Preface

Innovations that change established commercial paradigms occur in exponential 'S-curve'–shaped cycles typically spanning a quarter of a century. This empirical law derived from different industries and different periods observed since the Industrial Revolution suggests on the one hand that midterm technological futures are already written and on the other hand that the pace of progress is cognitively difficult to appreciate as disruptive discovery and radical innovation constitute exponential phenomena rather than incremental ones, whereas the human brain has been hardwired by evolution to perceive linear trajectories. Early and efficient adoption of disruptive technologies thus typically remains a challenging process. However, as the future of healthcare is already foreshadowed via early signals such as those captured in the intellectual property literature, a deep dive in scientific fundamentals of emerging pharmaceutical modalities enables one to appreciate the healthcare revolution unfolding today, which will dramatically change the healthcare of tomorrow.

Modern biotechnology was born via a series of landmark discoveries beginning in 1857 with publications by Louis Pasteur on his work on the lactic acid and alcoholic fermentations, followed by the observation in 1944 by Oswald Avery that DNA is the macromolecule that codes for information that transforms bacteria and the subsequent discovery of the structure of DNA in 1953 by James Watson and Francis Crick. These early advances set off an explosion of new discoveries around the DNA molecule including the deciphering of the genetic code in 1961 by Marshall Nirenberg, Har Gobind Khorana and Robert Holley, the isolation in 1967 of DNA ligases and the discovery and application of restriction endonucleases in 1968 by Werner Arber, Hamilton Smith and Daniel Nathans. These advances in turn enabled the development of techniques to manufacture monoclonal antibodies in 1975 by Georges Kohler and César Milstein. All these techniques combined with background know-how applied in virology were prerequisites for gene therapy to emerge after a rocky road of development over several decades, with the first successful approval of a gene therapy occurring in 2012 by the European Medicine Agency with Glybera (alipogene tiparvovec) to treat the single-gene disease reverse lipoprotein lipase deficiency. In parallel, the first successful embodiment of the idea of using live cells as therapeutics was the development of live attenuated vaccines first implemented by Louis Pasteur in 1879 to prevent chicken cholera caused by the bacterium *Pasteurella multocida*, building from the work of Edward Jenner who developed the first modern viral vaccine in 1796 to prevent smallpox caused by the Variola virus. It is also worth noting that the first tissue-engineered living dermal substitute of human

origin produced at the industrial scale and cryopreserved was approved by the US Food and Drug Administration in 2001, while the first approvals of allogeneic and minimally manipulated mesenchymal stem cell preparations including remestemcel-L were granted in South Korea, Canada and New Zealand a decade later. Cell-based therapies of human origins are rooted in haematopoietic stem cell– and mesenchymal stem cell–containing bone marrow transplantation first performed in 1957 by Edward Donnall Thomas, the discovery of transplantable stem cells by James Till and Ernest McCullough in 1961, the characterisation of mesenchymal stem cells by Arnold Caplan in 1979 and their applications to treat severe inflammatory diseases exemplified by the life-threatening acute graft-versus-host disease, the culture of embryonic stem cells successfully achieved in 1981 by Martin Evans and Matthew Kaufman and, in 2007, the reprogramming of adult cells into pluripotent stem cells by Shinya Yamanaka, with the first cell-based therapy in oncology being the dendritic cell vaccine preparation Provenge (sipuleucel-T), which was granted approval in 2010 for metastatic castrate-resistant prostate cancer. The T-body concept reported on in 1992 by Gideon Gross and Zelig Eshhar was born out of the similarity in the molecular structure and gene organisation of antibodies and the T cell receptor, with the inspiration to take the best of both worlds of T cells and of antibodies, that is, the native cell killing functions of T cells and the exquisitely precise targeting capabilities of antibodies. This concept became subsequently enabled by critical enhancements in the chimeric architecture to include costimulatory domains required to boost the efficacy of the engineered cells. The emergence of chimeric antigen receptor (CAR)-T cells culminated in 2017 with the approval of the CAR-bearing T cell preparation Kymriah (tisagenlecleucel) to treat relapsing or treatment-refractory acute lymphoblastic leukaemia and large B-cell lymphomas (the approval in the latter indication was granted in 2018) and that of Yescarta (axicabtagene ciloleucel) to treat relapsing or treatment-refractory large-B-cell lymphomas. Remarkably, CAR-T cells are products of the convergence innovation not only between gene therapy and adoptive (stem) cell–based therapies but also they are the unanticipated legacy of retroviral research following the AIDS epidemics. Notably, these products are also the outcome of the convergence between the therapy areas of immunology and oncology, which was signalled early on and notably by William Coley's work in 1891 with preparations of inactivated bacteria from different genera to treat cancer patients.

The approvals by the FDA or the EMA of Provenge, Glybera, Kymriah and Yescarta are critical milestones in healthcare in general and in gene-based therapy and cell therapy in particular, as these four products, irrespectively of their commercial fortunes, marked the advent by the regulatory authorities and practicing clinicians of, in that order, adult human live cell therapies, gene therapies and engineered human live cell therapies. This is a significant milestone as the 2010s marked the era of gene therapies for single gene hereditary diseases and of minimally manipulated adoptive cell therapies best represented by haematopoietic stem cells and mesenchymal stem cells. This was an important first step for ethical reasons, as the safety and efficacy of the novel approaches

had first to be solidly established and verified in robust clinical trials. Since 2017, engineered living drugs have not only made their debut in the armamentarium against cancer with the hope to ultimately render cancer a curable or chronic disease but also these approvals open the path for the implementation of recombinant live cells in other therapy areas and notably in indications where further therapeutic progress is vexingly slow, including, to name only a few, in cardiovascular diseases comprising heart failure, steroid-resistant inflammatory diseases including idiopathic pulmonary fibrosis or inflammatory bowel diseases and neurological indications including neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. As a result, enhancing the natural capabilities of other types of cells to be used as living drugs is a concept that has come of age, and the stunning progress achieved in 2017 using CAR-T cells constitutes a technological and regulatory big bang. This big bang is equivalent in long-term significance to 'Asilomar 1975', when modern genetic engineering was allowed to take off and enable modern industrial microbiology and biotechnology. The deep knowledge and tools achieved in nosotaxonomy, cytokine and signalling networks, artificial intelligence and virtual patients, big data harnessing including patient data, the 'million human genomes' projects ongoing over the globe, biomarker discovery and precision medicine platforms, body-on-a-chip or other preclinical models that recapitulate human biology in health and sickness, as well as therapeutic peptides or nucleic acids comprising aptamers and siRNAs, multivalent biologics and innovative small molecules, all these novel knowledge and dizzying array of novel technologies can now converge with gene-based and cell therapies with the view to arm or tailor them and make possible the development of more precise, more efficient and safer treatment algorithms, including by way of harnessing the power of combination therapies as demonstrated by the long-term management of human immunodeficiency virus infections that became possible only by the multitherapy approach. It is such revolutions in pharmaceutical paradigms that will ultimately enable practicing clinicians to achieve more ambitious clinical goals best exemplified by the success already met with stunningly high remission rates in otherwise incurable lymphomas.

Second Generation Cell and Gene-based Therapies is organised into four Parts to explore the past, the present and the future of the emerging field of regenerative medicine, as well as those of the cell and gene-based therapy industries, and particularly to highlight the significant biological advances that occurred during the 2010–2020 decade viewed from the perspective of clinical benefits and strategies for market access and capitalisation. Each chapter includes a Perspectives section in which the key messages and insights discussed are summarised. These perspectives aim at challenging the reader with forward-looking thoughts concerning the strategic directions of these fields relative to business or scientific issues or both.

In Part I of the book, the underlying scientific fundamentals of cell and gene-based therapies are examined. Cytotherapies of human origin comprise adult stem cells (haematopoietic stem cells, mesenchymal stem cells and tissue-specific stem cells such as

neural stem cells), pluripotent stem cells (embryonic stem cells and induced pluripotent stem) and cells of the immune system (comprising T cells, regulatory T cells, natural killer cells, macrophages, dendritic cells and virtually all other adult cell types and their subtypes). All these primary cellular types constitute a rapidly emerging class of pharmaceutical modalities that have advanced from the laboratory bench to the clinic in a typical technology S-curve similar to the one that had to be ridden to develop disease-modifying monoclonal antibodies. It is worth remembering here that biologics were faced with numerous clinical trial or commercialisation failures in the first years of their development or launch. An imperative for the regenerative medicine and advanced therapy medicinal product industry is to bring to clinical development the second wave of cytotherapies with tailored and enhanced efficacies built on robust safety attributes. An examination of the various approaches to achieve this second wave in parallel with the emerging gene therapy technology is presented throughout this book. A first tactical realisation of this framework is the success met with the development and deployment of gene therapies to tackle immunodeficiency-causing adenosine deaminase deficiency. Starting with a definition of gene therapy, the various molecular tools available to achieve the cloning of therapeutic genes and their deliveries are reviewed and punctuated by the development journey of Strimvelis (autologous CD34+-enriched cell fraction that contains CD34+ cells transduced with a retroviral vector that encodes the human ADA cDNA sequence). The therapeutic potential of cells of the immune system in autoimmune diseases and various cancers is subsequently revisited and exemplified by a deep dive in the therapeutic value of γδT cells. Building from this forward-looking review, a historic journey in the engineering and development of CAR-T cells and CAR-natural killer (NK) cells is conducted from the ideation of the necessary molecular architectures to the construction of the new pharmaceutical modalities at the laboratory bench, their manufacturing and logistics, their clinical translation and ultimately their delivery to patients. The opportunities and hurdles to overcome before achieving the full potential of live engineered human immune effectors cells are emphasised in two back-to-back chapters, not only regarding liquid cancers but also solid tumours. A second stream of this section is to discuss the use of first-generation pluripotent stem cell–derived cells in regenerative medicine through the lens of islet replacement therapy for diabetes. Hurdles in the clinical development of these new treatments are highlighted, notably including the merger between medical devices and cell therapy for achieving efficient delivery and encapsulation to better circumvent differentiation and safety issues including device retrievability, as well as efficacy considerations. Furthermore, emerging tools as critical enablers of the new medicine are detailed including in silico technologies and artificial intelligence, as well as editing technologies for the mammalian genomes. Particularly, the potential of TALENS, zinc finger nucleases and CRISPR/Cas9 systems to revolutionise preclinical and clinical science is reviewed including their practical therapeutic applications. In addition, latest advances in tissue and organ replacement are analysed through the prism of solid organ transplantation including an overview of replacement tubular structures and

three dimensional functional artificial organs. In addition, tentative industrial paths to standardise artificial organ generation are discussed along with commercialisation models. The journey further extends into a review of combining stem cells and materials notably to rewire the degenerative brain with the goal of achieving nerve tissue regeneration.

In Part II, the latest progress in translational medicine using advanced therapy medicinal products is surveyed, with an initial focus on the history and future trends in gene therapy clinical trials emphasising early failures caused by serious adverse events and how technical solutions were implemented to revive this field resulting in the first gene therapy being granted approval in 2012. In addition and building on the review of preclinical development of therapeutic T cell and engineering–enabling molecular biology techniques presented in the preceding section, CAR-T cell–focused clinical studies leading to the first approvals of such therapies are detailed. Similarly, the initial clinical studies with T cell receptor–engineered T cell therapy are presented to highlight the different biological attributes offered by this novel class of therapeutics. Completing the survey of clinical trials with engineered living drugs, an overview of translational studies with genetically engineered haematopoietic stem cells is proposed with a focus on treatments for genetic diseases such as sickle cell anaemia or β-thalassaemia major. What is more, the issue of reimbursement options in this particular market niche is addressed by assessing payer's impacts and value-based pricing.

In Part III, the next frontier in the landscape of cell and gene-based therapy is probed by assessing progress along several novel biological and therapeutic dimensions. First-generation allogeneic mesenchymal stem cells have provided significant scientific and clinical learnings for the industry. Looking beyond this horizon, further enhancements to this technology are discussed, from developing second-generation living drugs with enhanced efficacy attributes through a variety of technical means including genetic engineering, ex vivo conditioning using small molecules or enzymatic treatments, to developing derivative products such as therapeutic exosomes or mesenchymal stem cells used as delivery vehicles of a therapeutic payload such as an anticancer agent including a nucleic acid, an oncolytic virus or a cytokine synthesised and delivered in situ or either a small molecule loaded ex vivo or an antiinflammatory agent to create novel options in oncology and inflammatory diseases. The science of regenerative medicine remarkably provides novel frameworks to understand mammalian biology through fresh new angles, and the question whether senescence is reprogrammable is tackled using pluripotent stem cell paradigms deeply rooted in the tree of cellular filiation. Here, the concept of pluripotent stem cells is taken to another dimension as biological tools to serve as molecular 'can openers' for deciphering complex systems architecture, notably exemplified by deciphering the molecular basis of lithium therapy in bipolar disorders, which had escaped conventional molecular investigation. The space of novel therapeutic opportunities in oncology is likewise revisited, highlighting first how the potential of dendritic cells could be harnessed. This journey proceeds from the immune reaction cascade and a description of the basic biological attributes and the latest advances achieved in cancer

cell vaccines. A second forward-looking chapter highlights the challenges in treating solid tumours and particularly how to best make use of engineered immune effectors cells, with the suggested strategy being illustrated through the example of glioblastoma multiforme. Another glimpse into the future of healthcare is provided through an extensive review of combination therapy in solid tumour oncology by recapitulating the learnings of all the preceding chapters and notably how all the novel therapeutic modalities converge with emerging practices in healthcare including novel devices and big data crunching capabilities as well with conventional treatments to deliver on the ambitious vision to reinvent therapeutic regimens and algorithms. An ambitious path is proposed here on how the vision of precision oncology and long-term remission in solid cancers might be achieved.

In Part IV are coalesced perspectives in healthcare that are triggered by the unfolding revolution in advanced therapy medicinal products. Firstly, an ethical analysis is presented to generate axes of discussion regarding the societal acceptability of gene-editing technologies in adoptive T cell therapies for cancer. Secondly, a systematic framework is proposed to better grasp the development life cycles of cell and gene-based therapies. This is further implemented by bringing elements of response to the question of what could be the development directions for CAR-T cells, including a review of service-based business model or off-the-shelf blockbuster business model to define business sustainability concerns and possible answers. The question of how to finance the new wave of transformational oncology treatments is treated through the lens of strategic alliances and investment incentives for financial institutions and large pharmaceutical corporations. A brief landscape analysis of emerging technologies and of the biotechnology company ecosystem that develops them is provided, with the view to enable the assessment of fund raising and initial market capitalisation of these start-ups to explore whether those companies already have sufficient financial runway to bring the next generation of cancer treatment to the market and if in the foreseeable future, the public market trends remain open to continue to support the emerging segment of the oncology treatment arena. Importantly, the successful capitalisation on living drugs in oncology will fuel the momentum to innovate other cellular therapies for other therapeutic areas. In this journey that will redefine healthcare as we know it, as much if not more as the technology of monoclonal antibodies has transformed healthcare at the turn of the millennium, the role of governments in the commercial emergence of radical innovation is highlighted through the example of the initiative undertaken by the United Kingdom to catapult cell and gene-based therapies to the forefront of pharmaceutical sciences. Such initiatives are critical as it is one of the public sector's key roles to fund fundamental research and to build the development and manufacturing ecosystem as well as its value chains that will incubate the seeds of the radical innovations of tomorrow, as the private sector by itself is typically ill-equipped to achieve on its own such goals. In turn, these will translate into a virtual cycle of innovation and commercialisation by way of the successful adoption of

the novel technologies by the private sector, for the ultimate benefits of patients and of local economic communities. A pillar for the development of a flourishing commercial sector remains the implementation of solid intellectual property infrastructure supported by appropriate academic and commercial strategies; here, key patenting trends in CAR technologies are discussed as well as best practices in the field. Considering that advanced therapies in many cases have the potential to provide long-term remission or even cure with the delivery of a single dose, novel reimbursement and payments models are necessary that will mitigate the viewpoints of different stakeholders, ranging from incentives to innovate and affordability both at the level of national healthcare systems and private payers as well as at the level of patients. The cornerstone of the monograph lies in market access perspectives. These are first explored by way of a presentation of the commercial launch of the autologous CAR-T cell product Kymriah in multiple European jurisdictions, whereby, taking into account the voice of patients, the needs and responsibilities of the various stakeholders are discussed with the view to achieve an optimal value chain for the delivery of this lifesaving product. Last but not least, a thought-provoking synthesis of the overall perspectives presented herewith is made, whereby a strategic flow-chart is proposed to assess how to significantly move the needle of medicine such as to best offer to patients who need them pharmaceutical products with the highest relevance.

In a 5-year time window, the pharmaceutical industry has reached a tipping point that represents the accomplishment of half a century of technical prowess in modern biotechnology. The approvals of the first gene therapy and of the first engineered living drug that have provided true long-term remissions in otherwise incurable diseases are milestones that have changed healthcare forever. Like it is the convergence of immunology, virology, molecular biology, immune and nonimmune cell biology, adult and stem cell biology and oncology that carried the seeds of the revolution of immunooncology from checkpoint inhibitors to adoptive engineered immune effector cells, it is the convergence of different technological domains spanning biotechnology to information technology and device engineering that now represents the next iteration in the cycle of pharmaceutical innovation. The next frontier lies not only in scientific and technical advances for healthcare in oncology or immunology but also far beyond into the realms of treating incurable neurodegenerative or autoimmune diseases, debilitating strokes or fatal heart failures and metabolic diseases, and onto tissue replacement and organ reconstruction. To avoid the pitfalls of slow technology adoption observed during previous cycles of radical innovation, the rates of adoption of the new technologies measured in terms of market access can be accelerated by their proactive management, for the ultimate greater benefits of patients.

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