for dieters. Another study found that over nine years diet-soda drinkers gained nearly triple the abdominal fat – 8 cm – as those who did not drink diet soda. This may be puzzling but more long-term nutritional studies with a large number of volunteers will contribute to better understand the impact of our eating and drinking habits and, subsequently, to advice on what to eat and to drink (Oaklander, 2016).

As long as people do not overeat, whether what they eat makes a difference in terms of the risk of developing type-2 diabetes, is a matter of debate. A controversy has swirled around high-fructose corn syrups (HFCS) – a byproduct of maize used to sweeten processed foods and beverages. One study published in 2012 by Oxford University and the University of Southern California, suggested that HFCS consumption could increase the risk of diabetes. However, Diabetes UK, a charity that funds research, warned: "This study does not prove that HFCS causes diabetes. For example, it does not show that individuals with diabetes consumed higher levels of HFCS or that this consumption was the key factor leading to their condition." At the same time, Klurfeld et al. (2013) of the United States Department of Agriculture (USDA) Agriculture Research Service (Human Nutrition), Baylor College of Medecine (Houston), University of Central Florida, Orlando and Tufts University School of Medecine, Boston, published in the *International Journal of Obesity* the results of a study – partly-funded by the Corn Refiners Association – suggesting there was no evidence to pin the United States obesity epidemic on HFCS.

Stephanie Dunbar, director of nutrition at the American Diabetes Association, stated: "Our position is that people with, and at risk of diabetes, should limit or avoid intake of sugar-sweetened beverages (from any calorie sweetener including HFCS and sucrose) to reduce the risk for weight gain and worsening cardiometabolic risk profile." Clearly further research is needed, which is why Diabetes UK commissioned more work to assess the relationship between fructose intake and type-2 diabetes incidence. The research, carried out by L. Agius, professor of metabolic biochemistry at Newcastle University, showed that dietary fructose was a much powerful agent than glucose in causing fatty liver and changes in liver function, including the insulin resistance that increases the risk of developing type-2 diabetes. Since the starch in carbohydrate foodstuffs breaks down into glucose in the body, she concluded there was no evidence from long-term clinical

studies to support the assumption that low-fat, high-carbohydrate diets were healthier than ones low in carbohydrate and high in fat. This view has contributed to a rise in consumption of pasta, rice and potatoes (Daneshkhu, 2014).

In the meantime experts see great scope for preventive measures: from labeling foods to taxation. The WHO cited studies showing that in China increases in the price of unhealthy foods reduced intake, while in the United States cheaper healthy foods led to a 68% rise in consumption. The WHO added that moderate exercise of 150 minutes a week cut the risk of diabetes by 27% (Daneshkhu, 2014).

### *Another promising diet-and-lifestyle programme*

In the United States more than two-thirds of adults are overweight or obese. Since extra body fat is a major risk factor for type-2 diabetes, that means a lot of people are at risk of becoming diabetics. Still, some populations are at higher risk than others. African Americans, Hispanic and American Indians, for instance, have higher rates of the disease than whites. In 2012 diabetes cases – 90% of which were type-2 – cost the United States health-care industry *ca*. US$245 billion. And some of the larger price tags are for its complications. Type-2 diabetes can lead to blindness, kidney failure and nerve damage that can require foot or leg amputations. Taken together that has led researchers and physicians to look for better ways to reduce the number of people who develop the disease every year (Oaklander, 2016).

People with type-2 diabetes, who are often overweight, can experience extreme fatigue, blurry vision and sores. Though some people do not feel symptoms right away, the slow-growing but potentially debilitating disease can gradually damage their blood vessels and nerves. And even when it is well managed it requires constant vigilance: monitoring blood-sugar levels, counting carbohydrates, timing meals, taking multiple blood-sugar-lowering drugs and sometimes injecting one's abdomen with a syringe full of insulin. One out of three Americans will be diagnosed with diabetes by 2050, according to the Centers for Disease Control and Prevention (CDCs), and in 2015-2016 29 million people already had the disease (Oaklander, 2016).

For the better part of the past two decades new cases of type-2 diabetes shot up considerably each year, but that trend appears to be levelling off, according to a December 2015 CDC report. Meanwhile, data emerging from years-long studies indicate that exercise and changes in diet can dramatically reduce a person's risk of developing type-2 diabetes. "I think people intellectually know that eating healthy and being active is good for you, but I do not think they understand what an impact it has on preventing type-2 diabetes for those at high risk," stated Ann Albright, director of the Division of Diabetes Translational Research at the CDC (Oaklander, 2016).

Monica Peek, a primary-care physician and lead researcher of the South Side Diabetes Project in Chicago, is trying to figure out how to spread that message in a way that works in the real world. This project focuses on Chicago's best known black neighbourhood. Over time M. Peek's advice gained enough traction that those diet prescriptions are now at the heart of a novel study by the National Institutes of Health

(NIH) that is challenging the *status quo* of type-2 diabetes prevention and treatment (Oaklander, 2016).

M. Peek's project hosts cook-offs and offers diabetes-education classes as well as farmers'-market and grocery tours. All this is done in addition to a patient's standard treatment, which may include several kinds of medication administered by a physician at one of six medical clinics – two of which are run out of the nearby University of Chicago, where M. Peek is an associate professor of medicine. This type of programme, while not altogether new, is now winning the support of insurers, many of which are beginning to reimburse patients and organizations for lifestyle-based prevention programmes (Oaklander, 2016).

This is not the first time researchers have experimented with lifestyle as a way to prevent type-2 diabetes. In 2002 the George Washington University Diabetes Prevention Program Coordinating Center, a landmark NIH trial that lasted for three years, published its findings in the *New England Journal of Medicine* (*NEJM*). At 27 sites across the country researchers divided 3,234 overweight people with prediabetes into groups. Members of the lifestyle-intervention group ate less fat and fewer calories, exercised for *ca*. 20 minutes a day and aimed to lose *ca*. 7% of their body weight. Another group took metformin, a commonly prescribed glucose-lowering drug that is taken by millions of Americans. The third group took a placebo. The people in the diet-and-exercise group reduced their risk of developing diabetes by 58%. Lifestyle changes were especially impressive for older people; those 60 and older reduced their risk of diabetes by 71%. People who took metformin also saw a benefit, but they slashed their diabetes risk by only 31% - about half that of the lifestyle group (Knowler et al., and Nathan, 2002). "Those results really brought the issue to light that diabetes is not inevitable," stated David M. Nathan, chairman of the Diabetes Center at Massachusetts General Hospital (Oaklander, 2016).

In another randomized trial out of the Goldring Center for Culinary Medicine at Tulane University, a small number of people with type-2 diabetes were divided into two groups. One of the groups was taught how to prepare foods consistent with the diabetes-friendly Mediterranean diet; the other group was given basic nutrition instruction. After six months of follow-up the researchers saw significant improvements in the cholesterol and blood-pressure levels of the Mediterranean-diet group – and the changes lasted. David M. Nathan who ran the pioneering Diabetes Prevention Program study also saw lasting change. He and his colleagues followed their original groups for about 15 years. In the follow-up, published in November 2015 in *The Lancet Diabetes & Endocrinology*, type-2 diabetes incidence was reduced by 27% in the lifestyle intervention group, compared with the placebo group (Nathan et al., 2015).

Backed by substantial evidence that lifestyle changes work in preventing the development of type-2 diabetes – especially in those who are at high risk of the disease – the approach began to take hold. The CDCs now recognize more than 800 organizations across the United States that offer programmes in that vein. One of the most successful is the YMCA Diabetes Prevention Program, a one-year curriculum designed to help overweight adults with prediabetes prevent the onset of type-2 diabetes. *Ca*. 86 million Americans adults were estimated to have prediabetes, though

only 60% of them knew it. In weekly classes across all 43 States where the YMCA program is offered, people are coached on healthy ways to modify lifestyle. Those who finish the class lose an average of 5%-4% of their body weight by the end of the year (Oaklander, 2016).

And while no one is suggesting that diet and exercise alone can reverse type-2 diabetes, similar strategies can reduce the severity of the symptoms. A study of 5,000 people with type-2 diabetes showed that the same diet-and-lifestyle intervention used in the Diabetes Prevention Program improved their diabetes, blood pressure and cholesterol control, all while allowing people to use fewer medications than the control group. A major downside to the diet-and-exercise strategy is that it requires a lot of work over a long period of time. That means a consistently good diet and regular exercise – as well as addressing the barriers that make it challenging for people to stick to those healthy behaviours. Still, it can be an uphill battle (Oaklander, 2016).

In addition to nutrition tours and cooking classes M. Peek and her team distribute those doctor-signed prescriptions, with vouchers for farmers' markets or the food section of a Walgreens or for free fitness classes at nearby parks. For instance the South Side Diabetes Project teaches shoppers how to count carbohydrates, how to shop smart, e.g. for cereal 100% whole grain is best (but if you would not give up your favourite cereal, mix it with a more fibrous option, like bran); broccoli which are Cruciferous vegetables linked to lower levels of inflammation – good for diabetics, who are at higher risk for joint disorders; yogurt, choose Greek, which has less sugar and more protein than most sweetened kinds. So far the results from M. Peek's program are promising. A study in August 2015 found that the diabetes-education classes – central to Peek's program – were advancing not only patient knowledge about the disease but also their attitudes about taking charge of their health. Also leaders at the South Side Diabetes Project train the physicians and their teams at their six partner clinics on how to deliver care to patients of different cultures. Although the diabetes epidemic may be slowing ever so slightly, it is nowhere near over, and much remains to be seen about the best way forward. But M. Peek is stubbornly supportive of every person taking the first step: "You just have to care about people and that is something anyone can do," said M. Peek (Oaklander, 2016).

## Fitness programmes

In 2005 the United States Department of Veteran Affairs, conscious that an estimated seven in ten veterans were overweight or obese, launched a programme called *Move* to encourage weight loss through lifestyle change – a better diet alongside more exercise. In 2012 researchers published an analysis of the results: a dismal 5% of eligible candidates participated in the programme, and for those who did, its effectiveness was low, with participants losing 0.9 lbs more on average than non-participants after a year. The *Move* scheme was not aimed solely at combating diabetes, but what it found – the sheer difficulty of persuading people to change the way they live – is among the biggest challenges facing type-2 diabetes prevention programmes (Jacobs, 2014).

While meta-analysis of research shows that even modest weight reduction is the best way to reduce both the risk of developing type-2 diabetes and to fend off its most

devastating effects among those already diagnosed, achieving this is no simple matter. *In fact, no country in the world has managed to reduce obesity across all age groups* – although some have seen it reaching a plateau in one subset of the population or another. "One of the most challenging aspects of type-2 diabetes prevention remains the general application of positive results from clinical trials," wrote the authors of Diabetes UK's nutrition guidelines. "There are ongoing studies investigating different strategies in the community but at present there is little evidence in translation of the success of randomized controlled trials to public health." Part of the problem, Diabetes UK admits, is that while weight loss is the clear goal, no evidence supports one particular dietary strategy over another (Jacobs, 2014).

Jessica Apple, founder of the diabetes non-profit and online magazine ASweetLife.org, is a strong advocate of low-carb diet and believes organizations such as the American Diabetes Association are doing people a disservice. "The biggest problem is that people do not understand that whether you are eating a bag of candy or a big plate of pasta, your body is doing the same thing with it." She pointed to recipes recommended by the American Diabetes Association that replace butter – which has no impact on blood sugar – with apple sauce. "A low-fat diet is still held up as the model." That is not to say she believes adopting – or even advocating – such a diet is easy. Culture is one problem with many of the highest risk groups living in areas without easy or affordable access to healthy food. Moreover it is difficult to tell someone raised on rice and tortillas (Mexican maize crepes) to switch to Greek salads. The culture within the diabetes community can also play a role. J. Apple stated the mantra among many suffering from type-1 diabetes has long been: "I can eat whatever I want and cover it with insulin." "It is a point of pride," she added, but it is also a serious sticking point for any lifestyle programme (Jacobs, 2014).

The United Kingdom National Health Service *Five Year Forward View*, published in October 2014, aims to develop a national, evidence-based diabetes prevention programme. In the United States meanwhile a big effort has been underway since 2010 to scale up lifestyle programmes – aiming to reach the *ca*. 86 million (and rising) Americans with pre-diabetes (Jacobs, 2014). There is overall agreement (e.g. from the experience in the United Kingdom) about what interventions work in diabetes prevention and monitoring: opportunities for individuals to introduce gentle exercise in their lifestyle gradually, education sessions for newly diagnosed patients to learn how to self-manage their blood-sugar levels through diet, prescribed medicines and self-monitoring, and a series of regular tests and checks by a patient's physician. However these services are far from being universally available, for instance throughout England. Indeed, according to a national audit published in October 2014, between 2011 and 2013, the percentage of diabetics receiving eight crucial recommended care processes from their general practitioner – including blood sugar and pressure tests – actually fell to 62% (Gainsbury, 2014).

## Monitoring diabetes

When it comes to monitoring blood-sugar levels in diabetics, scientists, technologists and designers are exploring everything from devices that use light to measure glucose concentration through the skin to contact lenses equipped with miniature sensors and

radio antennas. One of the major advances has occurred some time ago: "We have had the basic advantage of people being able to test their own glucose with a certain degree of accuracy for a number of years," stated Matt Petersen, managing director of medical information at the American Diabetes Association. "That was the important step." The catalyst for development in diabetes monitoring came with two large trials in the 1990s. The trials – for both type-1 and type-2 diabetes – revealed that if patients maintained blood glucose at close to normal concentrations 24 hours a day, they experienced fewer complications. "There was no practical way that people could go to a clinic five times a day, so we needed these meters and they have revolutionized people's ability to manage the disease" (Murray, 2014).

The technology has evolved from what P. Petersen called a "glorified dipstick that changed colour to devices that generate enzymatic reactions creating an electrical current on a test strip that can be read by a meter. When Ideo, the design consultancy, started working on a new device – the contour USB – with Bayer, the German pharmaceutical group, the designers undertook an "empathy immersion", which helped them understand where a range of improvements could be made to monitoring devices. What Ideo's team discovered was that many of the meters on the market required users to carry around several pieces of equipment. The team therefore designed a device with a USB that plugged into any computer and automatically downloaded the software. The meter was also designed to recharge automatically whenever plugged into a computer. Finally the visual aspect of the device was important. Instead of producing something that resembled traditional medical equipment, the team designed a meter with a shiny black surface that looked more like a consumer gadget. Today the many meters on the market include backlit models and devices that wirelessly transmit data to mobile phones, to "talking" meters that announce the results for those with impaired vision. Also in widespread use are continuous glucose monitors. People using these insert a small sensor under the skin. The sensor, which needs to be charged about once a week, sends data on glucose concentration to a wireless monitor (Murray, 2014).

But monitoring blood glucose is not the only indicator of health. "Measuring blood glucose and insulin use are really important," stated Glenn Snyder, a principal at Deloitte Consulting. "But without also measuring patient-controllable things such as activity levels, calorie intake and body fat, you do not have a complete enough picture to enable a clinician or patient to manage their health." What tend to attract notice are projects such as Google's partnership with Novartis, the Swiss pharmaceutical company, to develop smart contact lens monitors. M. Petersen believes such developments remain some way off. But he said: "If a device combined that significant improvement in usability with greater accuracy, that would be a game changer" (Murray, 2014).

## Diabetes and tuberculosis

The relationship between diabetes and tuberculosis (TB) is not a new discovery. In 1,000 BC, the Persian philosopher and physician Avicenna discovered that phthisis (TB) caused complications in people with diabetes. But it was only in the past decade that researchers began to examine the phenomenon in such detail. They concluded that people with diabetes are up to three times as likely to contract TB. The multiple

is higher still for younger people. Today *ca*. 15% of adults with TB have diabetes – or more than 1 million people globally. A study in 2012 suggested the proportion was as high as 39% in Texas and 40%-45% in the South Pacific. Rising trends in obesity and diabetes risk such proportion increasing still further in the years ahead (Jack, 2014a).

Rapid urbanization and industrialization have contributed to a sharp convergence in lifestyles and diseases alike, with experts increasingly talking about a "double burden" in developing countries. The greatest global toll of diabetes is in China and India, which also have the highest numbers of TB sufferers. A 2012 study in Kerala, south of India, found 44% of those with TB also had diabetes. By weakening the human immune system diabetes makes people more susceptible to tuberculosis. Diabetics who have already been treated for TB have a higher chance of redeveloping the infection. And with an estimated 3 million people a year contracting TB but not diagnosed, there is a substantial danger that diabetics attending clinics will catch TB from others (Jack, 2014a).

It is less clear that TB makes people more susceptible to diabetes. But recent work suggests those with TB may prove both more difficult to diagnose with diabetes and more difficult to treat. Some tuberculosis drugs may interact differently in the body. When TB medicines are given to diabetics, they may require alternative treatments and higher doses over longer periods – in turn adding to the risks of toxicity and reducing the chance of a cure (Jack, 2014a).

While the fight against TB remains poorly funded by governments and donors, it has received far more attention since the start of the millennium than other diseases in many poor and middle-income countries. Now the debate is shifting to firming up links with diabetes, as part of an effort to integrate the treatment of various non-communicable diseases in low and middle-income countries. The problem is that treatment for diabetes, and with other chronic conditions, is poorly funded, requiring many patients to pay out of their own pocket. Diabetic specialists have also proved reluctant to diagnose TB. But attitudes are changing. When hundreds of researchers gathered in Barcelona in October 2014 for the annual meeting of the International Union Against Tuberculosis and Lung Disease, diabetes came up repeatedly. As a report presented at the meeting warned: "If we fail to act [against diabetes], the consequences could be catastrophic for health-care systems in areas that are impacted." Efforts by the Union, the World Diabetes Foundation and the World Health Organization (WHO) are bringing progress. The Indian and Chinese governments have run pilots for "bidirectional" screening and support between the two diseases, and India has changed its national policy as a result (Jack, 2014a).

More research and clinical guidelines, and practical experience, are required. Vouchers and support groups may be necessary to motivate people to attend clinics regularly. National screening and registers to identify and monitor patients could also be important. The surge in obesity risks slowing or even reversing the progress made in reducing the global burden of TB. As Antony Harries, a physician who long worked in Malawi, concedes : "With the lifestyle changes and cheap food of the urban poor, it is probably going to get worse" (Jack 2014a).

## Diabetes and Alzheimer's disease

In a trial known as the Elad study, Paul Edison of the Hammersmith Hospital in London is running one of several modestly funded research projects that have developed over the past decade and challenge a longstanding orthodoxy about the causes and best ways to tackle Alzheimer's disease. Currently a handful of older drugs have a marginal impact on slowing the progression of the disease for a few months at best. Most pharmaceutical industry research has focused on developing experimental treatments seeking to reverse impairment by tackling the beta-amyloid plaques that build up in the brains of patients. Yet P. Edison cautioned: "Most treatments that seek to clear the amyloid plaques have not worked. Antidiabetic agents have shown a significant effect in laboratory studies on memory and in reducing the abnormal protein that causes the problem." His study, partly funded by charities including the Alzheimer's Society, aimed to recruit 200 people in half a dozen study sites across the United Kingdom. Over 12 months, they will receive liraglutide (Victoza), one of several glucagon-like peptide1 (GLP-1) receptor agonists that are widely used for type-2 diabetes. Novo Nordisk, the Danish company that developed the drug and a global leader in insulin production, was also providing support for the study. Other research work has examined the role of older diabetic drugs such as metformin, inhaled insulin and more recent patented GLP-1 analogues produced by Eli Lilly and Sanofi (Jack, 2014b).

P. Edison's research builds on the work of academics, including Christian Holscher from Lancaster University, who recalls first being struck with the potential of the approach in the middle of the last decade. He had read several papers identifying a far higher risk of Alzheimer's in people who were diabetic. Past research has shown that the amyloid plaques in the brain associated with Alzheimer's are also present in diabetics. One study also suggested 85% of those with Alzheimer's disease also had type-2 diabetes, compared with 42% among those without dementia. Another study found that women without diabetes were twice as likely to have good cognitive function as those with the condition. In France a paper identified diabetes, alongside depression, as among the most important risk factors that could be influenced to reduce the risk of Alzheimer's. That has led some to dub Alzheimer's as "diabetes of the brain". "There is a theory that Alzheimer's is type-3 diabetes," said James Pickett, head of research at the Alzheimer's Society in London (Jack, 2014b).

It has long been known that diabetes affects the body – damaging blood vessels, the kidneys (kidney failure) and peripheral nerves. More recently, however, attention has focused on the illness's effect on the brain. Since diabetes drugs stimulate the pancreas to produce insulin, some studies have suggested that those who take them are less at risk of cognitive decline. This is because insulin, which allows cells to uptake glucose for energy production, seems to be important in activating new cell growth, also enhancing attention, memory and cognition. The problem with older generation diabetes drugs is that they also affect blood sugar concentration, making them unsuitable for those who are not diabetic. By contrast the newer GLP-1-type drugs do not affect glucose, offering scope for broader application in Alzheimer's disease. They also cross the blood-brain barrier, offering potential to be active in the brain. C. Holscher has tested the compounds in mice and noted cognitive improvement (Jack, 2014b).

"Amyloid plaques are probably involved in Alzheimer's and cause inflammation," C. Holscher stated. "But it may not be at the core of the disease, rather a side-effect and probably not the main reason for it." Much work is yet to be done in larger clinical trials of the different compounds to study their safety, efficacy and appropriate dosage – let alone to understand their mechanism. But given the setbacks with current mainstream approaches, the field offers potential – a fact reflected by the interest from the diabetic drug manufacturers themselves. AntiAlzheimer's drugs are failing to combat the disease because they are administered too late, once the condition has become irreversible. So it may be fruitful to explore their application far earlier – especially in groups at highest risk, such as diabetics (Jack, 2014b).

## Supplying affordable diabetes treatments to the developing world

### *Novo Nordisk's contribution*

Novo Nordisk, the Danish company that is the largest global supplier of insulin, has come a long way since August Krogh returned from Canada in 1922 after winning the Nobel Prize for Medicine or Physiology in 1920, bringing with him the rights to produce insulin, which had been discovered at the University of Toronto by Frederick Grant Banting and Charles Herbert Best in 1921 (F.G. Banting and J. MacLeod received the Nobel Prize for Medicine or Physiology in 1923). Thanks to Denmark's enormous pig-rearing industry the raw material for commercial insulin manufacture – porcine pancreas – was plentiful and formed the basis of Novo Nordisk's business. For more than a decade Novo Nordisk's sales have grown each year at double-digit rates, helping turn it into a US$115-billion behemoth that has overtaken Norway's Statoil as the Nordic region's biggest company by market capitalization. With the number of diabetes sufferers worldwide predicted to surge to more than 500 million in the next two decades, Novo Nordisk is on a rising trend. *Ca*. 24 million people use the company's insulin every day. But in poor countries – such as India where 1 million people died from diabetes in 2012 – with weak health-care infrastructure, tens of millions of people go without the drug (Crouch, 2014).

In response to these concerns Novo Nordisk announced it will strive to make its diabetes drugs available to 40 million people by 2020 – an increase of 33% in six years – by selling them at cost price. "We cannot change the demographic problem that people are getting older and more overweight," stated Mads Krogsgaard Thomsen, Novo Nordisk's chief science officer. "But we now have three generations of human insulin and the older varieties we can sell to the least developed countries for no profit." Under this policy the company offers so-called "human insulin" – the first generation of manufactured insulin – to governments in 48 of the poorest countries at a price that does not exceed 20% of the average for Europe, the United States, Canada or Japan. This does not guarantee that the savings are passed in full to sufferers, however; intermediaries take their cut in many countries and even hospitals increase the price of medicines as a source of revenue (Crouch, 2014).

The Access to Medicine Index which ranks pharmaceutical companies' efforts to improve availability of drugs in developing countries, notes with approval that Novo Nordisk supports its provision of non-patented human insulin with efforts to improve health-care infrastructure, logistics and education, and combat corruption, seeking to ensure that treatment in target communities is sustainable over the longer term. M.K. Thomsen stated he looked forward to even greater innovation, such as making insulin available in tablet form, based on new understanding of how such a fragile protein can be made sufficiently stable to withstand degradation by the stomach's enzymes and reach the bloodstream. "That will be a huge benefit for the patients and I believe we can make that happen in the next decade," he stated. Jenny Hirst, chair of the UK charity Independent Diabetes Trust (IDT), noted that for many people with diabetes, orally administered insulin would be an important step, but not a panacea. She would rather see Novo Nordisk spend more of its annual US$2-billion R&D budget on freeing diabetes sufferers from medication altogether, for instance through stem-cell research. Laurence Gerlis, a central London doctor and former medical director of Novo Nordisk, who is also a medical adviser to the IDT, had "deeply held misgivings" about the priorities of pharmaceutical companies in developing new forms of insulin. Yet he conceded that low-cost human insulin for poor countries is certainly saving lives. M.K. Thomsen explained profit from new medicines is what makes it possible for the older types of insulin – still very effective in combating the symptoms of diabetes – to be sold cheaply to the world's poorest. When insulin was first manufactured the life expectancy of a person with diabetes was 30 to 40 years shorter than for healthy people – now this is down to only seven or eight years, he stated (Crouch, 2014).

## *Sub-Saharan Africa*

Diabetes prevalence in sub-Saharan African is increasing and antidiabetic-medicines sales are also growing up. The continent can thus become a good source of revenue for big pharmas. Said Norou Diop, director of the antidiabetic centre Marc Sankalé, Dakar, Senegal, made the following comment: "Formerly, African people did not reach the age when diabetes is diagnosed. Nowadays, as they die less often from malaria or infectious diseases, such as tuberculosis, they increasingly suffer from diabetes, hypertension and cancers." According to this physician the number of diabetics in sub-Saharan Africa in 2014 amounted to 14 million and this figure would reach 45 million in 2035. The main cause of this rise is "some genetic susceptibility or predisposition and the lack of exercise due to a wide-ranging urbanization" (Hecketsweiler, 2014a).

The French big pharma Sanofi whose annual sales of antidiabetic drugs reached more than €6.5 billion in 2013, aims at conquering the new African market of this kind of medicines. It has therefore designed a strategy adapted to local conditions and focused on increasing the awareness of patients and training of physicians. In 2012 Sanofi created the so-called "diabetes clinics": the local hospitals give to Sanofi a room which the pharmaceutical company completely refurbishes with all the equipment needed for measuring glycemia, arterial tension, etc. Sanofi also trains the medical and technical staff. By the end of 2014, 24 "diabetes clinics" were expected to be working throughout Africa (Hecketsweiler, 2014a).

At the same time Sanofi launched an on-line training called "e-diabetes": every month several experts are invited to share their experience and *ca*. 2,000 physicians are connected to that kind of training monthly from 22 countries. Finally Sanofi is in close contact with the universities with a view to changing and adjusting the curricula and increasing the awareness of studies about the risks of untreated diabetes (e.g. cardio-vascular ailments, blindness, leg amputation). Sanofi has also lowered its drug prices (they are less expensive than in Europe) and offered a range of more basic products, e.g. insulin (Insuman), much cheaper than its blockbuster antidiabetic drug, Lantus. Sanofi intends to replicate this business model in the case of such diseases as hypertension or cancer (Hecketsweiler, 2014a).

## Great strides in developing new cures

### *Restoring beta-cell function*

Among diabetes sufferers those with the type-1 version are decidedly in the minority. In France they are 250,000 and in the United Kingdom, they number 400,000 – just a tenth of those with the more common type-2 version. The condition occurs when the cells in the pancreas that secrete insulin, known as beta cells, are attacked and destroyed by the body's immune system. The sufferers' ability to produce insulin is rapidly compromised and eventually eliminated (Pickford, 2014).

In October 2014 a group of researchers at Harvard University Department of Stem Cell and Regenerative Biology, Harvard Stem Cell Institute, published a paper showing they had created insulin-producing beta cells in the laboratory – and for the first time in the massive numbers needed to allow cells to be transferred into patients. A team held by Douglas A. Melton, a Harvard professor whose two children have type-1 diabetes, was able to grow 300 million cells at a time in a 500-ml flask, only one or two of which would be required to treat a patient. Derived from stem cells the beta cells were placed into diabetic mice and continued to produce insulin months after transplantation (Pagliuca et al., and Melton, 2014). A serious complication for all techniques in which new beta cells are inserted into diabetic patients is not only to keep the cells alive and functioning but to stop the body's immune system from obliterating them all over again. Even where patients with transplanted beta cells have their immune systems shut down by powerful drugs, aggressive lymphocytes have succeeded in destroying the new cells after weeks or months (Pickford, 2014).

Research led by Mark Peakman, a professor of clinical immunology at King's College London and Guy's & St Thomas' National Health Service Foundation Trust, has brought promising results. His team showed that certain immune cells appeared to protect the beta cells against attack from their more aggressive brethren. These cells were present in people who had a slow progression of the disease or siblings of diabetes sufferers who never developed the condition – a potential indicator of their importance for resisting type-1 diabetes (Kronenberg et al., and Peakman, 2012). After creating quantities of the peptides or proteins that stimulate the protective cells, M. Peakman and his collaborators administered them to subjects and found they had the ability to modify the immune response in a potentially protective way. He said the

effect was akin to that of a vaccine: "What a vaccine does is to reproduce an encounter with something in the immune system in a safe way. What we are doing is giving a peptide and hoping the immune response to that is beneficial for people at risk" (Pickford, 2014).

The treatment is not for those in whom the disease has fully progressed, since their beta cells cannot be revived. However, for groups at risk of getting it, or those who can be caught at the moment of diagnosis, when the beta cells retain some of their insulin-production abilities, it could prevent the condition from taking hold and allow the body to restore function. And for future treatments that rely on transplantation of cultured beta cells, it could help patients receive the cells without requiring immunosuppressant drugs. With a second generation of the vaccine ready for safety testing in type-1 patients M. Peakman is planning further trials over the period 2015-2017 (Pickford, 2014).

Another therapy is that developed by researchers led by Michael Haller, a pediatric endocrinologist at the University of Florida, who gave low doses of antithymocyte globulin (ATG) and granulocyte colony stimulating factor (GCSF) to 17 patients who had some beta-cell function remaining, and found their insulin-producing capability had not declined up to 12 months after treatment. "Quite remarkably and unexpectedly, patients with established type-1 diabetes may benefit from such combination immunotherapy," the researchers explained (Pickford, 2014).

Another line of research consists of transforming embryonic pancreatic glucagon-producing cells into beta cells. The team of Patrick Collombat, in charge of the diabetes' genetics team of the French National Institute for Health and Medical Research (INSERM) and University of Nice Sophia-Antipolis (Valrose Biology Institute), south-east of France, has been working on mice, with a view to regenerating insulin-producing cells. They have first discovered two genes that control the insulin-producing capacity of cells; then, when they activated one of the two genes, called Pax4, they were able to transform pancreatic glucagon-producing cells into beta cells; this could cure a chemically-induced diabetes in genetically modified mice. These results were published on 15 July 2013 in the *Developmental Cell* journal (Al-Hasani et al., and Collombat, 2013). They must now be validated in humans. The next step consists of finding a pharmaceutical that mimicks the effects the gene Pax4 in order to massively transform healthy glucagon-producing cells into beta cells. In this research P. Collombat and his team are working with the Max-Planck Institute of Göttingen, the Broad Institute (Harvard University and Massachusetts Institute of Technology) and Novo Nordisk. The American Juvenile Diabetes Research Foundation is providing €5 million to the work carried out by these various research centres. "The development of a medicine will take five to ten years," warned P. Collombat, who hoped to publish the results of current research in 2016 (Santi, 2014b).

### *Stem-cell therapy*

The discovery in 1998 of a way to extract and grow human embryonic stem cells (hESCs) was hailed as the dawn of an era of "regenerative medicine" in which new

cells could be implanted into patients to replace diseased or missing tissues. Diabetes was immediately seen as one of the top prospects for regenerative treatment, if new insulin-producing beta cells could be produced to replace the ones that disappear in type-1 (and some type-2) patients. Reliable cell therapy for diabetes is in prospect at last (Cookson, 2014). See Noguchi (2010).

In October 2014 two crucial developments occurred. ViaCyte, a privately owned biotechnology company based in San Diego, initiated the world's first clinical trial of a diabetes treatment based on hESCs. On the other hand, an academic research team at Harvard University published the first evidence that hESCs can generate fully functional beta cells in the huge quantities required for such treatment to reach the medical mainstream. Richard Insel, chief scientist of the Juvenile Diabetes Research Foundation, which helps fund both projects, stated that together they represent a historic advance towards cell therapy for type-1 diabetes, an autoimmune disease.

The lead product of ViaCyte programme, called VC-01, consists of "pancreatic precursor cells" – essentially immature beta cells – being implanted under the skin. They are encapsulated in a semi-permeable membrane designed to protect them from immune attack and prevent vascularization (the growth of unwanted blood vessels through the implanted cells). If VC-01 works in humans as well as it had in animal tests, the pancreatic precursor cells will differentiate and mature after implantation. After a few months they will be fully functioning beta cells, producing not only insulin but also other hormones that are important for healthy metabolism and regulation of blood-sugar concentration. ViaCyte expected to enrol 40 patients for the study who will live with the implanted product for up to two years. As with any phase-1 trial the primary aim is to assess safety but clinicians will also evaluate the efficacy of VC-01 in replacing the lost insulin-production function central to type-1 diabetes. "To our knowledge, this is the first time that an embryonic stem cell-derived cell replacement therapy for diabetes has been studied in human subjects, and it represents the culmination of a decade of effort by the ViaCyte team and our supporters at the California Institute for Regenerative Medicine," stated Paul Laikind, CEO of ViaCyte (McCormack, 2014).

The second above-mentioned project is led by Douglas A. Melton. In contrast to the immature pancreatic cells produced by ViaCyte, which need to develop further inside the patients before they are fully active, the Harvard team discovered how to convert human pluripotent stem cells (hPSC) into potentially unlimited numbers of fully mature beta cells *in vitro* – which might make a more effective treatment (Pagliuca et al., and Melton, 2014). Commenting on the research, Chris Mason, professor of regenerative medicine at University College, London, stated: "A scientific breakthrough is to make functional cells that cure a diabetic mouse, but a major medical breakthrough is to be able to manufacture at large enough scale the functional cells to treat all diabetics … If this scalable technology is proven to work in both the clinic and in the manufacturing facility, the impact on the treatment of

diabetes will be a medical game-changer on a par with the effect of antibiotics on bacterial infections." D.A. Melton is working with colleagues at the Massachusetts Institute of Technology (MIT) on an implantation device that, like the ViaCyte system, protects the implanted cells from the recipient's immune system (Cookson, 2014).

The first immune attack problem – recognition of the implanted cells as foreign material – could be overcome by using the human pluripotent stem cells (hPSC) technique rather than hESCs, making the beta cells from stem cells that originate from the patient. But that leaves the risk of autoimmune attack. One possibility is simply to replace the implanted cells when they are no longer working. Without clinical data it is hard to estimate how long they would last inside the patient but replacement every year or so might be necessary (Cookson, 2014; Motté et al., 2014; Agulnick et al., 2015).

## Conclusions

Indeed, diabetes is the "silent epidemic of the 21st century." Obesity as a result of changing lifestyles and food habits is helping to generate a global epidemic, especially with respect to type-2 diabetes. Even if lifestyles do become healthier – still a very big if – the incidence of diabetes will keep rising in the United States and Europe because people are living longer and old age is itself a risk factor. With the number of diabetes sufferers worldwide predicted to surge to more than 500 million, in poor countries with their insufficient health-care infrastructure, tens of millions of people do not have access to insulin; in India, *ca*. 1 million people died from diabetes in 2012. The top priority in combating the disease must be prevention: from labeling foods to taxation, in addition to exercise and nutrition education. Regarding the treatments of diabetes, steps are afoot to supply affordable treatments to the developing world, while great strides are expected in the measuring devices of blood glucose and insulin, as well as in the replacement of beta cells by new ones derived from stem cells.

Health-care systems can be disrupted, unless proper attention is given to avoid unnecessary expenses. For instance, at £10 billion, the annual cost to the United Kingdom National Health System (NHS) in England of treating 3 million people diagnosed with diabetes is already huge – some 10% of the total budget. If the 11 million people estimated in 2014 to be at high risk of developing the disease over the next decade do go on to acquire it, the burden could spell catastrophe for the already cash-strapped system. No wonder diabetes – particularly type-2 – was highlighted in the five-year strategy published by the NHS England chief executive Simon Stevens. He warned that without medical reductions in the demand for hospital-based health services, the NHS will face a multi-billion pound funding deficit by the end of the decade. "We are spending more on bariatric surgery [such as gastric banding] than we are on ways to help people stay healthy," S. Stevens stated. But bariatric surgery is just the tip of the iceberg of escalating costs and serious health

complications stemming from poorly managed diabetes. Between 2006 and 2011 the number of diabetics in England experiencing cardiac failure more than doubled. The numbers suffering a stroke or kidney failure increased by 87% and 77% respectively, while those developing blindness rose by almost two-thirds. This rapid increase in the number of diabetes sufferers being hospitalized through medical emergencies meant that of the £10 billion spent a year on patients with diabetes, only £2 billion was spent directly treating their diabetic condition. The rest is swallowed up by the costs of treating the life-threatening complications which are the consequence of a failure to keep a patient's diabetes under control (Gainsbury, 2014).

There is little disagreement across the NHS that what is needed is a radical shift of focus and resources from hospital-based care to prevention. Both preventing type-2 diabetes itself by detecting those most at risk of developing the condition and persuading them to take up healthier lifestyles; and preventing those already with the condition going on to develop even more serious and expensive complications. There is agreement too about what interventions work: opportunities for individuals to introduce gentle exercise into their lifestyle gradually, education sessions on how to self-manage blood glucose levels through diet, prescribed medicines and self-monitoring, and a series of regular tests and checks by a physician (Gainsbury, 2014). Finally, in both industrialized and developing countries, the mantra should be: combating diabetes is combating obesity and overweight.

# OBESITY

## Obesity prevalence

It has been estimated that, if the current epidemiological trends are confirmed, the number of overweight adults (whose body mass index is over 25 kg/m$^2$) would reach 2.7 billions in 2025 across the world, compared with 2 billions in 2014. Severe obesity (i.e. adults with a body mass of 35 kg/m$^2$ or more) would afflict 177 million adults, compared with 98 million in 2014-2015. In France, for instance, 3.4 million adults would suffer from severe obesity in 2025, compared with 2.5 million in 2014. These figures have been communicated on Monday 11 October 2015 – the World Day of Obesity.

Between 1980 and 2014 the prevalence of obesity worldwide has more than doubled. Affecting both wealthy and poor countries, adults and children obesity and overweight are now occurring in *ca*. 30% of humankind, according to a vast study published in 2014 by *The Lancet* (Ng et al., 2014). Human body weight standards are defined through the body-mass index (BMI) calculated as weight in kilograms divided by height in meters squared. Overweight is diagnosed when the BMI is equal or superior to 25 kg/m$^2$, while obesity corresponds to an index equal or superior to 30 kg/m$^2$. In both cases this anomaly is due to an energy disbalance between the intake of kilocalories and those spent by the body. The main causes are, on the one hand, the consumption of foodstuffs containing too many calories and fats, and on the other, the lack of exercise and a sedentary lifestyle. The implications of overweight and obesity are cardiovascular diseases, diabetes, muscle-skeleton ailments and cancers (breast, colon and uterus).

Regarding Europe the WHO launched a serious warning on 6 May 2015 during the European congress on obesity, held in Prague, Czech Republic, where was presented a modelization by Laura Webber (UK Health Forum) and Joao Breda (WHO Europe Regional Office). Obesity and overweight are affecting both men and women in the majority of the 53 countries of the Europe Region, as defined by the WHO (it includes Eastern Europe and Central Asia). In most of these countries the spread of obesity has not slowed down since 2010. With a 68% rate Belgium and Bulgaria were the countries where the female population was mostly affected by overweight in 2010. They were expected to be in the same position in 2030, with at that time a record proportion of 89%. With respect to obesity Moldavia (28%) was in the first position, followed by Russia and Turkey (27%), and 20 years later, they will be outpaced by Ireland, where

the percentage of obese women was expected to rise from 23% to 57%. Irish men with overweight, representing 76% of the whole population in 2010, would make up 90% of the whole population in 2030, just ahead of Uzbekistan and Iceland. Turkey which was topping the countries with regard to male obesity (36%) in 2010, would be largely outpaced in 2030, despite its proportion of 51%, by Kazakhstan (from 11% to 74%) [Benkimoun, 2015d].

In the United Kingdom one-third of the female population (and not one-fourth in 2010) would be obese in 2030. Also 64% of them would be overweight, compared with 59% in 2010. The proportion of obese men would rise from 26% in 2010 to 36% in 2030, while that of overweight men would increase from 70% in 2010 to 74% in 2030. The situation was also expected to worsen in Greece, Spain, Austria and the Czech Republic. France also follows a similar trend. The proportion of overweight women would rise from 43% in 2010 (28$^{th}$ rank in Europe) to 58% (15$^{th}$ rank) in 2030. Regarding men the proportion would rise from 54% to 66%. With respect to obesity the change that is expected would be quite prominent among women (16% to 29%), while it is also serious among men (14% to 25%). By contrast, if the prospects are correct, the Netherlands would be in a better position than most of the European countries. The proportion of obese and overweight men would decrease from 2010 to 2030: 49% compared with 54% in the case of overweight, and 8% compared with 10% in the case of obesity. The proportion of overweight women would remain stable (around 43%), while obesity would decrease from 13% in 2010 to 9% in 2030 (Benkimoun, 2015d).

It should be underlined that the research team which has been working on this project, has used data coming from various sources in the 53 Europe Region countries. The availability and quality of these data vary throughout the region, and, in some countries, the researchers could not rely on complete and updated figures. They were not able to present comprehensive results for the 53 countries. On the other hand we are dealing here with projections (estimates) which inherently have their range of uncertainty. According to Joao Breda of the WHO Europe Region Office, "the study should be used with caution, because its size was relatively modest and it was based on the available national data that would not eventually reflect the most recent estimates of the WHO that are always under study." Furthermore these projections are based on a scenario where the policies for the prevention of, and struggle against, obesity would not be modified. The situation prevailing in 2010 does not necessarily correspond to the present one. "The measures currently taken could make the predictions unrealistic, and, in some European countries, the trend is towards stabilization thanks to preventive actions, some of which are successful, e.g. regarding children obesity," stated Joao Breda in a press release by WHO-Europe. Laura Webber (UK Health Forum) insisted on the fact that "there is an urgent need for policies aimed at reversing the trend of obesity increase (e.g. one 11-years-old child out three is obese or overweight); although there is not a miracle recipe to fight obesity, governments should make more efforts in order to restrict the commercialization of unhealthy food and to make quality foodstuffs more affordable." In other words, as stated by Paul Benkimoun, the obesity epidemic is not something unavoidable (Benkimoun, 2015d).

## Americans shift their diet towards less calorie intake

There is no perfect way to measure Americans' calorie consumption. But three main sources of data about diet all point in the same direction. Detailed food diaries tracked by government researchers, data from food bar codes and estimates of food production all show reductions in the calories consumed by the average American since the early 2000s. Those signals, along with the flattening of the national obesity rate, have convinced many public-health researchers that the changes are meaningful. Since the mid-1970s, when American eating habits began to rapidly change, calorie consumption had been on near-steady incline. "I think people are hearing the message, and diet is slowly improving," stated Dariush Mozaffarian, the dean of the Friedman School of Nutrition Science and Policy at Tufts University, Boston. Barry Popkin, a University of North Carolina professor who has studied food data extensively, described the development as a "turning point in US diets." The eating changes have been the most substantial in households with children (Sanger-Katz, 2015).

There is no single moment when American attitudes toward eating changed, but researchers point to a 1999 study as a breakthrough. That year, researchers from the Centers for Disease Control and Prevention (CDCs) published a paper in *The Journal of the American Medical Association* (*JAMA*) that turned into something of a blockbuster. The paper included bright blue maps illustrating worsening obesity rates in the 1980s and the 1990s in all 50 States. Researchers knew the obesity rate was rising, but Ali H. Mokdad, the paper's lead author, stated that when he presented the maps at conferences, even the experts were amazed (Mokdad et al., 1999). A year later he published another paper, with a similar set of maps, showing a related explosion in diabetes diagnoses (Sanger-Katz, 2015).

Shortly afterward the surgeon general, David Satcher, issued a report – *Call to Action to Prevent and Decrease Overweight and Obesity* – modeled on the 1964 surgeon general's report on tobacco. The 2001 report summarized the increasing evidence that obesity was a risk factor for several chronic diseases, and underlined that controlling children's weight should be a priority, to prevent the onset of obesity-related illness. By 2003 60% of Americans stated they wanted to lose weight, according to Gallup, up from 50% in 1990 and 35% in the 1950s. The President Barack Obama's administration has increased pressure. The Affordable Health Care Act, passed in 2010, required chain restaurants to publish the calorie content of their meals. The federal government also changed requirements, making school lunches healthier, although the effort created some backlash (Sanger-Katz, 2015).

Several cities have gone further. Philadelphia subsidizes produce purchases. New York limits the kind of food available in day-care centres. Berkeley, California, in 2014 became the first city in the United States to tax sugar-sweetened beverages. The evidence for the effectiveness of these interventions is mixed, but their popularity reflects public health officials' emphasis on diet and obesity. The anti-obesity public-health campaigns have focused on one subject more than any other: beverages. Antisoda messages hit their target. The average American purchased *ca*. 40 gallons (≈ 160 liters) of full-calorie soda a year in 1998, according to sales data from the

industry trade publication *Beverage Digest*, analyzed by the Center for Science in the Public Interest. That fell to 30 gallons in 2014, about the volume that Americans bought in 1980, before the obesity rates took off (Sanger-Katz, 2015).

Outside of beverages there are few clear trends. Experts who have examined the data stated the reductions did not mean that Americans are flocking to farmers' markets and abandoning fast food. Consumption of fruits and vegetables remains low; consumption of desserts remains high. Instead people seem to be eating a little less of everything. The calorie reductions are seen across nearly every demographic group, but not equally. White American families have reduced their calorie consumption more than African American and Hispanic families. Most starkly families with children have cut back more than households with adults living alone. These calorie reductions appear to be good news, but they do not mean the end to the obesity epidemic: *ca*. a third of American adults were still considered obese in 2014, putting them at increasing risk of diabetes, heart disease and cancer. Americans are still eating far too few fruits and vegetables and far too much junk food, even if they are eating somewhat less of it, experts said. Kevin Hall, a researcher at the National Institutes of Health (NIH), estimated that for Americans to return to the body weights of 1978 by 2020, an average adult would need to reduce calorie consumption by 220 kilocalories a day (Sanger-Katz, 2015).

## Personalized nutrition

David S. Ludwig, pediatrician and researcher at Children's Hospital, Boston, and director of the New Balance Foundation for Childhood Obesity Prevention, Clinical Research and Care at Children's Hospital, authored with Dawn Ludwig the book *Always Hungry? Conquer Cravings, Retrain Your Fat Cells and Lose Weight Permanently*, published on 5 January 2016 by Grand Central Publishing (304 pp.). They wrote: "Some people eat as little fat as possible to lose weight and stay healthy, while others avoid carbohydrates. A vegan diet (with no animal product) and the paleo diet (with lots) both have enthusiastic devotees. One popular diet encourages intermittent fasting, another frequent small meals. Who is right?" In November 2015 an Israeli study of personalized nutrition was heralded by a media frenzy (Zeevi et al., 2015). The study suggested that dieters may be mistakenly eating a lot of some foods, like tomatoes, that are good for most people, but bad for them. And it raised the possibility that an individualized approach to nutrition could eventually supplant national guidelines meant for the entire public. But in fact it was known for a long time that people respond differently to specific foods based on their genetic make-up, past health and other factors. What does this mean when it comes to obesity?

Unfortunately, standard diets typically fail to produce long-term weight loss. But those average outcomes mask tremendous individual variability. For instance, while a clinical trial published in 2005 of 160 adults randomly assigned to the Atkins, Ornish, Weight Watchers and Zone diets reported modest results in all groups after one year, individuals in those groups experienced weight changes ranging from a loss of 35 pounds or more to a gain of 10 pounds or more. This variation is commonly attributed to behaviour. Some people are simply more motivated and compliant with

their assigned diet than others. But suppose the people who did poorly on the low-fat Ornish diet would have done well on the low-carbohydrate Atkins diet because of their biological make-up, and vice-versa? If we knew that ahead of time we could assign everyone the diet that is best suited for him or her. This is what the Israeli study intended to explore. Investigators at the Weizmann Institute of Science, Departments of Computer Science and Applied Mathematics, Molecular Cell Biology and Immunology, Rehovot and Tel Aviv Sourasky Medical Center, used specialized devices to monitor continuously the blood sugar of 800-person cohort. The data showed that blood sugar after meals varied among the participants in ways that could not be explained by what they ate alone. The researchers devised a machine-learning algorithm that integrates many dietary and non-dietary characteristics – including body weight, blood sugar first thing in the morning and even gut microbiota – to predict more accurately what would happen to blood sugar after a specific person ate a specific food. Since high blood sugar after eating is strongly associated with the risk of type-2 diabetes and heart disease, the study results now have people talking about whether a computer app might someday help us prevent chronic disease. Should you eat tomatoes, apples or chocolate? Input your characteristics and get a personal prescription for optimal health (Zeevi et al., 2015; Ludwig, 2015).

Blood sugar is not the only way to predict an individual's predisposition to obesity-related problems. Insulin may be an even more powerful determinant. The pancreas releases insulin after a meal and that hormone directs incoming calories into storage sites in the liver, muscle and fat tissue. A few hours later, insulin levels fall and calories re-enter the bloodstream for use by the body. This is why people with type-1 diabetes who receive excess insulin predictably gain weight, whereas those treated with too little insulin invariably lose weight, no matter how much they eat. The amount and timing of insulin release after a meal differ substantially from person to person. To assess this difference researchers give volunteers a bottle of glucose to drink and measure their insulin levels 30 minutes later – the test is called the "insulin-30" level (Ludwig, 2015). In a study published in *The Journal of the American Medical Association* (*JAMA*), D.S. Ludwig, Cara B. Ebbeling et al. (2007) of Children's Hospital, Boston, randomly assigned 73 young adults to 6, 12 and 18-month diets that were low either in fat or in processed, fast-digesting carbohydrates (called a low-glycemic load diet). They found that individuals with high insulin-30 did better on the low-glycemic load diet – losing 10 pounds more than they did on the low-fat diet. This suggests that people with this characteristic should really focus on cutting highly processed carbohydrates out of their diet. Individuals with low insulin-30, on the other hand, lost about the same amount of weight on both diets.

In another study published in 2015 in the journal *Obesity*, D.S. Ludwig and his colleagues found that people with high insulin-30 lost more muscle and less fat on a standard low-calorie diet. Their metabolisms also slowed the most when they lost weight, which means they would be likely to regain the weight in the long term. The good news is that a person's susceptibility to weight gain may not be assured. After a month on a low-carbohydrate diet the high insulin responders were able to tolerate more carbohydrates without their metabolisms slowing down so much (although we do not know how long this effect lasts) [Hron et al., 2015].

Many other biological factors – genetic and acquired – undoubtedly influence humans' response to diet. But in the end the Israeli study provides little reason to believe that a complicated 21st-century technology will work any better than a simple dietary prescription pioneered decades ago. There is no need for an app to control post-meal blood sugar; we just need to eat fewer processed carbohydrates. Analyses of larger groups of people followed carefully over many years, like the Nurses' Health Study, suggest that many cases of diabetes and heart disease can be prevented by adhering to a few straightforward dietary practices. For this reason it is critically important that the Dietary Guidelines for Americans 2015, released by the Department of Agriculture, reflect the latest science. They should jettison the traditional emphasis on low-fat diets, which we now know have no special benefit for body weight or general health, and focus more on the quality of the carbohydrates we are eating (Ludwig, 2015). While personalized medicine has become well established in clinical practice, personalized nutrition is not yet ready for practical application in the clinic. But this field of research may help explain why people respond so differently to diet based on biology. In this way personalized nutrition may build upon, rather than substitute for, national dietary guidelines (Ludwig, 2015).

## McDonald's setback and a new approach to fast food

According to the data published on 23 January 2015 McDonald's net profit in 2014 amounted to US$4.76 billion (or €4.24 billion), a 15% decrease, compared with 2013. During the last quarter of 2014 the profit even plunged 21%. This was due to a 2.3% reduction in the annual sales which plummeted to US$27.44 billion. Here also the reduction was higher during the last quarter of 2014: - 7.3% (Girard, 2015a).

### *A deep crisis of trust and the company new strategy*

McDonald's is facing competition from two groups of rivals: on the one hand, Burger King, KFC (Kentucky Fried Chicken). Wendy's, Taco Bell or Subway, which multiply promotion sales with low prices aiming to attract people who want to spend less on food; on the other, Starbucks, Chipotles or Panera attract young people. Just like Coca-Cola, the other icon of junk food, McDonald's is less attractive to American consumers, who are more conscious of the obesity risk and are wishing to ban from their menus burgers and XXL soda cups. Even in China – a very sensitive market – McDonald's has gone through a deep crisis of trust. One of its suppliers was caught up because it had sold outdated chicken meat. The suspected factory, based in Shanghai, which also supplied Japan, was closed down, but the lack of trust remains (Girard, 2015a).

In Japan, in December 2014, sales fell down by 21%. Globally it is in Asia-Middle East-Africa where the sales decreased by 4.8% during the last quarter of 2014. In Europe the sales reduction was less marked: -1.1%. Also McDonald's had to close down several of its restaurants in Russia – a decision made by the Russian authorities, officially further to health-safety controls. France was no exception, due to the weakness of the market during the last quarter of 2014 and also to a harsher competition from Burger King and from the bagel chains. Only the United Kingdom was not so much affected (Girard, 2015a). Confronted with such situation McDonald's

unveiled in December 2014 its offensive strategy in the United States, with a new CEO at the helm, Mike Andres, the second chief executive in less than two years; he was appointed in October 2014 with a view to fostering the sales of McDonald's subsidiary in the United States. He announced the simplification of the company offer: from16 to 11 menus. But the most important change was to propose to the American clients to choose themselves their burger and other food items through a computer screen. This was considered a way to suit everybody's tastes. McDonald's American subsidiary noted that the first positive signals of its strategy were being registered: its sales rose 0.4% in December 2014. This reverse trend that McDonald's had not acknowledged since October 2013 was stressed by analysts. McDonald's also decided to limit the number of new openings and it estimated that its investments in 2015 were to reach US$2 billion (Girard, 2015a).

### *Presence in sub-Saharan Africa*

In sub-Saharan Africa McDonald's has been present in South Africa since 1995 with 200 restaurants, but not so much outside the arcoiris nation, because the markets are considered too risky. Only its rival, KFC, has opened outlets in English- and Portuguese-speaking countries. However the demand for fast-food restaurants is fuelled by sprawling urbanization and the emergence of a middle class that wishes to eat quickly and near to the workplace. For instance in Nigeria where city-dwellers reached the number of 73 millions in 2014, local fast-food restaurants have become iconic in 20 years: in Lagos, Mr Bigg's, MamaCass, Tantalizers, Sweet Sensation or Chicken Republic have followed the American model with respect to their management, but their menus include local recipes, with an emphasis on chicken, less costly and easier to store. The success of Mr Bigg's (160 restaurants, a subsidiary of the United Africa Company of Nigeria, listed on Lagos' stock exchange) is linked to its meat pie, made from the traditional meat of southern Nigeria. At MamaCass (19 restaurants) one cannot find hamburgers, but Nigerian meals like the jollof rice – the Yoruba greasy rice. The key to success of Nigerian fast-food restaurants is a flexible logistics with a short circuit linking them to local producers, so as to guarantee the products freshness (Le Bec, 2015).

In the sub-Saharan French-speaking cities there exists fast food, but not many chains. In Abidjan, Côte d'Ivoire, for instance, the French oil giant Total built a partnership with the *Société ivoirienne de production animale* (Ivory Coast Company for Animal Production), in order to change the situation; their chain called Tweat and launched at the end 2014, expected to open nine restaurants, five of them in Abidjan, by the end of 2015. The first fast-food outlets were to be set up in the gasoline service stations of Total, but Tweat is supposed to open outlets in downtown areas, firstly in Côte d'Ivoire and thereafter in countries of West Africa (Le Bec, 2015).

## Fast-food companies in North Africa

In North Africa Morocco and Tunisia have attracted fast-food investors. In 2015 there were 39 McDonald's restaurants in Morocco, compared with seven outlets of Burger King and one of Quick; in 2018 the figures were expected to be 50, 22 and a few

ones of Quick, respectively. Quick is a French company whose main market is Europe, where it is facing the harsh competition by McDonald's and Burger King, among others. Quick belongs to Qualium Investissement, a subsidiary of the *Caisse des Dépôts et Consignations* – an important public financial group. Quick, like its rivals, is trying to be more present in Asia, the Middle East and Africa. Morocco and Tunisia were considered a priority destination, with two restaurants to be opened in Casablanca and Rabat before the end of 2015, as well as in Tunis. Both countries "are dynamic economies with an important young population having very strong ties with Europe; this is an attractive factor for international fast-food brands to be settled there," stated an analyst of the sector (Ballong et al., 2015).

After setbacks in 2003 in Morocco and in 2007 in Algeria one is wondering whether the new offensive of Quick will be more successful than before. The group's turnover in 2014 fell down to €1.029 billion, a 4% decrease, and consequently a new international development executive was appointed in September 2014: the Frenchman Thierry Rousset who was hired from the American rival Burger King, where he was in charge of the markets in Turkey, southern Europe and Africa. With a solid experience in fast food and franchise, he started his career at McDonald's and thereafter worked for Subway. His strategy is to make alliances with local partners with a perfect knowledge of the markets and solid financial capacities. Quick adjusts its development strategy to each market in an optimal way. For instance in Tunisia Quick partner is the family conglomerate Hachicha, involved in agrifood, chemistry and house electrical appliances. In this way the group ensures that "all the meat served is certified halal" and that rather soon the offer will be identical to the French one. The same approach will be followed in Morocco, with local suppliers that comply with Quick requirements (in 2014-2015, fish, beef and chicken meat were still imported from France) [Ballong et al., 2015].

By contrast with Algeria where none of the large fast-food groups is present, Morocco has attracted Quick interest, while its two American rivals, McDonald's and Burger King, are competing. McDonald's has been present in Morocco since 1992 and is the uncontested leader on the market. Burger King set up its first restaurants in December 2011; as mentioned above its objective is to run 22 restaurants in 2018, compared with 7 in 2015. This will need a €25-million investment, according to the company's management, and Burger King will benefit from synergy with the chain of commercial malls, Almazar. McDonald's reaction has been quick: it will invest €22.7 million in order to have a network of 50 restaurants across the country in 2018. For its comeback Quick chose to partner with a heavy-weight multiprofessional holding, Tenor Group, involved in new technologies, real estate, insurance and education. This partnership was expected to make the competition between the three fast-food brands even harsher, with the hope that it would benefit the Moroccan consumer who is very fond of burgers (Ballong et al., 2015).

In Algeria there are few international fast-food brands, compared with the high number of local fast-food restaurants, e.g. 7,000 in the country's capital Algiers in 2012 – 3.34% of all the city's businesses. Quick attempt in 2007 was a failure. Analysts underline that although Algeria looks attractive, there are many difficulties, such as the requirement to

produce everything locally, the need to obtain an authorization from the Bank of Algeria for any transfer of royalties and the high cost of business agreements. Consequently the potential investors have to rely on smart solutions, e.g. "disguized franchise". This is the case of three American fast-food brands – Dal's Burger, Gold'n' Brown and Granada Pizza – which were registered in Algiers in November 2013. The franchiser made partnerships with an Algerian investor and three foreign ones from Jordan, Yemen and the United Arab Emirates. In fact the director of the fast-food restaurants under the above-mentioned three brands reckoned that this business required an industry which did not yet exist in Algeria (Rondeleux, 2015).

## Fast-food companies in France

In France McDonald's is the dominant fast-food company with more than 1,340 restaurants and an overall annual turnover (including that of the company McDonald's France and that of the franchised units) of €4.6 billion in 2014. And the company is still growing: +2.6% in 2014 on the French market (Jacquin, 2015). Burger King – the world's third-largest fast-food group – had in France only 50 restaurants by the end of 2015. Olivier Bertrand, the CEO of the Groupe Bertrand who owns the exclusive license of Burger King in France (franchise), had announced before the summer of 2015 that he wanted to speed up the expansion of Burger King across the French territory and he mentioned the opening of another 60 fast-food units in 2016. Moreover he announced on Monday 28 September 2015 that his group had been negotiating the purchase of Quick – a chain of fast-food restaurants, created in 1971 in Belgium, that became international in 1980 through its restaurants in France first, thereafter in Luxembourg, the Netherlands and Hungary. The exclusive negotiations were being carried out with Qualium Investissement. The latter has been trying for years to sell Quick, with 509 fast-food restaurants in France, Belgium and Luxembourg, and an annual turnover of €1.03 billion in 2014. Burger King – whose Whopper is its emblematic hamburger – which returned to France in 2013 after being absent for 15 years, is therefore challenging McDonald's through the purchase of Quick 400 restaurants that existed in France in 2014. The purchase of Quick by Burger King aims at changing its development scale in France where it was not so well represented compared with its competitors. While French Quick fast-food units will progressively adopt the American brand, the others, especially those located in Belgium, where are the inventors of Giant and Quick N'Toast food items, will keep their brand and their menus (Jacquin, 2015).

The price for the purchase of Quick by Burger King was not revealed by the Groupe Bertrand, but according to some sources it was over US$700 million. Analysts considered that such a transaction was quite risky, because the French group was buying a company twice bigger than itself and because fast-food was not until then at the core of its business. This family firm (Groupe Bertrand) owns coffee and tea brands, as well as a renowned café in Paris; it signed with Burger King a licensing agreement in 2013. It should be underlined that in the fast-food industry the exploitation model is the franchise. Both Burger King and McDonald's do not own the restaurants that bear their brand; this allows a development which does not need too much capital. Marketing, purchase policies, advertisement campaigns are implemented by the fast-food group,

while staff expenses and real-estate investments are born by the franchised. Quick, the European brand for the hamburger, was bought in 2006 by Qualium Investissement from the Belgian billionaire Albert Frère. It has suffered from the harsh competition existing on the fast-food market; its sales decreased by 4% in 2014 and 2015 did not seem to be a good year either. This certainly had an impact on the price paid by Burger King to acquire Quick. Also for the 19,000 employees working for Quick it will not be easy to work now under Burger King (Jacquin, 2015).

## Novel foods: *in vitro*-made meat

The idea to make meat from animal cell cultures was that of Willem van Eelen who could not withstand the fact of killing animals in order to meet humans' carnivorous appetite. Aged 92 in 2014 this former entrepreneur gathered a few life-sciences researchers in 2004 with a view to working on his project. After a colleague left the group because of health problems, Mark Post replaced him. A physician, M. Post was working at that time on the cardiovascular system. He indicated that "the other biologists were participating in the project with totally different objectives: some wished to better understand the behaviour of muscular stem cells, while others were interested in creating muscle for medical applications;" as far as M. Post was concerned he found that the production of meat *in vitro* was an excellent idea. M. Post was in charge of the research unit on physiology at the University of Maastricht and he became the specialist of *in vitro*-made meat (Barnéoud, 2014).

In August 2013 he showed an artificial hamburger to the public during a taste-event in London. M. Post is an environmentalist who eats organic products and travels in train, and in his view "to make meat *in vitro* aims at alleviating the impact of intensive livestock husbandry on the environment, through reducing the emissions of greenhouse-effect gases (methane) as well as the production of nitrates (from manure)." Furthermore, being a physician having a major concern about food safety (remembering the scandals about bovine-meat production in the 1990s), he considered that "it is possible to better control the quality of foodstuffs, when the latter are produced in the laboratory." M. Post therefore ventured in the area of novel foods with the help of Sergueï Brin, co-founder of Google, who is very much concerned about animal suffering. S. Brin invested €1.1 million in M. Post's laboratory (Barnéoud, 2014).

The artificial burger is made of 30 billion cells and its manufacture lasted nine weeks and relied on the use of 30 culture dishes (the size of a shoe box) and a large incubator. Bovine serum, antibiotics and fungicides were also used. Total cost amounted to €250,000. Jean-François Hocquette, a director of research at the French National Agricultural Research Institute (INRA, French acronym), criticized M. Post's project: "To allocate so much money to a long-lasting and hypothetical project, when it is urgent to feed a growing population, seems to me an error. Moreover, if the whole chain of production is scrutinized, from the production of antibiotics to the quantity of electricity consumed by the incubators, it is impossible to assert that *in vitro*-made meat will be less polluting, or healthier than meats from livestock." M. Post reckoned the existence of these hurdles and made a strong advocacy for more research. In his unit four researchers were working full time on *in vitro*-made meat. He used liquid

cell cultures instead of culture dishes, with the advantage of reducing laboratory space to make the artificial meat, as well as the number of manipulations. This leads to less contamination and consequently to use less antibiotics. In the medium term *in vitro*-made meat was expected to be produced in containers with a volume of 25,000 liters (Barnéoud, 2015).

The taste of the artificial burger must also be improved. M. Post indicated that "we are cultivating fatty cells in order to make the meat less dry." It remains to be seen whether the consumers would accept what has been dubbed as the "frankenburger." Maybe, in ten years, we shall see a label like "meat from a living animal," said M. Post who recalled that we have seen the proliferation of "bio" labels or those put on products commercialized by small producers from the developing world in order to help them ("solidarity"). M. Post who was threatened by livestock farmers, is convinced that *in vitro*-made meat will be on the plates of consumers within a decade; and thereby it could replace a small proportion of the meat that is increasingly consumed worldwide (Barnéoud, 2015).

## Creating new plant-based foodstuffs

The overall consensus about combating obesity and overweight, as well as the associated diseases such as type-2 diabetes, is based on persuading people at risk (or even without risk) to adopt a healthier lifestyle, that includes not only exercise, but also different food habits and consumption. A healthier and cheaper food, and just as satisfying as meat, egg, dairy and other animal-based products, could be derived from plants. The idea of making such products is attracting entrepreneurs and venture-capital firms who think that the traditional food industry is ripe for disruption, because it is inefficient, inhumane and in need of an overhaul. The companies have different approaches but they share the ambition of creating new plant-based food, with a much lower environmental impact than animal-based products from the livestock industry. "Animal farming is absurdly destructive and completely unsustainable. Yet the demand for meat and dairy products is going up," said Patrick Brown, founder of one such startup, Impossible Foods, based in Redwood City in the heart of the Silicon Valley. It raised US$75 million to develop plant-based meat and cheese imitations (*The Economist*, 2015c).

According to the United Nations data livestock uses *ca*. 30% of the world's ice-free landmass and produces 14.5% of all greenhouse-effect emissions. Making meat also requires supplying animals with vast amounts of water and feed: in the United States, producing 1 kg of live animal weight typically requires 10 kg of feed for beef, 5 kg for pork and 2.5 kg for poultry. Yet between now and 2050, the world's population is expected to rise from 7.2 billion to over 9 billion people – and the consumption of meat to grow along with it. It is a big challenge, but also an economic opportunity: "Anytime you can find a way to use plant protein instead of animal protein there is an enormous efficiency in terms of the energy, water and all sorts of other inputs involved – which translates at the end of the day into saving money," stated Ali Partovi, a San Francisco-based entrepreneur and investor in technology startups, such as Dropbox and Airbnb, as well as half-a-dozen sustainable food companies (*The Economist*, 2015c). The

problem or difficulty is that many people shun vegetables and prefer to eat meat or dairy products. The solution is therefore to mimic the taste of meat and other animal-derived foods with plants and take the animal out of the equation. This has become a reality. In addition to Impossible Foods, Beyond Meat, which makes plant-based chicken strips and beef "crumbles", is selling its products in stores. As is Hampton Creek whose eggless mayonnaise has become a bestseller at Whole Foods Market, a big American chain (*The Economist*, 2015c).

### *Addressing wider population groups than vegetarians*

Food giants already offer a variety of meat and dairy alternatives that many vegetarians and vegans buy. What is different with this new approach is that the startups are not targeting the small percentage of the population who largely live on a plant-based diet already. They target people who like to consume meat and dairy products, and that means replicating the meaty, cheesy or creamy flavours and textures that so many people crave. This is also different from making meat in a laboratory using tissue engineering, which involves culturing cells taken from live animals and creating muscle. Modern Meadow, a New York company, is working on this technology (see p. 368). The business has attracted a fair share of famous venture-capital firms and investors, including Kleiner Perkins, Google Ventures, Andreessen Horowitz, Khosla Ventures, Bill Gates and others. If the companies they are backing succeed the returns could be massive. The United States beef industry alone is worth US$88 billion. And even for condiments, such as mayonnaise, the market value totals US$2 billion. But these are high-risk endeavours and some of them might fail, cautions Michael Burgmaier of Silverwood Partners, an investment bank involved in dozens of food and beverage deals. The basic question is, he said: "Is the consumer ready for some of these products?" (*The Economist*, 2015c).

Patrick Brown of Impossible Foods thinks they are. He is the inventor of a DNA chip now widely used in gene-expression analysis and his firm has been developing meat and cheese imitations from plants for three years. For meat the aim is to recreate its key components (muscle, connective and fat tissue), using suitable plant materials. The company first product, a hamburger patty, already looks and cooks like meat, and will taste as good or better by the time it reaches the shops, P. Brown promised. To do this he had assembled a team largely made up of molecular biologists and biochemists, as well as some physicists; only a few members of his staff had a background in food science or had culinary training. In the company laboratories scientists break down plant materials and extract individual proteins with functional properties that can, for instance, make foods firm up or melt down during cooking or baking (*The Economist*, 2015c).

The company Impossible Foods also spent a lot of time working out what gives meat its unique flavour. According to P. Brown, the secret to a burger taste is hem, a compound found in all living cells, e.g. in hemoglobin or chlorophyll. It is especially abundant in blood and muscle tissues as myoglobin. It also gives a burger its red colour. During the cooking process hem acts as a catalyst that helps transform the amino-acids, vitamins and sugars in muscle tissue into numerous volatile and flavourful molecules, he explained. To create the meaty flavour in its burger patties the company uses a hem

protein equivalent to one found in the roots of legumes (leghemoglobin). In terms of nutrition the patty protein content may be slightly higher than that of a conventional burger and have at least as many micronutrients. Because it is made from plants it will not contain any traces of antibiotics, hormones or cholesterol. The company hoped to start selling its burger before the end of 2015 (*The Economist*, 2015c).

### *Achieving the flavour qualities in the new foodstuffs*

Beyond Meat, based in Southern California, was also studying the components of meat to emulate its texture and flavour. The firm's flagship product, Beyond Chicken Strips, has been on sale since 2013 and has a surprisingly authentic feel when eaten. When several Whole Foods Markets accidently sold mislabelled chicken salads with the company plant-based strips, there were no complaints. Only when an employee discovered the mix-up after two days were the salads officially recalled. The product texture is the result of years of research at the University of Missouri and it can now be created in a process that takes less than two minutes. An extruder rapidly heats, cools and pressurizes a mixture of protein and other ingredients into a structure that mimics the fibrous tissue of muscle. The company's most recent product, the Beast Burger, was released in February 2015. It has more protein, more iron and is overall more nutritious than actual meat burgers (*The Economist*, 2015c).

But marketing plant-based burgers to carnivores is not easy. Beyond Meat is therefore building the brand with images of vitality, fitness and health. In promotions it is using athletes: David Wright, captain of the New York Mets baseball team, has already signed up. In return he got a small stake in the company. Still under development is what may be Beyond Meat most ambitious product – a raw ground beef equivalent which would be offered in supermarkets meat sections right next to actual beef. Due for release in 2016 it can be cooked and moulded into a meatloaf or meatballs, or even supplied to fast-food chains to make burgers (*The Economist*, 2015c).

San Francisco-based Hampton Creek has replaced eggs with plant proteins in the products it has released so far. Its Just Mayo and Just Cookie Dough were distributed in 2015 in 30,000 stores, including Kroger and Walmart. Other items in the works included a ranch salad dressing, a scrambled egg alternative and pasta. The goal is to create products that make it easy for people to choose sustainable plant-based over conventional items. "Change happens by making something so delicious and so affordable, everyone chooses it," stated the firm CEO, Josh Tetrick. To accomplish this Hampton Creek assembled a team that includes experts in biochemistry, bioinformatics and food science along with a number of chefs. Scientists extract and isolate proteins from plant materials and conduct basic biochemical studies in order to understand their characteristics and possible applications for a variety of foodstuffs. The promising ones are tested in recipes in the company's bakery and culinary sections to see how they perform (*The Economist*, 2015c).

By 2015 Hampton Creek had analyzed more than 7,000 plant samples and identified 16 proteins that might prove useful in food applications. Several are already being used in its commercial food products, including a type of Canadian yellow pea in its

mayonnaise. The team of scientists are looking for proteins with functional properties such as foaming, gelling and moisture retention. Mayonnaise, for instance, requires a substance that binds the right amount of oil with water to create a stable emulsion. For its version in stores the company tested more than 1,500 different formulations. Dan Zigmond, the former lead data scientist for Google Maps and now Hampton Creek vice-president of data, was in charge of simplifying the process of finding useful proteins. There are an estimated 400,000 plant species in the world, each of which may have tens of thousands of proteins. To search this vast number more efficiently his team are feeding data the company has already gathered into machine-learning models, which are designed to predict which types of proteins could be useful in specific food applications without having to go through all the biochemical tests (*The Economist*, 2015c). In December 2014 Hampton Creek announced its latest funding round of US$90 million, bringing its total raised to US$120 million. The company has been successful with the products it already sells. However it is not trying to make a burger patty from scratch with plants, as Impossible Foods is trying to do, and it was expected to release a scrambled-egg replacement (*The Economist*, 2015c).

Perhaps the most radical approach to disrupting the food industry comes from Soylent, whose beverage is designed to be a complete substitute for food and not just one of the many diet drinks or nutritional supplements. Sold as a powder to be mixed with water it contains all the ingredients needed for sustenance, stated Rob Rhinehart, Soylent founder. It also eliminates the need for planning meals, cooking and cleaning afterwards. The name originates from the science-fiction novel *Make Room*! *Make Room*! in which people in an overcrowded, apocalyptic world, live on foods made of soy and lentils. The company moved from the San Francisco area to Los Angeles in late 2013 in search for cheaper office space. Some users of the first version of the beverage complained of flatulence because of the high fibre content. That drawback was largely addressed by changing the carbohydrates blend and adding some digestive enzymes. Soylent 1.3 has a smoother texture than the original, a more neutral taste and its omega-3 fatty acids come from algae as opposed to fish oil (*The Economist*, 2015c). As of mid-February 2015 Soylent had a four-to-five-month backlog for new orders. Customers subscribe online to receive monthly shipments with a "meal" costing *ca*. US$3. According to Rob Rhinehart, who himself uses Soylent for *ca*. 80% of his dietary needs, his company was already profitable and used a US$20-million cash infusion to expand production and sales (*The Economist*, 2015c).

## *Cultural hurdles*

Not everyone may want to separate eating into utility versus pleasure. P. Brown of Impossible Foods does not believe a compromise is necessary: "I do not see any reason why you cannot have it all – the best tasting food, healthiest, best for the planet and most affordable." But even if the scientific hurdles of making plants taste like meat and other animal-based products overcome, the bigger obstacle these companies face may be cultural. People have been eating meat and having meals together for thousands of years. Meat in particular is not only prized for its taste but also perceived as a force of vitality, strength and health. A study by the Humane Research Council, an

animal advocacy group, stated most vegetarians and vegans, about 2% of American population, go back to eating meat eventually. In the future that may not be an option. Whether out of necessity or choice Silicon-Valley food-biotechnology startups' vision of a big shift to plant-based foodstuffs may be inevitable (*The Economist*, 2015c).

## Role of intestinal microflora

Karine Clément, a professor of nutrition and director of the Institute for Cardiometabolism and Nutrition (ICAN, French acronym) at La Pitié-Salpêtrière hospital in Paris, has made an outstanding contribution to understanding the role of intestinal microflora in cardiovascular and metabolic diseases, including obesity. She published the results of her research work in *Nature* on 29 August 2013 (Le Chatelier et al., Clément and Ehrlich, 2013). In November 2014 she was awarded, with Dusko Ehrlich, professor at the National Agricultural Research Institute (INRA), the Health Prize of the French magazine *La Recherche*. K. Clément is the coordinator of an international research consortium called Metacardis – a European project initiated in 2012 – at the INSERM. Some 2,000 patients were involved in the studies carried out by K. Clément and the consortium (Santi, 2014a).

The intestinal microflora or microbiota is considered like an organ and is made of one hundred thousand billions of bacteria, i.e. one hundred times the number of human body's cells. Those persons who have a less diversified intestinal microflora are more susceptible to metabolic diseases, such as diabetes, obesity, liver dysfunction, blood anomalies and cardiovascular illness, and they tend to put on weight. The sequencing of the genomes of these bacteria has shown that the more diverse the microflora, the lower risk for metabolic diseases. Trials were carried out in rodents and humans regarding transfers or grafts of intestinal microflora, with a view to understanding the role of this microflora in the etiology of diseases, and particularly of obesity (Santi, 2014a).

K. Clément underlined the fact that obesity is a multifactorial disease. This was the approach she wanted to develop when she was an intern, by the early 1990s, in the medicine and nutrition department of Paris Hôtel-Dieu hospital, under the leadership of Bernard Guy-Grand and Arnaud Basdevant. "These two physicians have invented the medicine of obesity, with a psychological and environmental dimension; it was not just a question of food intake, but the lifestyle and history of people are as important as the biological approach," narrated K. Clément. "At that time weight problems were approached in a simplistic way, you are overweight, you eat too much – and this is still true among some practitioners. But in fact it is a much more complex issue," she added (Santi, 2014a).

Along with her medical internship K. Clément prepared in 1992 a Master degree in diabetes genetics, then a PhD at the Centre for the Study of Human Polymorphism (CEPH, French acronym), whose directors were Daniel Cohen and Philippe Froguel at Saint-Louis hospital in Paris. She reckoned she had been lucky to be part of the team which discovered one of the first genes involved in diabetes. The team showed that three obese young women belonging to the same family had blood levels of

leptin (a hormone that regulates satiety) six to ten times higher than those observed in individuals of similar body weight. Genetic anomalies inhibit the leptin receptor and hinder the biological role of the hormone. One of the three young women who had been tested was a patient treated by K. Clément. The discovery of genes involved in rare forms of obesity was published in *Nature* (Clément et al., 1998).

In 2002 K. Clément, after having been distinguished by the INSERM *Avenir* (Future) programme, identified with Jean-Daniel Zucker – an engineer working on the analysis of complex data – the role of inflammatory phenomena is the fatty tissue of obese people. In 2010 her project to create the ICAN was approved. She now coordinates within this institute 14 research teams, with a total of 120 researchers. The objective of the whole programme is to associate research with clinical work (70,000 patients are annually seeking medical consultations at La Pitié-Salpêtrière hospital departments of obesity, nutrition, diabetes and cardiology). Transversal team work is precisely needed for better understanding the interactions occurring in tissues, cells and organs, and therefore the etiology of multifactorial diseases such as obesity (Santi, 2014a).

Nestlé, the Swiss giant agrifood company with €80.35 billion in annual turnover, has invested in the knowledge of microbiota (intestinal microflora). Since 2015 "the group was engaged in a partnership with the American biotechnology company Seres Therapeutics," reports Greg Behar, director-general of NestléHealthSciences, the subsidiary that deals with the prevention of ageing and nutritional therapies. At the beginning of 2016 this partnership yielded US$120 million (or €107.79 million) to Nestlé. On 20 April 2016 the Swiss company participated in raising funds for Enterome, a French biotechnology startup, for a total of €14.5 million. This startup was founded by Pierre Béléchard in order to develop diagnostic tools and treatments for the Crohn disease – a chronic inflammation of the digestive tube (Garnier, 2016).

## Antiobesity drugs

On 19 December 2014 the European Medicines Agency (EMEA) authorized the commercialization of a new antiobesity drug, called Mysimba, produced by Orexigen Therapeutics, an American biotechnology company based in La Jolla, near San Diego, California. This is the only drug of this startup whose stock equity was estimated at more than US$700 million (or €572.4 million). Mysimba contains two active principles: naltrexone which is utilized in the treatment of alcohol and opium-derived drugs addiction; and bupropion that is prescribed as an antidepressant drug and also to help people stop smoking. After several years of hesitation due to the likely occurrence of heart attack the FDA approved the commercialization of Mysimba in September 2014. In its press release the EMEA indicated that Mysimba will be available only through prescriptions for obese or overweight adults, in so far as they have other risk factors like hypertension or a high amount of cholesterol. The EMEA recognizes that "uncertainties still remain regarding the long-term impact on the cardiovascular system." Consequently the patients treated with Mysimba should be examined by their physicians after 16 weeks and the treatment should be terminated if they had not lost at least 5% of their initial weight (Hecketsweiler, 2014k).

In 2012 the EMEA had rejected the request for commercialization of another antiobesity drug, called Qsiva, produced by the American firm Vivus, because of its long-term cardiovascular side-effects. In France one single antiobesity drug and treatment are authorized: Xenical, produced by the Swiss pharmaceutical group Roche. It contains orlistat, a substance that limits the absorption of lipids, and it is delivered only through medical prescriptions. The commercialization of another treatment/drug also containing orlistat and called Alli, that had been sold without prescription (over the counter) since 2009, was suspended by its manufacturer GSK in 2012. According to the data provided by Celtipharm, Alli has been popular when launched, with more than 186,000 boxes sold in May 2009; thereafter sales have steadily declined. In three years less than 900,000 boxes were sold. Xenical followed the same curse: in May 2008 220,000 boxes used to be sold per month, while five years later less than 50,000 boxes were being sold (Hecketsweiler, 2014k). But the most striking failure was that of Acomplia, a drug launched by Sanofi in 2006. It was expected that its sales would reach billions of euros, but it was withdrawn from the market in 2008 upon the request of the EMEA. The data reviewed by the EMEA showed that Acomplia duplicated the risks of depression, compared with a placebo. These side-effects are certainly related with Acomplia action on the brain's cannabinoid-receptors (Hecketsweiler, 2014k).

With respect to the authorization of Mysimba the French independent journal *Prescrire* ("Prescribe") deplored the decision made by the EMEA regarding a drug that contains bupropion, a molecule that is close to amphetamins. "The health authorities should draw the lessons of past health disasters, particularly of those concerning antiobesity drugs (which reduce the patients' appetite) that were thereafter withdrawn from the European market because of serious undesirable effects," underlined the journal. The latter recalled the scandal of Mediator (benfluorex, also a molecule close to amphetamin) – an antidiabetes drug widely prescribed because of its anti-appetite properties, before being withdrawn from the market in 2009. Mediator which causes serious lesions in heart valves may be responsible in the long term for 2,100 deaths, according to a judiciary expertise (Hecketsweiler, 2014k). *Pharmacovigilance* is here of major importance because the pharmaceutical groups are very much interested in the huge market of antiobesity drugs and will continue to search for new treatments, despite past and current setbacks. According to the United States Centers for Disease Control and Prevention (CDCs) – the equivalent of the French National Institute for Health Vigilance (*Institut national de veille sanitaire*) – 80 million adults were obese in the United States in 2014, i.e. *ca*. one-third of the whole population. The cost for the health national system was estimated at US$147 billion (or €120.2 billion) in 2008 (Hecketsweiler, 2014k).

# NEURODEGENERATIVE DISEASES

## Alzheimer's disease: the hope for more efficient drugs

An experimental drug for Alzheimer's disease is being developed by Biogen IDEC, an American company created in November 2003 as the merger of two global biotechnology leaders, Biogen Inc. and IDEC Pharmaceuticals corporation. It is based in Cambridge, Massachusetts. In a phase-1 clinical trial, designed to look at safety of the drug (not the effect on cognition), it was shown that the drug sharply slowed the decline in mental function. The report by Biogen researchers on 20 March 2015 revived hopes for an approach to therapy that experienced up to that date repeated failures. The drug called aducanumab (and before known as BiiBo37) could lead to sales of billions of dollars a year if the results from the small-trial users were replicated in large trials that Biogen IDEC hoped to begin in 2015. Aducanumab is an antibody that binds to, and may reduce beta-amyloid plaques in the brain, that are widely believed a cause of the dementia in Alzheimer's disease. However other drugs designed to prevent or eliminate plaque have failed in large trials, raising questions about what role the plaque really plays. Johnson & Johnson and Pfizer abandoned a drug they were jointly developing after it showed virtually no effect in large trials. Eli Lilly and Roche, on the other hand, are continuing to test their respective drugs despite initial failures. Researchers stated that the drugs might work if used early enough, when the disease's symptoms are still mild (Pollack, 2015c).

Biogen IDEC tried to increase its chances of success by treating patients with either mild disease or so-called prodromal disease, an even earlier stage. It also enrolled only patients shown to have plaque in their brains using a new imaging technique. In some trials of other drugs some of the patients turned out not to have plaque which could have been one reason the trials were not successful. The results reported on 20 March 2015 were for 166 patients, who were randomly assigned to receive one of several doses of the drug or a placebo. The drug both reduced plaque and slowed cognitive decline, and higher doses were better than lower doses. That is a sign that the effects seen were from the drug (Pollack, 2015c). On one measure of cognition, a 30-point scale called the mini-mental state exam or MMSE, those receiving the placebo worsened by an average of 3.14 points over the course of a year. The decline at one year was only 0.58 point for those receiving the highest dose and 0.75 point for a middle dose. The difference with a placebo was statistically significant for both doses. The difference with placebo exceeded 2 points that some analysts had said would represent a good result. On another measure of both cognitive and

the ability to function in daily tasks, patients in the placebo group worsened by an average of 2.04 points at one year. Those receiving the highest dose of the drug had a decline of only 0.59, a statistically significant difference (Pollack, 2015c).

A major side-effect of the drug was a localized swelling in the brain, known as ARIA-E. This has been seen with other drugs in this class, although the rate for aducanumab seemed higher. However both Rachelle S. Doody, director of the Alzheimer's Disease and Memory Disorders Center at Baylor College of Medicine, Houston, and Samuel Gandy, director of the Center for Cognitive Health at Mount Sinai Hospital in New York, stated that the swelling often does not cause symptoms and probably can be managed by watching for it and reducing doses. There were not discontinuations from this side-effect among patients taking a middle dose. And that middle dose also seemed somewhat effective in slowing cognitive decline. These results were presented in Nice at the 12th International Conference on Alzheimer's and Parkinson's Diseases and Related Neurological Disorders (AD/PDTM, 18-22 March 2015) [Pollack, 2015c].

There has been a lot of excitement, among researchers and journalists alike, in the lead-up to a lecture given on 22 July 2015 at the Alzheimer's Association International Conference (AAIC), in Washington, D.C. The lecture was entitled "Delayed-start analyses of up to 3.5 years in the phase 3 solanezumab expedition program in mild Alzheimer's disease," and was presented by Hong Liu-Seifert, Pail S. Aisen et al. That meant that the researchers presenting the paper, who work for Eli Lilly (Liu-Seifert et al., 2015) and at the University of California, San Diego, La Jolla (Paul S. Aisen), thought they had come up with something which slows down the illness progression. This is another monoclonal antibody, called solanezumab by its inventors, that sticks to beta-amyloid plaques. When Eli Lilly tested the drug in 2012 they found little evidence of success – except in those with mild, early-onset Alzheimer's, for whom there were hints that the progression of the disease had been slowed. But by extracting this group of patients from the rest and concentrating on them the firm scientists discovered something more hopeful. The 1,322 qualifying patients were divided into two groups. One lot were put on solanezumab immediately. The others were given a placebo for the trial first 18 months, and thereafter switched to solanezumab which they have been taking for two years (by mid-July 2015). In cognitive tests that use a quantitative scale of dementia effects, those in the delayed group fell behind the others in the months when they were on the placebo. Once they switched to the drug their rate of decline slowed to match that of those who had been on treatment since the beginning. The monoclonal antibody appeared therefore to be slowing the disease progress (Liu-Seifert et al., 2015; Siemers et al., and Liu-Seifert, 2016). This result which is not a cure, may, however, point the way to one: perhaps a different antibody, or a combination, would have a greater effect (*The Economist*, 2015g).

## Multiple sclerosis

In the field of neurology multiple sclerosis is a peculiar disease. According to Jean Pelletier, of the university hospital la Timone in Marseille, this "disease is an area of very significant change in the neurosciences; its treatment has been drastically modified by new diagnostic and therapeutic tools." His colleague, Catherine Lubetzki of La Pitié-Salpêtrière hospital in Paris, could not agree more. In 2014-2015 new treatments administered orally are available, while several clinical trials will be initiated with a view to repairing the myelin that wraps nervous fibres (Rosier, 2014b).

## *A chronic disease, difficult to predict and to treat*

Considered as the second cause of invalidity or handicap in adults (after casualties), multiple sclerosis has many symptoms, that vary from one patient to another, and even in the same patient over time. In France the estimates of the number of patients suffering from this neurological disease were *ca*. 80,000 in 2014. It is distinguished from the other diseases of the nervous system, due to the unique conjunction of three factors. First, because it is diagnosed between 20 and 40 years in 75% of the cases: "this disease appears at the beginning of the adult period and has therefore an impact on all living projects of the patients, e.g. weddings, pregnancy, first employment or job, first loans to buy an apartment," testifies Caroline Papeix, a neurologist at Paris La Pitié-Salpêtrière hospital and author of the book *La sclérose en plaques : S'informer pour mieux se soigner* (Multiple Sclerosis. To Be Informed in Order to Be Better Treated, 2011, Odile Jacob ed., Paris, 160 pp.). The second factor is that multiple sclerosis can benefit from a broad range of diagnostic and therapeutic advances; in addition to the immunomodulators (beta-interferons and copolymers) physicians can use new more effective treatments, but we lack data on the innocuity of these new drugs in "real life" and among a large population of patients. The third factor that makes multiple sclerosis singular is its uncertainty. "Despite these major advances, we have to deal with a chronic disease for which we have no means to predict, individually, the reaction to the treatment, nor the long-term prognosis," stated Olivier Lyon-Caen, a neurologist at La Pitié-Salpêtrière hospital (Louapre et al., and Lubetzki, 2013; Rosier, 2014b).

In multiple sclerosis myelin – which normally accelerates the nervous influx and protects the neuron – is attacked by the patients' immune cells. This destruction is followed by the formation of "plaques" in the brain and spinal cord. When these plaques affect the optic nerve, vision can be impaired. When the spinal cord is concerned the patient feels a weakness or fatigue in his/her muscles, pins and needles in the arms and legs. "This is a polymorphic disease per excellence," wrote Jean-Martin Charcot in 1868 at La Pitié-Salpêtrière hospital; he was one of the first neurologists to describe the disease (Rosier, 2014b). In 85% of the cases the disease progresses through outbreaks: the patient shows symptoms that last for a few days or weeks, then spontaneously disappear, leaving a residual handicap in one-third of the cases. Severe outbreaks are treated with corticoids. In 15% of the cases the disease becomes more serious progressively, without outbreaks. There are also benign forms of the disease. Further to the advances in brain imagery via magnetic resonance multiple sclerosis can be diagnosed in one single test. "This results in saving two years in the treatment of the illness, compared with the 2000s, and therefore in proposing an earlier therapy," underlined Jean Pelletier. "The magnetic resonance imagery can also accelerate the development of candidate drugs, thanks to evaluating their effects on the number of plaques …" "The plaques that result from the destruction of myelin are just the tip of the iceberg. Non-conventional magnetic resonance imagery shows the submerged part, i.e. reveals that multiple sclerosis is a diffuse disease of the nervous system," added J. Pelletier, whose centre, in Marseille, was the only one in 2014 to use magnetic resonance imagery with sodium (Dubois-Dalcq et al., and Lubetzki, 2008; Rosier, 2014b).

## *Therapeutic means: a pluridisciplinary approach*

Immunomodulators are "first front" treatments which are administered via injections. Two new drugs, natalizumab (injected) and fingolimod (taken orally) have become available in France since 2007 and 2011, respectively. These are "second front" treatments: more active for the prevention of outbreaks, they also have a risk of potentially serious secondary effects. They are used in the severe forms of the disease. These long-term treatments are effective in preventing the handicap that grows with the outbreaks. But there is no randomized study which would enable the researchers to be sure that these treatments reduce the handicap in the long-term. In the fall of 2014 two new "first front" treatments were expected to become available: teriflunomide and fumaric acid, to be taken orally. Another long-term drug, alemtuzumab, received by the end of 2013 the authorization to be commercialized in the European Union, as a "second-front" treatment. However the FDA refused to give such an authorization because of the high risk of serious secondary effects (Louapre et al., and Lubetzki, 2013; Rosier, 2014b).

In 2011 a French Observatory for Multiple Sclerosis was set up: 40,000 French patients are being monitored with respect to their treatments so as to compare the efficacy of the latter. Research is also focused on the repair of the destroyed myelin. "Around the plaques formed after the destruction of myelin, there exist precursors of cells that synthesize myelin. And we have identified several pathways related with the migration of these cells towards the lesions as well as with their capacity to regenerate the destroyed myelin," indicated Brahim Naït-Oumesmar of the Brain and Spinal Cord Institute (National Institute for Health and Medical Research, INSERM, Paris La Pitié-Salpêtrière hospital). A very first clinical trial on the regeneration of myelin was being carried out in 2014; it was using an antibody, called anti-Lingo, targeted against a molecule that inhibits the repair of myelin. Several hundred patients were involved in four trials concerning the repair of myelin, according to Catherine Lubetzki (Tepačević et al., and Nait-Oumesmar and Lubetzki, 2014). The congress BRAINS, organized by mid-February 2014 by Novartis, summarized the data relating to these trials and, in particular, highlighted the role of microglial cells – immune cells which play an active role (that has been neglected until then) in the irreversible lesions of the axon (Rosier, 2014b).

The pathway of stem cells is also explored: in 2013 the team of Steve Goldman published the results of their experiments on the brain of mice suffering from a myelin disease; they grafted cellular precursors of oligodendrocytes which produced myelin. The grafted cells originated from human pluripotent cells. There is still this "new frontier" to get through: the progressive forms of multiple sclerosis against which current medicine cannot do much. "An international consortium was created in order to find ways to control these progressive forms of the disease," indicated C. Lubetzki (Piaton et al., and Lubetzki, 2009). A few trials were initiated: in the 18 March 2014 issue of *The Lancet* a small study suggested that a statin may improve the patient's capacity of movement (Chataway et al., 2014). On 5 April 2014 a foundation called *Aide à la recherche sur la sclérose en plaques* (ARSEP, French acronym for Support for Research on Multiple Sclerosis) organized a meeting of patients, physicians and researchers,

with a view to reviewing the progress made and also the treatments' limitations. One thing is certain: the treatment of multiple sclerosis must be multidisciplinary; in other words the patients have to be followed by a team of physicians who monitor the effects of the various therapies or approaches. For instance symptomatic treatments that help patients in their daily life should not be neglected, e.g. a new drug, fampridine, that increases the walking distance of certain patients; or Sativex, made of cannabis, available in 2015, against muscular cramps (Rosier, 2014b).

Another enigma of multiple sclerosis that challenges neurologists is why the disease affects women more than men. In the 1950s the sex ratio was two women for one man; nowadays it is three women or even four women for one man. Besides several assumptions regarding the role of genetic and environmental factors, there is a controversial explanation: oral contraception. It is known that pregnancy protects against multiple sclerosis outbreaks, while post-delivery reactivates them among one-third of the patients. However all the studies carried out on the possible role of contraception have been negative. But an American study presented at the end of April 2014 to the congress of the American Academy of Neurology, suggested a 35% increase in the risk of developing multiple sclerosis under oral contraception; this result is to be taken with great caution, according to Thibault Moreau of the university hospital in Dijon, centre-east of France (Rosier, 2014b).

## Prions

Stanley Prusiner who discovered the prions had to struggle against at least three dogmas which were taught to generations of students. An infectious pathogen should belong to one of the following categories : viruses, bacteria, fungi or parasites. It must contain genetic material, DNA or RNA. And the three-dimensional structure of a protein is dictated by the sequence of its amino-acids. The prions indeed do not respond to these three assertions. They are made of a protein having an abnormal structure and therefore they become pathogens causing neurodegenerative diseases such as Creutzfeldt-Jakob disease (CJD), "madcow disease" or bovine spongiform encephalitis (BSE). In Parkinson's and Alzheimer's diseases abnormal proteins causing brain degenerescence are also found. Stanley Prusiner received in 1994 the Albert-Lasker Prize for Basic Research in Medicine and the Nobel Prize for Medicine or Physiology in 1997 (Benkimoun, 2015c).

To his opponents who considered him as heretic he methodically brought the evidence through his publications that the pathogen which was searched was a protein and not a virus of a new kind, as he himself thought for some time. He mentions in his autobiography published in 2014 by Yale University Press, *Madness And Memory: The Discovery of Prions – A New Biological Principle of Disease*, the "human weaknesses" that were at the origin of the attacks against him and the uncivilized behaviour of his opponents. For instance Carleton Gajdusek, author of a pioneering work on kuru, a spongiform encephalopathy identified among tribes of Papua-New Guinea and probably caused by the consumption of human brains, claimed that he was the discoverer of prions. S. Prusiner recalled that his colleague was known for this kind of claims and behaviour. In fact the world of science is not free from jealousy. Stanley

Prusiner commented: "In the business world, when you make a breakthrough, all those who have shares or stock options become richer. In the realm of science, when somebody makes a breakthrough, five others feel belittled." Regarding the media he acknowledged the positive attitude of, for instance, Lawrence Altman, a physician and journalist writing in the *New York Times*, while in the same newspaper Gina Kolata supported those who doubted about S. Prusiner's results (Benkimoun, 2015c).

Stanley Prusiner is born on 28 May 1942 in Des Moines, Iowa. His relatives from the father and mother side had to leave Russia because of antisemitism and measures against the Jews, and to emigrate to the United States. At the University of Pennsylvania Sidney Wolfson persuaded him to engage in medical studies. He moved to the University of California, San Francisco. He worked at the National Institutes of Health (NIH), Bethesda, Maryland, and in September 1972 (he has been already working for three years at NIH) he was called to examine a patient who later on was diagnosed with Creutzfeldt-Jakob disease (CJD). That was the beginning of his research work, starting with mice which were used as models for studying scrapie (another disease caused by prions in sheep). Although many of his colleagues tried to discourage him S. Prusiner stubbornly carried out his research. He said he was lucky, because who makes an important discovery is not the one who was expected to do so. "I call that the Obama effect, while referring to his victory over Hillary Clinton during the Democrat primary elections in 2008," S. Prusiner remarked with amusement (Benkimoun, 2015c).

S. Prusiner also had an excellent intuition that contributed to the success of his theory: giving a very appropriate name to the protein he discovered. From the two key words, "protein" and "infection", he designed the word "prion", which was widely accepted and used. His objective now is to develop a treatment against the diseases caused by prions, because unfortunately the majority of drugs against neurodegenerative diseases did not provide a cure. S. Prusiner is collaborating with the Japanese pharmaceutical holding Daiichi Sankyo in order to try to meet this long-term challenge. He also expressed his concern about the difficulties encountered by young researchers in funding their work: "We, the scientists, are unable to build up a system where the best young researchers can be recruited and their work funded. At the end of the day to carry out research depends less on the quality of the scientist than on his/her capacity to find a source of funding" (Benkimoun, 2015c).

# HUMAN REPRODUCTION MEDICINE

## Contraceptives for the developing world

### *Birth-control pill*

Scientists had long known that high concentrations of estrogen and progesterone inhibited ovulation. But synthesizing them from animal or plant extracts had proved expensive and ineffective for use as oral contraceptives. The synthesis by Carl Djerassi and his colleagues, George Rosenkranz and the student, Luis E. Miramontes, was economical and effective for oral use. All three names went on the patent (McFadden, 2015).

Carl Djerassi who died in San Francisco at the beginning of 2015 at the age of 91 was working in 1951 at a small pharmaceutical laboratory in Mexico City, where he first synthesized a progestin, called norethindrone, which became the key ingredient for the oral contraceptive known as "the pill". It was on 15 October 1951 – one of those dates recorded for posterity – a year before others developed similar compounds in other laboratories. Carl Djerassi arrived in America as World War II engulfed Europe, a 16-years-old Austrian Jewish refugee who, with his mother, lost their last US$20 to a swindling New York cabdriver. He wrote to Eleanor Roosevelt, asking for assistance, and obtained a college scholarship. Carl Djerassi became a brilliant researcher who taught at universities for five decades, wrote books, plays and 1,200 scientific articles. He obtained a patent on the first antihistamine. Working on human fertility the team that synthesized norethindrone had to carry out five years of trials to demonstrate the safety of the chemical compound and its effectiveness in inhibiting pregnancy. Even then drug companies were reluctant to market the pill, fearing boycotts of their products by religious groups and others opposed to birth control (McFadden, 2015).

In the 1960s, however, the pill – based also on pioneering work by M.C. Chang, Gregory G. Pincus, John Rock and others, and technically known as the combined oral contraceptive pill – was developed and marketed by various drug companies. They included Syntex, where C. Djerassi and his colleagues had worked. The use of the pill spread rapidly, producing vast economic and social effects. It gave women an unprecedented control over fertility, separating sexuality from procreation. It let couples plan pregnancies and regulate family size, and women plan education and careers. It also generated debates over promiscuity and the morality of birth control. The Roman Catholic Church, in particular, emphasized its bans on artificial contraception (McFadden, 2015).

The pill made C. Djerassi wealthy and something of a celebrity as he moved through a series of careers as a professor of chemistry, an insect-control entrepreneur, an art collector, a rancher, an author of science novels and non-fiction books, a poet, a playwright and the founder of an artists' colony in California. "Yes, I am proud to be called the father of the pill," he told Nicholas Wroe of *The Guardian* in 2000. "But identifying scientists is really only a surrogate for identifying the inventions or discoveries. I am certain that if we did not do our work, then someone else would have come along shortly afterwards and done it," (McFadden, 2015).

Carl Djerassi was born in Vienna on 23 October 1923. His parents were physicians. His father specialized in treating venereal diseases before penicillin. He emigrated to the United States in 1939, settling in upstate New York. He attended Tarkio College in Missouri, then earned a bachelor's degree with honours in chemistry at Kenyon College in Ohio in 1942, when he was not quite 19. In 1945, he earned a doctorate at the University of Wisconsin and became a naturalized American citizen (McFadden, 2015).

## *A contraceptive for the developing world*

Depo-Provera, an injectable contraceptive given once every three months, is a popular choice of women in developing countries who value the convenience and discretion of not having to take a daily birth-control pill. But the injections are out of reach for many more women because they live in rural areas that are too far from a health clinic to make the treatments practical. A major collaboration between Pfizer and several global aid groups is aiming to change that by providing funding to make a new version of the drug – redesigned with developing countries in mind – available to 69 nations throughout Africa, Asia, Latin America and Eastern Europe. The new product, called the Sayana Press, is a single-use syringe designed to be portable and easy to use. Depo-Provera is typically injected into the muscle by health-care workers who must first draw the drug into a syringe from a glass vial. The new product was reformulated into a lower dose and used an existing device, called a Uniject system, that looks like a plastic bubble with a short needle attached. It is injected under the skin by squeezing the bubble and can be administered with minimal training (Thomas, 2014).

The product is already being used in several African countries, but Pfizer stated it planned to expand distribution through a financial partnership that would allow the product, which typically costs *ca*. US$1.50 a dose, to be sold to health-care institutions in those countries for about US$1. Several aid groups, including the Bill and Melinda Gates Foundation and the Children's Investment Fund Foundation, as well as the United States Agency for International Development (USAID), will help subsidize the cost and assist in introducing the drug in countries around the globe. Women will most likely receive the product free or at a reduced cost. Chris Elias, president for global development at the Bill and Melinda Gates Foundation, stated the Sayana Press could be an important new choice for the estimated 225 million women worldwide who would like access to contraception but do not have it. "Family-planning is an important priority for us and this is expanding the range of methods," he stated (Thomas, 2014).

Pfizer declined to comment on the revenue it expected to generate from sales of Sayana Press, but John Young, president of Pfizer Global Established Pharmaceuticals, said it was not seen as a major revenue driver for the company. Depo-Provera sells to health institutions in those countries for about UScents75 a dose. Amitasrigowri S. Murthy, an assistant professor at New York University Langone Medical Center in the department of obstetrics and gynecology, did not work on the project, but stated the Sayana Press carried advantages because injecting Depo-Provera into muscle requires the skills of a knowledgeable health-care worker. With the new product "you press it and it injects it," she said. "It is similar to insulin" (Thomas, 2014). Fiona Walugembe who is overseeing the introduction of the Sayana Press in Uganda through the Program for Appropriate Technology in Health (PATH), funded by the Bill and Melinda Gates Foundation, stated that women were eager to hear about the new product. About a third of Ugandan women have no access to family-planning options, she underlined. Many women like Depo-Provera because they do not have to tell their husbands that they are using a contraceptive. Now they will not have to make a long trip four times a year to receive the injections (Thomas, 2014).

## Oral contraceptives and cancer

Since their commercialization in the early 1960s (they were authorized in France in 1967) oral contraceptives have markedly contributed to medical advance: the remarkable efficacy of the "pill" resulted in avoiding non-desired pregnancies and in providing a solution to the scourge of clandestine abortion. There has been also a social and anthropological progress, because, thanks to the pill, women could separate sexuality and reproduction, and make decisions about when and how many pregnancies they will have. In France, for instance, oral contraceptives have become in 50 years the preferred contraceptive means of women: 70% of women under the age of 35 years using contraception had adopted the oral pill, according to a survey made in 2011, and one woman out of two between 15 and 49 years of age in 2010. Since 2013 this proportion has decreased down to 41% of women between 15 and 49, further to the polemic on the pills of the third and fourth generation.

Those called of second generation are pills that contain a different progestative compound. These "minidosed" pills were marketed in a very aggressive way by the pharmaceutical groups, but they were not more efficient than the former ones and had more side-effects, e.g. risks of stroke and thrombophlebitis. The sales of these pills have dropped but that decrease was to a large extent compensated by the sale of pills with higher safety and by other means of contraception (implants and sterilets). The controversy which surged had the merit to recall some facts. Although oral contraceptives are a pharmacological means of prevention of an event which is not a disease, and are prescribed to women in the absence of a pathology, they are nevertheless medicines. As such they have beneficiary effects, but also side-effects or risks.

In 2005, in addition to possible side-effects on the cardiovascular systems, the International Centre for Research on Cancer raised concerns and controversies when it listed hormonal contraceptives among carcinogenic products. The centre experts

considered that oral contraceptives could slightly increase the risk of breast, cervix and liver cancers. By contrast they underlined the protective effect of combined hormonal contraceptives (estroprogestative) against endometrium, ovary and colorectal cancers. A study published on 5 August 2015 by a British team of researchers confirmed the protective role of oral contraceptives against endometrium cancer: after five years of taking the pill, the risk of cancer is reduced by one-fourth and this protective effect lasts at least during 30 years after interrupting the use of oral contraceptives. These are good news that contribute to the necessary information of women who take the pill. It is also good for the new generations who are questioning oral contraception to understand and realize the advantages offered by an efficient means of contraception which, although not being the only means, has brought in a revolution in women's perception of their sexuality and maternity.

## *In-vitro* fertilization with three genetic parents

Every year *ca*. 100 children are born in the United Kingdom with seriously damaged cell mitochondria – the organelles which produce cell energy. Mitochondria are different from other cellular components; they are the distant descendants of free-living bacteria that, in the deep evolutionary past, became symbiotic with the cells they now inhabit. As such they contain their own tiny genomes, separate from the main human genome that resides in the nucleus. Those infants who are born with the mitochondria defect, often die. Those who survive live a debilitating existence in which the tissues of their bodies – brains, muscles, nerves and the rest – are chronically short of energy they need to function. There is no cure. But there might be a way to help the roughly 2,500 women whose future children would be at risk. On 3 February 2015 the House of Commons voted, by 382 to 182, to make the United Kingdom the first country in the world to permit "mitochondrial donation", a form of *in-vitro* fertilization (IVF) designed to ensure that those children can be born free of the condition (*The Economist*, 2015b).

It was a free, unwhipped vote because, despite the potential benefits, the issue is contentious. The procedure involves transplanting the nucleus from an egg cell (ovocyte) with damaged mitochondria into another one ovocyte with healthy ones. That second ovocyte is provided by a donor. A baby created via mitochondrial donation will thus have genes from three persons – its mother, its father, and the women who donated the ovocyte with healthy mitochondria (*The Economist*, 2015b). The promoters of this "IVF with three parents", i.e. the DNA of the nuclei of both parents (the ovocyte and sperm cell) and the mitochondrial DNA of the donor's ovocyte, have underlined the fact that the newborn will have the genetic traits of his father and mother (which are encoded by the nucleus genome/DNA) [Bernard, 2015].

Those who supported the vote by the House of Commons highlighted that their proposal was based on the results of 15 years of research and seven years of consultations. They indicated furthermore that mitochondrial donation will be authorized only when there is a "significant risk" of serious handicap or illness for the child to be born, and after a decision made by an independent authority (Bernard, 2015). There is indeed

a small slice of mitochondrial DNA from the donor that is transferred: humans have *ca*. 22,000 coding genes and mitochondria have 37, all of which are concerned with metabolic energy production; therefore mitochondrial DNA from the donor should not affect traits like eye colour, height or personality which will be influenced by the child's parents in the usual way. But since all the mitochondria in a person's body are descended from the original few in the egg, women born via the procedure will pass the modifications to their children in turn (*The Economist*, 2015b).

"An important option will be offered to women, i.e. to become a mother without fearing a serious mitochondrial defect that could affect her child," claimed Robert Meadowcroft, in charge of an association that struggles against muscular dystrophy or Duchenne's disease – a rare genetic disease (Bernard, 2015). The strongest opposition to mitochondrial donation came from traditionalists and the religious – a weakening lobby in a country that is becoming ever more secular and one that in this case was divided. The Church of England (Anglican) supports the idea in theory, although it wants more research, and some clergymen have spoken out against. The Roman Catholic Church is opposed, as it is to all forms of IVF. Britain's Muslims are split. Polls suggest that a majority of the public, once informed about the procedure, are in favour (*The Economist*, 2015b). In France, René Frydman, a renowned pioneering researcher in *in-vitro* fertilization (and he helped conceive and deliver the first child born in France from IVF), expressed a critical comment: "With this germinal therapy, the genome is modified; 1% of a genome from a donor is introduced, and we do not know what may be its impact," (Bernard, 2015).

In response to the opposition from several quarters a patriotic rhetorics spread through the United Kingdom. Alison Murdoch, head of the Fertility Centre at Newcastle University where the mitochondrial donation technique was developed, stated : "In an area that is very demanding from researchers the United Kingdom has shown to the world how to proceed." "All the members of the House of Commons who fear that the country may lose its advance in a scientific domain where we are the best in the world, must vote the text," demanded Polly Toynbee, of *The Guardian*, on the morning of the House of Commons meeting. "Otherwise, the United States will take hold of our first rank," she added (Bernard, 2015).

Clinics in the United Kingdom (after the final vote by the House of Lords) will need a license from the Human Fertilization and Embryology Authority, which regulates fertility medicine, as well as specific permission to treat each couple. Any children born as a result will be followed up, to check for unforeseen complications. Doug Turnbull, a scientist at the University of Newcastle who led much of the research into mitochondrial donation, reckoned that *ca*. 150 families a year could benefit. But he thinks that, while the technique is new and relatively untried, only a minority will go for it. Much will depend on whether the National Health System (NHS) agrees to pay, as it does for some but not all IVF patients. Yet the ranks of British early adopters could be swollen by couples from overseas (*The Economist*, 2015b).

# STEM-CELL THERAPIES : NEW PROSPECTS

## Clinical trials with induced pluripotent cells (iPS) in Japan

Shinya Yamanaka created the University of Kyoto Center for iPS Cell Research and Application (CiRA) in 2010, after having discovered the induced pluripotent cells in 2006. In 2012 he was awarded the Nobel Prize for Medicine or Physiology. In 2014 the results of the first and, at that time, unique, clinical trial with iPS carried out in Japan were known. Masayo Takahashi, an ophthalmologist working at the Riken Institute in Kobe, used the skin cells of patients suffering from macular degenerescence associated with age and she was able to transform then into retinian cells. About ten months were necessary in order to obtain cells that could be implanted in the patient's retina. The yield of this kind of transformation is very low: a very few per cent; but the retinian cells are easy to identify and moreover they do not proliferate more than eight times, which limits the risks of cancer (Thivent, 2014).

The iPS are cells which are transformed into embryonic cells and thereafter they can be assigned another differentiation into a specialized cell or tissue. "The main advantage of these iPS compared with embryonic cells is that they originate from the patient. Consequently there is no need for applying an antirejection treatment after implanting the transformed cells," explained the Japanese ophthalmologist. One year after the surgery the patients had no difficulty in adopting their grafts and an improvement in their visual capacity was even observed. Six patients were to receive such grafts in two years, and similar clinical trials with iPS were expected to be launched in Japan. That was at least the suggestion made by Jun Takahashi, professor at the CiRA and the companion of Masayo Takahashi. He could improve the capacity of movement of monkeys suffering from Parkinson's disease thanks to the grafting of dopaminergic neurons derived from iPS. He was hoping to initiate clinical trials within two years (Thivent, 2014).

Beyond these two examples of using iPS in clinical trials Japan has become a country where there is a real enthusiasm for this kind of cells, because they do not raise the legal or ethical issues related with the use of embryonic cells, which is prohibited in Japan. "Shinya Yamanaka has been able to convince the Japanese government very early about the interesting properties of iPS, and this explains why Japan has an edge over many other developed countries," explained John De Vos of Saint-Eloi hospital in Montpellier, south-east of France. Another example of Japan's advance is the publication on 25 September 2015 in the *Annual Review of Genetics* of the work by S. Yamanaka and his co-workers at the CiRA on the editing of the genome of an iPS

cell in order to treat congenital disorders (Hotta and Yamanaka, 2015). "This approach opens extraordinary prospects," said Annelise Bennaceur-Griscelli, a specialist of stem cells working at the French INSERM (Thivent, 2014).

Furthermore Japan has decided to create an iPS cell-bank so as to produce at a lower cost tissues that are needed by, and compatible with, the majority of Japanese population. "With iPS, the patient can be both the donor and receiver. The difficulty is that it is not economically possible to produce and store iPS for each individual. This will be too expensive. That is why we intend to select a few donors whose cells could be tolerated by the largest number of people," explained Peter Karagiannis of the CiRA. The goal is to identify in ten years ten cell lines that could respond to the needs of 30% of Japanese population. But at the same time, Japan, France, the United Kingdom and United States are thinking of creating a common bank of iPS cells in order to produce tissues which can be accepted (tolerated) by the majority of the populations concerned. "According to an English analysis, 150 cell lines would be needed for producing tissues tolerated by 95% of Caucasian populations," indicated A. Bennaceur-Griscelli. France has one of the most important iPS cell-bank at INSERM. It is called Ingestem, set up in 2012 and involving five research units; it is coordinated by A. Bennaceur-Griscelli, who stated: "We store a total of 250 iPS cells, but all of them are derived from donors suffering from genetic diseases. This cell collection is only used for research purposes (e.g. to test therapeutic compounds) and it cannot be used in a clinical context." Several interesting molecules have already been identified thanks to this young cell-culture collection, e.g. in the treatment of progeria (Thivent, 2014).

While in France the first clinical trial using embryonic cells to treat heart lesions (due to infarctus) has been authorized in 2013 (see p. 391), "there remain many uncertainties about iPS cells," said Sophie Jarriault of the Institute of Genetics, Molecular and Cell Biology in Strasbourg. "The efficiency of cell-reprogramming methods remains very low, which means that we are still very far from understanding the nature of these cells comprehensively, or even the nature of cell identity," she added. In the opinion of Masayo and Jun Takahashi of the Riken Institute and CiRA, this is not a real issue, because the history of medicine contains a wide range of treatments whose exact action is badly known. A. Bennaceur-Griscelli recalled that "iPS are reprogrammed cells through a modification of the genome of the initial cell. They behave like embryonic stem cells, but their innocuity has still to be demonstrated" (Thivent, 2014).

## Stem-cell therapy for heart failure

In heart failure the heart cannot properly perform its pumping role so as to supply blood and oxygen to the different body's tissues and organs. This pathology affects 1% to 2% of the French population and it increases markedly after the age 75 years. It has been estimated that more than 22,000 deaths a year are caused by heart failure. The disease has multiple causes, including heart attack or infarctus which leads to the death of the heart-wall cells (necrosis) and the alteration of the function of the left ventricle. Medical treatments can be efficient in the mitigation of heart failure and, in extreme cases, heart transplantation can save patients (Benkimoun, 2015a).

## *Grafting embryonic stem-cell-derived cardiac muscle cells*

Since the mid-1990s Philippe Menasché, a cardiac surgeon at the European Georges Pompidou hospital in Paris, and his team have been working on developing a cure consisting of grafting in the region affected by the heart attack cells that have the capacity of contracting like the normal heart muscle. Their first attempt in June 2000 consisted of directly injecting into the necrotic area stem cells taken from the thy of the patient. Due to ethical reasons, because we were dealing with an experimental trial, the patient also had a coronarian bypath so as to better irrigate the heart muscle itself. The results from a small-scale study in France and thereafter from a European study were disappointing. A cell from the thy muscle is not similar to a heart-muscle cell and does not contract exactly in the same way (Benkimoun, 2015a).

P. Menasché and co-workers pursued their work through the development of their own experimental transformation of embryonic stem cells into heart-muscle cells. These stem cells are derived from embryos obtained after an *in-vitro* fertilization. In 2007 P. Menasché and Michel Pucéat of INSERM were able to demonstrate that these human stem-cell-derived cardiac cells, when injected into the hearts of rats suffering from heart failure, pursued their successful differentiation into heart-muscle cells. After the trials carried out in laboratory animals the collaboration between P. Menasché and the teams of Jérôme Larghero (of Saint-Louis hospital in Paris) and Valérie Vanneaux, a specialist of cell culture and transformation, aimed to initiate human trials. The researchers have been working together since 2000 and in May 2013 the National Agency for the Safety of Medicines and Health Products approved an experimental protocol for six patients. The latter should comply with three criteria: to suffer from a severe alteration of the function of the left ventricle; to have had a heart attack; and to present an indication for a coronarian bypath. The approved protocol systematically included this bypath at the same time as the cell therapy (Benkimoun, 2015a).

## *Human clinical trials*

The French researchers have been able to create a bank of pluripotent embryonic stem cells that can differentiate into all the human body's cells. After being defrozen the cells are again multiplied, and during four days they are transformed into cardiac cells – at least 50% of the stem cells could differentiate into heart-muscle cells. Those cells which did not differentiate are thereafter eliminated so as to obtain a sample containing at least 95% of differentiated cells that can be injected. The elimination of undifferentiated cells is carried out in order to avoid the formation of tumours. Moreover P. Menasché, J. Larghero and co-workers have designed a better device than the simple injection of cells: a 20-$cm^2$ patch that is made of precursors of cardiac cells mixed with fibrinogen – the protein of the blood plasma which is a coagulation factor. A few drops of thrombin (an enzyme) transform the fibrinogen into fibrin, the last step of blood coagulation. The patch is therefore a kind of mesh or "scaffolding" where are located the precursors of cardiac cells. The patch is stitched on the area affected by the heart attack (Benkimoun, 2015a).

A first patient, 77-tyears old, and complying with the three above-mentioned criteria, accepted the experimental treatment. Although the surgery was carried out successfully, the patient died, probably because he was affected by several pathologies (obesity, diabetes, kidney failure). Another eight patients were not accepted. Finally a 68-years-old woman suffering from a severe heart failure and from diabetes underwent the surgery and could later on come back home. She was thereafter able to walk and its clinical condition improved. This result can also be due to the coronary bypath, at least in part. On 16 January 2015 P. Menasché and J. Larghero reported their results to a meeting of the French Society of Cardiology. At this stage the purpose of the trial was to check the safety and feasibility of cell therapy, and not so much its efficiency. However the success of this first trial of heart cell therapy was encouraging. The application of the patch to another five patients and the long-term follow-up of the trials will certainly shed light on the efficacy of this innovative way of treating heart failure (Benkimoun, 2015a).

## Differentiation of stem cells into sperm cells

Spermatogenesis, or the production of sperm cells in testis, is a complex process the duration of which is 62 to 74 days in humans. It starts at puberty and continues during the whole adult life. For the last 30 years many research teams have tried to obtain sperm cells *in vitro*. Some successful results have been achieved in rodents and published in scientific journals (Cabut, 2015d).

### *In-vitro production of human sperm cells from germinal stem cells*

By mid-September 2015 researchers of a startup in the French city of Lyon, Kallistem, announced they had succeeded in producing human sperm cells in the laboratory; to that end they started from germinal stem cells or spermatogonia. This startup is a young offshoot of Lyon Institute of Functional Genomics (a collaborative research unit in which participate the French National Scientific Research Centre or CNRS, the National Agricultural Research Institute or INRA, Lyon's Higher Teacher Training School or ENS, and the University of Lyon-I). Kallistem used a communication strategy that raised polemics among the members of the scientific community. On 5 May 2015 the startup made a press release to announce the successful production of human complete sperm cells *in vitro*. The press release underlined that this was "a first world event", as well as "a real outstanding biotechnological results." But there was no scientific evidence presented and the researchers did not respond to the media. In fact they could not speak because the startup filed a patent request by the end of 2013. The patent describing the whole experiment, called Artistem, was published on 25 June 2015, and the startup (which is raising funds) decided to make public not new results, but a few precisions on the experiments carried out and the technology used (Cabut, 2015d).

"We have achieved this complete spermatogenesis in rats, monkeys and humans, and we have shown that the sperm cells obtained are morphologically normal," underlined Marie-Hélène Perrard, a researcher at the CNRS and co-founder of Kallistem. Philippe

Durand, a former research director at the INRA and scientific director of Kallistem, stressed that the designed culture pattern is novel, because "it allows the conservation of the 3D structure of the seminiferous tubules (i.e. the area of the testis where spermatogenesis takes place), as well as the close relations between reproductive cells and the feeding cells." He also explained that human spermatogenesis had been achieved using testis tissues of men who had been castrated or who underwent surgery after a treatment that upset their fertility (Cabut, 2015d).

Isabelle Cuoc, president of Kallistem, hopes that "in five years, two or three pilot centres will be able to propose a solution to patients." This kind of therapy may be theoretically used to preserve the fertility of teenagers who have not yet reached puberty and who have received a gonadotoxic treatment (radio- or chemotherapy), mainly because of a cancer. It may also be used in mitigating some male infertilities that are not treated by the currently available technologies. Kallistem researchers estimated the number of these individuals between 15,000 and 120,000 (Cabut, 2015d).

### *Reluctance from the scientific community*

Kallistem researchers' fellow scientists have not been fully convinced by the announcements made by the Lyon startup. Thus Louis Bujan, a specialist of reproduction medicine and director of the research unit on human fertility at the University of Toulouse III, stated: "We are eagerly awaiting a peer-reviewed scientific publication. Until then, it is difficult to give an opinion." Isabelle Rives, president of the French Federation of the Centres for the Study and Conservation of Human Eggs and Sperm (CECOS, French acronym), who is working in the same area at the university hospital of Rouen (north of Paris) – she has published several articles on successful spermatogenesis achieved *in vitro* in mice, starting from pre-puberty testis, fresh or defrozen – has shown reluctance with respect to Kallistem results (Cabut, 2015d).

It is true that for Kallistem research team the road is still a long one. First it must be checked, in animals, that the sperm cells which have been obtained using the technique developed by Kallistem, are of good quality and functional, that their fertilizing capacity is satisfactory and that, therefore, they can lead to normal and viable rodents. In case clinical trials are necessary with the sperm produced *in vitro*, Kallistem researchers will have to abide by the regulatory measures of the French National Agency for the Safety of Medicines and Health Products. They run the risk of facing a big obstacle: "The issue will be raised about the compatibility of this kind of work (*in vitro* spermatogenesis) with the prohibition by the law on bioethics of the creation of human embryos for research purposes," explained Dominique Royère, of the French Biomedicine Agency (Cabut, 2015d).

# GENOME ENGINEERING AND ORGAN TRANSPLANTS

Most organs that are nowadays transplanted are harvested from dead people. Shortfalls in the number of volunteer donors, the difficulty of obtaining the consent of grieving relatives and a reduction in most countries of the rate of fatal road casualties (the most reliable source of healthy organs), mean that there is a lack of them. Thousands die each year while on waiting lists for transplants. Researchers have therefore long sought ways to boost supply of organs to be transplanted (*The Economist*, 2015k).

## Difficulties in transplanting animal organs into humans

One idea is to harvest animal organs. In 1984 an American child lived for three weeks after receiving a baboon heart intended as a stopgap until a human donor could be found (unfortunately, one was not found in time). Conversely human organs have been transplanted into animals for the purpose of research. For instance Brazio and Woodall (2015) described the transplantation and growth of human fetal kidneys in rats. Until now, though, two technical problems have hindered the routinely transplantation of animal organs into people. One is that the recipients' immune system must be persuaded to tolerate a big chunk of foreign tissue. The other is that swapping tissues between species risks swapping diseases, too. This second problem may soon be addressed, if George Church of Harvard Medical School has his way. He and his colleagues described in *Science* (11 October 2015) how genetic engineering can now be used to eliminate one of the most worrying types of pathogen that might be spread via transplants ( Yang et al., and Church, 2015).

## Experiments with porcine cells and using genome editing

The animal most commonly suggested as a donor is the pig. Pigs are roughly the size of human beings. Their physiology is reasonably well understood. And millennia or centuries of experience mean they are easy to breed. But their DNA is full of retroviruses, known specifically as porcine endogenous retroviruses or PERVS. The genes of these viruses hitch a lift from one pig generation to another as an integral part of the porcine genome, whence they can break out and cause infection. And tests in laboratories suggest that, given the opportunity, they can infect human cells as well. The existence of PERVS, then, has been one of the main obstacles to transplanting pig organs into people. And G. Church and his colleagues thought PERVS ideal candidates to test the genome-editing technology, CRISPR-Cas9. G. Church and his fellow researchers analyzed the genetic sequences of one family of PERVS, with a view to deleting them

(or inactivating them) using CRISPR-Cas9. They found that the sequence of the gene which lets the virus integrate itself into its host's DNA is the same from one virus strain to another. That allowed them to program a CRISPR-Cas9 system to look for this particular sequence and chop it out of the genome (*The Economist*, 2015k).

The porcine kidney cells G. Church used for his experiments has 62 PERVS embedded in their genomes. He and his colleagues tested their endonucleases on several lines of these cells. In the most responsive they managed to snip out all 62 copies of the integration gene. Since PERVS rely on this gene to infect human cells as well as porcine ones, deleting it should stop them jumping into human hosts. Tests in Petri dishes showed that the modified pig cells did not infect human cells grown alongside them. And, despite the extensive editing made to their DNA, those pig cells seemed unharmed by the procedure. The editing work would need to be done to sex cells, or their precursors, if actual lines of "clean" pigs were to be bred for use as organ donors. But this is still a striking result. Not only does it demonstrate that it is possible to cleanse animal cells of unwanted viruses, thus helping remove one of the big barriers to cross-species organ transplants; it also shows the power of a genetic-engineering technique that has existed for only three years (*The Economist*, 2015k). See Part Two.

# PRECISION MEDICINE INITIATIVE

## Precision or personalized medicine

On 30 January 2015 the president of the United States, Barack Obama, made a series of remarks about innovation in medicine: "We have to invest in innovation, we have to nurture innovation. We have to encourage it and make sure that we are changing it in ways that are most productive. And that is especially true when it comes to medicine. After all, when American researchers developed a vaccine for polio, a program created by Congress helped to distribute it. A federally funded study helped American doctors discover the risk factors for heart-disease. Grants from the National Science Foundation (NSF) and National Institutes of Health (NIH) supported the early experiments that led to the invention of the magnetic resonance imaging (MRI) …"

"So, Francis, Dr Collins here, helped lead the Human Genome Project. And one study found that every dollar we spent to map the human genome has already returned US$140 to our economy. There is a huge economic stake in us tapping this innovation …" "And that is why we are here today. Because something called *precision medicine* – in some cases, people call it personalized medicine – gives us one of the greatest opportunities for new medical breakthroughs that we have ever seen. Doctors have always recognized that every patient is unique, and doctors have always tried to tailor their treatments as best as they can to individuals. You can match a blood transfusion to a blood type. That was an important discovery. What if matching a cancer cure to our genetic code was just as easy, just as standard?"

"And that is the promise of precision medicine – delivering the right treatments, at the right time, every time to the right person. And for a small but growing numbers of patients, that future is already here. Eight out of ten people with one type of leukemia saw white blood-cell counts return to normal with a new drug targeting a specific gene. Genetic testing for HIV/AIDS-affected patients helps doctors determine who will be helped by a new antiviral drug, and who will experience harmful side-effects."

"Wearable electronics make it easier than ever to record vital signs from your blood sugar to your heart rate. Electronic medical records let doctors and researchers across the country collaborate more closely than ever before. And more powerful computers help us analyze data faster than ever before. So if we combine all these emerging technologies, if we focus them and make sure that the connections are made, then

the possibility of discovering new cures, the possibility of applying medicines more efficiently and more effectively so that the success rates are higher, so that there is less waste in the system, which then means more resources to help more people – the possibilities are boundless. *So the time is right to unleash a new wave of advances in this area, in precision medicine, just like we did with genetics 25 years ago*."

## Precision Medicine Initiative, launched by the United States government

"And that is why the budget I sent this Congress on Monday will include a new *Precision Medicine Initiative* that brings America closer to curing diseases like cancer and diabetes, and gives all of us access, potentially, to the personalized information that we need to keep ourselves and our families healthier. First, we are going to work with the National Cancer Institute. We want to find the genetic factors that can lead to cancer. Second, we are going to work with the Food and Drug administration (FDA) to develop new approaches for evaluating next-generation genetic tests. The way we approve a new gene-sequencing technology is going to be different from the way we approve a new pacemaker or prosthetic device. And we need to make sure that our approach reflects the difference in technology. Third, we are going to work with the National Institutes of Health (NIH) to create a research group of one million volunteers. And just like analyzing our DNA teaches us more about who we are than ever before, analyzing data from one of the largest research populations ever assembled will teach us more about the connections between us than ever before. And this new information will help doctors discover the causes, and one day the cures, of some of the most deadly diseases that we face. So if we have a big data set, a big pool of people that is varied, then that allows us to really map out not only the genome of one person, but now we can start seeing connections and patterns and correlations that help us define exactly what it is that we are trying to do with respect to treatment. And finally, we are going to make sure that protecting patient privacy is built into our efforts from day one."

## Foundation of a genuine health-care system

"So the Precision Medicine Initiative we are launching today will lay the foundation for a new generation of life saving discoveries. But in order for us to realize its potential, I am asking more hospitals, and researchers, and privacy experts to join us in this effort. And I am asking entrepreneurs and non-profits to help us create tools that give patients the chance to get involved as well. Because we want every American ultimately to be able to securely access and analyze their own health data, so that they can make the best decisions for themselves and their families. And ultimately, this has the possibility of not only helping us find new cures, but it also helps us create a genuine health-care system as opposed to just a disease-care system. Part of what we want to do is to allow each of us to have sufficient information about our particular quirks that we can make better life decisions. And that, ultimately, is making sure that we have got a system that focuses on prevention and keeping healthy, not just on curing diseases after they happen."

Thus in January 2015 the federal government launched a US$215 million Precision Medicine Initiative: to help build a database of health information about 1 million Americans; and to support research at the National Cancer Institute. That funding alone however is not nearly enough to usher in the new era of custom cancer care. For this idea to succeed every personalized therapy a doctor tries must be pooled to build a massive research tool that all physicians can share. That is the goal of the American Society of Clinical Oncology (ASCO) which announced it is creating a registry of patients who take drugs that are approved for a cancer other than the one for which they were cleared by the FDA. "We want to gather that information and see what happens to those patients, even if they are not in a clinical trial," stated Julie Vose, chief of hematology and oncology at the University of Nebraska Medical Center and president elect of ASCO (Park, 2015a).

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**About the book**

Part One highlights the current considerable flow of financial resources towards medical biotechnology. We are acknowledging a real boom of the bioindustry and a "golden era of DNA." Such unprecedented situation does not seem to be a bubble that would burst, because the "science has never been so good and the pace of medical advances has never been so fast." Rocketing start-ups have been created and some of them have become big pharmas due to large initial venture-capital investments and to the commercialization of their innovative drugs. Also, there has been a frenzy of mergers and acquisitions in the pharmaceutical and medical-biotechnology sectors, as well as an increasing involvement of the giant information-and-communication technology groups (GAFAs) in life sciences and biomedicine. The latter are competing for dominance in the next era of computing and for harnessing unparalleled troves of data in order to offer new services, including the handling of patients' personal data; they also want to seize the opportunities arising from the predictable great impact on biomedical and clinical research of the combination of human genomics with the wide-ranging applications of information-and-communication technologies.

Part Two deals with the current and foreseeable impact on human genomics of the ever faster and cheaper sequencing of genomes, as well as with genome editing and its derived implications for biomedical research, development and innovation. Some of the huge amount of data produced by genome sequencing are being correlated with diseases. Also, the soaring market of genetic tests designed for the large public responds to requests from individuals and society; however, their interpretation raises bioethical issues and call for an open societal debate.

We have also moved from genome reading to genome editing or to "gene surgery". The discovery and rapid adoption and use, since 2013, of the CRISPR-Cas9 technique may have profound consequences on gene therapy and the cure of some genetic defects or diseases. However, the majority of researchers call for a moratorium on any experiment that aims to edit the genome of a human embryo to be implanted in the womb with subsequent genetic modifications in the offspring. Investors, although cautious, have shown interest for the technology, as well as for the current boom of synthetic biology.

Part Three reviews current achievements and prospects in medical biotechnology: vaccines and vaccination issues, with emphasis on the vaccines against Ebola, dengue, influenza and papilloma viruses; towards eradicating tuberculosis, malaria (control of mosquito populations); genetic and rare diseases; new weapons against cancers; cardiovascular diseases; diabetes/obesity; stem-cell therapies; and the development of precision medicine and of a genuine health-care system.

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