Industrial Perspective on Immunotherapy

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Abstract

Immunotherapy has revolutionised oncology and represents a fast-growing area of new drug products in anti-cancer therapy. Patients can now benefit from an expanded landscape of treatment options for several tumour types. The value of cancer immunotherapy is well-established thanks to the clinical success following regulatory approval of several immunomodulators and cellular immunotherapies, and both the private and the public sector are investing to provide patients with improved immune-based agents and to extend the indications of already marketed products. Although recent achievements offer the best promise for successful treatment, innovators in the field of cancer immunotherapy still face many challenges toward commercialisation that could be mitigated by a smart drug development strategy.

Keywords

Immunotherapy · Industry · Tumour heterogeneity · Clinical development · Intellectual property · New indications · Resistance to treatment · CAR-T cells · Monoclonal antibody · Biomarker · Patient-centric approach

Abbreviations

Acronym	Definition
ADCC	Antibody-dependent cell-mediated cytotoxicity
AE	Adverse event
AI	Artificial intelligence
AIDS	Acquired immune deficiency syndrome
APC	Antigen-presenting cells
ATMP	Advanced therapy medicinal product
B2M	β2-microglobulin
B-ALL	B-cell precursor acute lymphoblastic leukaemia
BCMA	B-cell maturation antigen
BMS	Bristol–Myers Squibb's
CAR	Chimeric antigen receptor
CAT	Committee for Advanced Therapies
CD	Cluster of differentiation
CDC	Complement-dependent cytotoxicity
CEA	Carcinoembryonic antigen
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CSF1R	Colony-stimulating factor 1 receptor
ctDNA	Circulating tumour DNA
CTLA4	Cytotoxic T-lymphocyte-associated protein
DARPins	Designed ankyrin repeats
DC	Dendritic cells
DLBCL	Diffuse-large B-cell lymphoma
dMMR	Deficient mismatch repair
EBV	Epstein–Barr virus
EGFR	Epidermal growth factor receptor
EMA	European Medicines Agency
Fc	Fragment crystallisable
FDA	Food and Drug Administration
GITR	Glucocorticoid-induced TNFR-related protein
GMP	Good manufacturing practise
GPC3	Glypican-3
HBV	Hepatitis B virus
HER2	Receptor tyrosine-protein kinase erbB-2
HLA	Human leukocyte antigen
HNSCC	Head and neck squamous cell carcinoma
HPV	Human papilloma virus
HSV	Herpes simplex virus
HTA	Health technology assessment
ICI	Immune checkpoint inhibitors
ICOS	Inducible T-cell costimulator
IDO	Idoleamine-2,3 dioxygenase
IgG	Immunoglobulin G

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IL	Interleukin
INFAR	Interferon-alpha receptor
INF-γ	Interferon-γ
IP	Intellectual property
LAG3	Lymphocyte-activation gene 3
MA	Marketing authorisation
mAbs	Monoclonal antibodies
MAGE	Melanoma-associated antigen
MDSC	Myeloid-derived suppressor cells
MHC-I	Major histocompatibility complex I
MRD	Minimal residual disease
MSI	Micro satellite instability
MUC-1	Mucin 1
NGS	Next-generation sequencing
NIH	National Institutes of Health
NK	Natural killer
NSCLC	Non-small cell lung cancer
PASS	Post authorisation safety study
PD1	Programmed cell death protein 1
PDL1	Programmed cell death protein ligand 1
PGE2	Prostaglandin E2
PRO	Patient-reported outcome
PSCA	Prostate stem cell antigen
PSMA	Prostate-specific membrane antigen
PTA	Patent term adjustment
R&D	Research and development
RCC	Renal cell carcinoma
ROS	Reactive oxygen species
RSV	Respiratory syncytial virus
SME	Small- and medium-sized entrepreneurs
SPC	Supplementary protection certificate
STAT3	Signal transducer and activator of transcription 3
STING	Stimulator of interferon genes
TCR	T-cell receptor
TGF-β	Transforming growth factor beta
TIL	Tumour-infiltrating lymphocytes
TLR	Toll-like receptor
TMB	Tumour mutational burden
TME	Tumour microenvironment
TNBC	Triple-negative breast cancer
TNF	Tumour necrosis factor
Treg	T regulatory cells
TRIPS	Trade-Related Aspects of Intellectual Property Rights
USPTO	United States Patent Office
VEGF	Vascular endothelial growth factor
WT1	Wilms tumour protein
WTO	World Trade Organization

1 Introduction

1.1 What Is Cancer Immunotherapy?

Immunotherapy can be defined as a therapeutic procedure aiming to stimulate or suppress the immune system in order to fight a broad range of diseases including infections and cancer.

The idea of exploiting the individual's immune system to fight disease dates back to the last centuries and it has been widely explored in the field of vaccination. However, before the formal proof of concept that cytotoxic responses could be redirected to destroy malignant tissues, the application of immune-based therapeutic agents to the field of cancer has lagged behind other therapeutic options, such as chemotherapy and radiotherapy.

In this scenario, the description of the durable responses in metastatic melanoma elicited by Ipilimumab (a blocking antibody binding to the checkpoint inhibitor cytotoxic T-lymphocyte-associated protein – CTLA-4) [1] and the efficacy of a monoclonal antibody binding to the checkpoint inhibitor programmed cell death protein 1 (PD-1) [2] represented the dawn of a new era in the treatment of cancer.

The above-mentioned unanticipated clinical observations revealed that, by targeting the so-called checkpoint inhibitors, it was possible to reinvigorate the inherent ability of the host's immune system to efficiently eradicate cancer. Based on these findings, "Yervoy" (Ipilimumab), developed by Bristol–Myers Squibb, was the first cancer immunotherapeutic agent receiving regulatory approval in the United States. It was soon followed by "Opdivo" (Nivolumab), an anti-PD-1 monoclonal antibody, developed by Ono Pharmaceutical, which received regulatory approval in Japan and later by in the United States.

The discovery of immunostimulatory monoclonal antibodies (mAbs) [3] was more recently followed by the description of adoptive T-cell therapy, which was pioneered by Steven Rosenberg [4], Zelig Eshhar [5], Carl June and Michel Sadelain. Globally, these approaches triggered a revolution of the paradigms of clinical cancer management. Since then, cancer immunotherapy has emerged as a clinically beneficial alternative to conventional treatments for a variety of oncologic malignancies, including melanoma [1, 6, 7], hematologic malignancies – such as refractory Hodgkin lymphoma – [8] non-small cell lung cancer (NSCLC), ovarian cancer [9], prostate cancer, kidney cancer, bladder cancer [10], head and neck squamous cell carcinoma (HNSCC) [11], and renal cell cancer (RCC) [12]. This is acknowledged

by the numerous U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) approvals of new therapeutic entities and by the rapid expansion for existing agents [13].

Notwithstanding the ground-breaking effect of the emerging field of cancer immunotherapeutic agents on patients care, it should be noted that the seminal discoveries at the basis of cancer immunotherapy date back more than 25 years ago. The route from bench to bedside of the first wave of cancer immune-based agents, such as Ipilimumab, followed drug discovery and development timelines similar to those of canonical drug entites [14].

Indeed, the discovery of the immune checkpoint inhibitor PD-1 dates back to the seminal observations of Dr. Tasuko Honjo at Kyoto University in 1992, whereas the checkpoint inhibitor CTLA-4 was discovered in 1994 by Dr. James P. Allison, through his work at the University of California Berkeley and Memorial Sloan Kettering Cancer Center in New York. The major impact of these discoveries and, in general, of the emerging field of cancer immunotherapy has been acknowledged by the award of the Nobel prize for physiology or medicine in 2018 [15] to Allison for the discovery of CTLA-4 [16] and to Honjo for the discovery of programmed cell death protein 1/programmed cell death protein ligand 1 (PD-1/PD-L1) [17].

The launch of a first generation of cancer immunotherapies based on the pioneering discoveries of Honjo, Allison, and other researchers, including Lieping Chen and Gordon Freeman, is thus a success story of translation of basic science research into clinical treatment. Since then, there has been a tendency toward shortening the clinical development and approval process of cancer immune-based agents [14].

2 How Does Cancer Immunotherapy Work?

The seminal discoveries by Honjo and Allison revealed that interfering with the regulatory mechanisms of the immune system can prove fundamental to treat cancer. In order to fully understand the broad clinical activity, the durable response rates and the distinct characteristics of immune-based agents, it is thus fundamental to provide a detailed explanation of the immunological circuits which they rely on. This analysis is of crucial importance to appreciate the factors that differentiate cancer immunotherapy from traditional cytotoxic or targeted therapies.

Similarly to what is described in the context of immunisation, different immunotherapeutic strategies are available, which comprise passive and active approaches. Passive cancer immunotherapy consists of enhancing existing immune response to tumour cells, while active immunotherapy, by interfering with precise regulatory circuits, directs immune cells to attack tumour cells.

In order to fully comprehend the mechanism of action of immune-based agents, it is crucial to familiarise with a pillar of the adaptive immune response, that is, the concept of self/non-self-discrimination. The discrimination of self/non-self proceeds thanks to the selective recognition of antigenic peptides displayed on the cells' surface bound to major histocompatibility complex I (MHC-I). Antigen recognition occurs via the T-cell receptor (TCR) of Cluster of Differentiation-8+ (CD8)+ T lymphocytes. CD8+T lymphocytes, together with natural killer (NK) cells, are endowed with the ability to kill sister cells as a mechanism of defence for eradicating or controlling intracellular pathogens and tumours.

During their development and maturation, T cells are selected in order to be able to recognise foreign antigens and become able to perform immune-mediated surveillance of the host. Thus, the repertoire of self-reactive TCRs is general very low, although the escape of self-reactive TCRs is associated with autoimmune disorders such as type 1 diabetes and multiple sclerosis.

However, in order to ensure that a targeted immune response is mounted selectively against foreign antigens, the adaptive immune system also developed an additional regulatory circuit, that is represenTeed by the requirement of a second positive signal in addition to TCR triggering. This concept represents the core of current immunotherapy and is generally referred to as co-stimulation.

T-cell co-stimulation needs to be analysed as a "social" phenomenon that occurs in a complex inter-cellular and receptor-dense environment. Regulatory circuits of the immune system comprise a plethora of molecular and cellular actors [18, 19], including but not limited to T regulatory cells (Treg), checkpoint inhibitors, immunomodulatory cytokines, such as Interleukin-10 (IL-10). These mechanisms have evolved to counterbalance activation stimuli that, if not restrained, could lead to deleterious, mis-targeted immune responses.

It should be noted that lack of proper stimulatory signals may lead to T-cell anergy and T-cell exhaustion. These represent a state of T-cell dysfunction that is typical of many chronic infections and cancer, characterised by poor effector function, sustained expression of inhibitory receptors and a transcriptional state distinct from that of functional effector or memory T cells [20]. The ultimate outcome of this form of T-cell dysfunction is the inability of the adaptive immune system to eradicate an infection or a tumour.

Positive co-stimulation occurs thanks to the fine-tuned action of several receptors expressed on the surface of T cells and antigen-presenting cells (APCs). Binding of CD40 on the surface of APCs to CD40L (CD154) on the T-cell surface stimulates the expression of CD28 and B7 (either B7.1 or

B7.2) by the T cell and APC, respectively. Interaction between CD28 and B7 (mainly CD80 and CD86) is the "second signal" required for efficient T-cell activation and survival.

CD28-B7 binding is also crucial to regulate the intensity of the T-cell response, as it stimulates CTLA-4 (CD152) expression on the T-cell surface. Indeed, CTLA-4 is the competitor of CD28 and its engagement to B7 – which is characterised by a considerably higher affinity compared to CD28–B7 interaction – fully suppresses T-cell response. It is clear that the balance between the contrasting signals triggered by CD28 and CTLA-4 is crucial to fine-tune adaptive immune responses [21].

Accordingly, blocking CTLA-4 with an anti-CTLA-4 antibody such as the above-mentioned Ipilimumab allows efficient interaction between B7 and CD28, thus reinvigorating T-cell responses. It should be noted CTLA-4 is a valuable target also for the treatment of autoimmune disorders, where suppression of CD28-mediated immune responses is pursued; a CTLA-4-immunoglobulin (Ig) fusion protein, named Atabacept, has been successfully employed to interrupt CD28-B7.2 interaction. Actively binding B7-2 with a CTLA-4-Ig fusion protein, like Abatacept, interrupts the interplay between CD28 and B7-2 and thus suppresses CD28-mediated T-cell activation.

Additional regulatory circuits involve T-cell-expressed receptors, such as ICOS (inducible T-cell co-stimulator) and PD-1. PD-1 (PDCD1 or CD279) is expressed on activated T cells, while the expression of its ligand PD-L1 is limited to epithelial and endothelial cells in homeostasis. PD-1/PD-L1 interaction dampens T-cell activation thus protecting PD-L1+ cells. As several cancer cell lineages evolve to escape immune responses by expressing PD-L1, it is straightforward that the use of anti-PD-1 antibodies – such as nivolumab and pembrolizumab – and anti-PD-L1 antibodies – such as atezolizumab, avelumab and durvalumab – can interfere with PD-1/PD-L1 binding and thus sustain T-cell responses.

It is clear that, while cancer cells evolve to take advantage of – and even hijack – the regulatory mechanisms that ensure the safeguard of self tissues, cancer immunotherapy intervenes by releasing inhibitory checkpoints to favour anti-tumour cytotoxic responses [22].

Immune-based agents may be classified as "passive" and "active" based on their ability to engage the host immune system. However, this classification should be applied with a certain degree of plasticity due to the complexity of the drug–host–tumour interaction [23]. In this context, it is widely accepted that the immune checkpoint inhibitors described beforehand represent the archetype of active immunotherapy. Conversely, adoptive T-cell therapies may be classified as passive cancer immunotherapy. It should be noted that passive agents (including tumour-targeting mAbs) often rely on the host immune system in order to achieve their anticancer activity and may de facto constitute active forms of immunotherapy [23].

The development of therapeutic vaccines targeting tumour antigens to arrest cancer progression and preventing recurrence, an example of active immunotherapy, has delivered very little to clinical practise so far [24, 25]. Conversely, adoptive T-cell therapies – exploiting either tumour-infiltrating lymphocytes (TIL) or chimeric antigen receptor (CAR) T cells – have shown remarkable potential.

Isolation and *ex vivo* culture of autologous TILs, followed by perfusion with exogenous IL-2 to patients that are rendered lymphopenic by suitable preconditioning regimens have shown outstanding durable responses [26]. TILs, which are isolated from autologous tumour tissue or from draining lymph nodes, are able to recognise tumour antigens though their native TCR; this allows a broader reactogenicity, which is not restricted to a single human leukocyte antigen (HLA) haplotype and thus prevents unexpected off-target toxicity [27]. Current preclinical and clinical evidences suggest that TILs infiltration could be amenable to broad clinical application.

Alternatively, by genetic manipulation of autologous T cells giving rise to CAR T cells, it is possible to redirect cytotoxic responses to any tumour antigen. Re-infusion of CAR T cells is generally preceded by lympho-depleting chemotherapy to allow *in vivo* expansion of the infused CAR T cells. Engineered CARs encompass a transmembrane receptor, usually consisting of a single-chain antibody domain and intracellular signalling domains. CAR T cells including an anti-CD19 antibody domain and the intracellular signalling domains of CD3ζ, together with additional signalling domains, such as the ones of CD137 or CD28, are able to develop cytotoxic responses toward a target cell population expressing CD19, consisting of B lymphocytes.

Tisagen-lecleucel and axicabtagen-ciloleucel have received FDA approval in 2017 and EMA approval in 2018 for the treatment of relapsed or refractory paediatric B-cell precursor acute lymphoblastic leukaemia (B-ALL) and adult diffuse-large B cell lymphoma (DLBCL) [28, 29]. Importantly, "real-world" CAR T-cell therapy efficacy has been confirmed by independent evaluation by several academic research centres in the United States, supporting remarkable clinical benefit [30].

3 The Value of Cancer Immunotherapy

The cost of cancer care represents one of the fastest growing areas of healthcare-related spending in the United States [31] and globally. It is estimated that due to increased demand for oncology care by an aging population, prolonged survival of cancer patients and changes in oncology practise pattern incorporating newer, more sophisticated treatment options,

the total cost of cancer care is going to exceed $175 billion after 2020 [32].

The introduction of cancer immunotherapy to current oncology practise had a profound and multi-level impact on cancer-related expenditure and still represents a revolution for the current value models.

Indeed, the value of a pharmaceutical product needs to be assessed not only from the scientific and clinical standpoint but also from an economic perspective, in a similar way as the health technology assessment (HTA) is evaluated. This means that the social, economic, organisational, and ethical issues of a health intervention or health technology need to be analysed. Specifically, the value of cancer immunotherapy should be evaluated considering its effect on mortality and morbidity, on the patients' quality of life, on the potential reductions in the use of other healthcare interventions and on the cost of the intervention itself [31]. All of these factors present an undeniable economic effect.

As detailed in the previous sections, immune-based agents stimulate cancer eradication through the activation of a pluripotent immune system rather than by inhibiting individual molecular pathways. This, in addition to immunological memory, is associated with long-term benefit in a proportion of patients, some of which can be cured of metastatic disease.

Existing frameworks of value evaluation still fail to capture the positive effects of immunotherapy on a patient's quality of life. Crucial aspects in favour of the value of cancer immunotherapy are the long-term treatment-free survival following treatment with immunotherapy, resulting in dramatic improvements of the patients, as well as that of their family and communities lives, including their returning to productive work. These effects can often be recorded through patient-reported metrics of health. It is also worth noticing that responders to immunotherapy do not need additional subsequent treatment. Additionally, compared to alternative oncologic treatments, the rates and severity of adverse events (AE) are significantly lower [7, 33]. If correctly managed, these AEs can be resolved in few weeks with immunomodulating agents, such as corticosteroid treatment, without interfering either with therapeutic activity or with the patient's wellbeing [31].

Based on these evidences, the value of cancer treatment with immune-based agents should be evaluated in view of anticipated savings in the future accompanied by a dramatic improvement of the quality of life of oncologic patients [31, 34].

Reconciling the reward to innovators who bring new drugs to the market in a field where research and development presents unique challenges needs to be considered side by side to the unique clinical benefits and the "value of hope" offered by cancer immunotherapy. In this context, a patient-centric model is required to negotiate with payers the value of immunotherapy keeping in mind the inherent challenges related to the complexity of the current healthcare fiscal environment and the resulting call for sustainability.

4 The Current Landscape of Cancer Immunotherapy

A landscape analysis of the most recent clinical trials, publications, and patents in the field of cancer immunotherapy reveals an overall growth in this area, which is still characterised by a significant lag time between academic discoveries and industrial applications [35], wherein academic centres lead in target identification, target validation, and early-phase clinical trials, often with sponsorship from the National Institutes of Health (NIH); nonetheless, the last two decades have seen a substantial increase in the involvement of industrial partners, whose expertise can contribute to scale-up for clinical delivery [36, 37].

The same analysis can illustrate a topographical localisation of R&D focusing on immunotherapy, revealing that the field is predominantly US-centric, with more than 70% of the relevant patents of the field granted to US applicants. However, more recently China is also emerging not only as a lead market but also in the clinical landscape, due to the higher number of clinical trials, especially in the CAR T-cell space [30, 36]. The reduced number of CAR T-cells trials in the European Union should be addressed by the scientific community and by local healthcare policy makers [30, 38].

As it was previously described, the term cancer immunotherapy encompasses a wide range of different therapeutic agents. Currently, the most widely exploited agents are immune checkpoint inhibitors (ICI), antibodies or fusion proteins evoking antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC), often with modifications within the antibody's Fragment crystallisable (Fc) – the portion of the antibody responsible for effector functions – bispecific antibodies or fusion proteins, cytokines, adjuvants, NK cells, dendritic cells, TILs and CAR-T cells [39].

Checkpoint inhibitor, cytokines and adjuvants can be generally defined as immunomodulators. At least one representative of each of these drug products has received regulatory approval by the FDA (Table 1), mostly for advanced or treatment-resistant cancers, although immunomodulators' approval as first-line options is emerging.

Checkpoint inhibitors are by far the most widely represented class of agents. They are generally conventional antibodies, although antibody-drug conjugates and bi- or tri-specific antibodies are emerging as a second generation of immune checkpoint inhibitors. Of note, based on market records, checkpoint inhibitors mAbs are now competing with the previous generation of mAbs, some of which have dominated the scenes from 2000 onward, such as adalim-

Table 1 FDA-approved immunomodulators. Checkpoint inhibitors, cytokines and adjuvant are listed. Immunoglobulin G (IgG), interferon-alpha receptor (INFAR), Toll-like receptor (TLR)

Checkpoint Inhibitors		
Atezolizumab	"Tecentriq"	Anti-PD-L1, IgG1
Avelumab	"Bavencio"	Anti-PD-L1, IgG1
Cemiplimab	"Libtayo"	Anti-PD-1, IgG4
Durvalumab	"Imfinzi"	Anti-PD-L1, IgG1
Ipilimumab	"Yervoy"	Anti-CTLA-4, IgG1
Nivolumab	"Opdivo"	Anti-PD-1, IgG4
Pembrolizumab	"Keytruda"	Anti-PD-1, IgG4
Cytokines		
Aldesleukin	"Proleukin"	Genetically modified IL-2
Interferon alpha-2a	"Roferon-A"	Agonist of IFNAR1/2 pathway
Interferon alfa-2b	"Intron A"	Agonist of IFNAR1/2 pathway
Peginterferon alfa-2b	"Sylatron", "PEG-Intron"	Agonist of IFNAR1 pathway
Adjuvants		
Poly ICLC	"Hiltonol"	TLR ligand

Table 2 FDA-approved CAR T-cell therapies

CAR-T cells		
Axicabtagene ciloleucel	"Yescarta"	Anti-CD19
Tisagenlecleucel	"Kyrmriah"	Anti-CD19

umab (anti-tumor necrosis factor (TNF)) "Humira" and infliximab (anti-TNF) "Remicade", "Remsima", "Inflectra", rituximab (anti-CD20) "Rituxan", "MabThera", bevacizumab (anti-Vascular endothelial growth factor (VEGF)-A) "Avastin", trastuzumab (anti-HER-2/neu) "Herceptin", or palivizumab (anti-respiratory syncytial virus (RSV)) "Synagis" [40].

The main target that has been explored so far is PD-1, but immunomodulators under evaluation in clinical settings include agents directed to several immunological pathways. Pharmaceuticals targeting chemokine receptors aimed at promoting migration and recruitment of immune cells (e.g. CXCR4) or agents activating co-stimulatory pathways, such as CD40, OX40, ICOS and CD137, hold great promise. In parallel, therapeutic agents blocking immune cells suppression, such as CD73, Lymphocyte-activation gene 3 (LAG3), idoleamine-2,3 dioxygenase (IDO) and glucocorticoid-induced TNFR-related protein (GITR), are currently under clinical evaluation. An alternative approach aims to target CD47, a "don't eat me signal" on tumour cells to promote immune-mediated cancer cells clearance. As a second generation of anti-cancer adjuvants, alternative Toll-like receptors (TLRs) and stimulator of interferon genes (STING) ligands are undergoing clinical evaluation, together with agonist of the signal transducer and activator of transcription 3 (STAT3) pathway.

In parallel to the evaluation of additional targets, a further stream of preclinical and clinical research is focused not only on improving the structural and functional features of already available immune-based agents but also to develop structural alternatives thereof. This is evident from the structural modifications to the Fc portion of checkpoint inhibitor mAbs, such as atezolizumab, durvalumab, wherein the Fc was engineered to avoid ADCC. Moreover, as it is apparent from the list of approved checkpoint inhibitors (Table 1), which are characterised by a different antibody isotype, also the evaluation of the natural functional features of different antibody isotypes may prove valuable to fine-tune the desired therapeutic activity. Furthermore, the design of antibody mimetics, such as designed ankyrin repeats (DARPins) [41], Affibodies, and Anticalins, could provide therapeutic agents with improved characteristics.

Among immunomodulators, Interferon alpha-2b has received FDA approval as adjuvant therapy for patients with high risk of melanoma recurrence, paving the way for additional approvals for cancer immunoprevention [42, 43]. A parallel preventive approach, although effective only in specific cancer types, is represented by preventive vaccines directed to viruses characterised by an oncogenic potential, namely Human Papilloma Virus (HPV), such as "Cervarix" and "Gardasil", and Hepatitis B Virus (HBV) such as "HEPLISAV-B". Therapeutic vaccines are still lagging behind prophylactic vaccines: only Sipuleucel-T ("Provenge"), a vaccine composed of autologous stimulated dendritic cells, has received regulatory approval for prostate cancer.

Following the breakthrough approval of the first two CAR T-cell targeting CD19-expressing B cells (Table 2), the "adoptive therapy" landscape is characterised by substantial clinical research aiming to extend the available targets. Further strategies under investigation to treat B cells malignancies involve targeting of CD22, CD30, CD33, CD123 (also known as IL-3R), B-cell maturation antigen (BCMA), and Epstein–Barr virus (EBV)-related antigens. Alternatively, currently investigated adoptive therapies are directed to different haematological and solid malignancies. In one approach, antigens expressed only in cancer cells are targeted, such as carcinoembryonic antigen (CEA), melanoma-associated antigen (MAGE), cancer/testis antigen 1 also known as LAGE2, LAGE2B or NY-ESO-1, and tyrosine-protein kinase transmembrane receptor ROR1. Alternatively, antigens overexpressed by malignant cells are targeted, such as epidermal growth factor receptor (EGFR), the disialoganglioside GD2, glypican-3 (GPC3), receptor tyrosine-protein kinase erbB-2 (HER2), mesothelin, mucin 1 (MUC-1), prostate stem cell antigen (PSCA), prostate-specific membrane antigen (PSMA) and Wilms tumour protein (WT1).

FDA-approved oncolytic therapy treatment options are so far restricted to T-VEC ("Imlygic"), a modified Herpes sim-

plex virus (HSV) that infects tumour cells and promotes their destruction. Current preclinical and clinical research is focused on evaluating additional virus platform that could be applied to anti-cancer therapies, including Adenovirus, Reovirus and Picornavirus. Remarkably, although the potential of oncolytic virus technology has been explored early in time, the low number of patents in the field suggest that the development of this technology has been slower compared to other subfields of cancer immunotherapy [35]. A similar trend can be observed for cellular vaccines, whose clinical trials had been widely sponsored by industries until 2012. Since 2013, cellular vaccines trials have significantly declined, coinciding with an increased interest to CAR T cells [36].

5 Current Trends

The most promising developments of the fast-evolving field of cancer immunotherapy that will be dealt in detail in the following section are the present focus on combinational therapy aimed at providing synergistic anti-tumour effects, the expansion of current immune-based therapies to new therapeutic indications and the identification of predictive and prognostic biomarkers. A comprehensive overview of additional advances, including the discovery of new checkpoint inhibitors and immunosuppressive mechanisms [44], progresses in the field of T-cell trafficking to tumours [45] and the characterisation of non-synonymous mutations giving rise to neoantigens [46] is provided elsewhere [25].

5.1 Combination Therapy

It is acknowledged that treatments targeting a single molecular cancer pathway have only limited efficacy in most cancers. The results obtained with such a reductionist approach can be significantly improved by administering drug combinations that target multiple mutations and cancer pathways [43, 47].

Combination therapy is thus arising as a new land of opportunities in oncology for multiple reasons. First, the activity of different agents acting on different cellular and molecular targets, potentially with a synergistic effect, is often significantly higher compared to the single agents per se. In parallel, combination therapy can reduce the duration of the treatment, thus limiting the insurgence of treatment-resistant cancer clones and, importantly, reducing the costs and AEs associated with treatment. Additionally, it has been estimated that immunotherapy combinations may actually be less expensive than single agents if they work faster [25].

The idea of combining different immune-based agents arose soon after it was evident that checkpoint inhibitors PD-1 and CTLA-4 use slightly different mechanisms of action; the combination of the first-generation cancer immunotherapies targeting those receptors showed remarkable synergistic anti-tumour effects and has been investigated by more than 250 clinical trials so far [48]. Considering that several immunomodulatory agents have received regulatory approval and the resulting almost infinite number of combinatorial treatment regimens [30], careful preclinical and early-clinical assessment should be performed before clinical testing to avoid the selection of a combination of agents showing antagonistic effect [49] or having positive effects at the expense of safety concerns [7, 50, 51]. Simultaneous targeting of multiple pathways including CTLA-4, PD-1/PD-L1 blockade, transforming growth factor beta (TGF-β), CD40 and ICOS is expected to bring promising clinical results [30] and is generally perceived as the most potent engine for oncology progress [25, 52, 53].

Most frequently, the combined immune-based agent are branded products marketed by different pharmaceutical companies. Agreements aimed at the joint-development of combination therapies may present several advantages. On the one hand, patients can benefit from new therapeutic options undergoing clinical trials and becoming available in due course; on the other hand, the output of R&D pipelines can be maximised.

Not only can immune-based agents be combined between themselves but even combination with standard of care therapies, such as chemotherapy and radiotherapy, have been showing outstanding clinical efficacy. In general, successful combination regimen relies on the use of checkpoint inhibitors and co-stimulatory agents of various nature, provided that a baseline immune response towards tumour neoantigens is present [46]. Several exogenous strategies, such as vaccination and adoptive T-cell transfer, may be employed to create a baseline anti-tumour response [25], which can be supported by several means. Possible combination strategies are focusing on removal of inhibitory signals, by means of acting on checkpoint inhibitors or depleting Tregs, and supply of costimulatory signals, such as by blockade of CD137, CD40 and OX40, together with the manipulation of the tumour microenvironment, for instance, by interfering with TGF-β and by IDO inhibition [25].

In this regard, chemotherapy and radiotherapy were known to exert their antineoplastic effect by triggering TLR4-mediated activation of the innate immune system due to apoptotic cancer cell death [54]. This in turn activates the T-cell compartment of the adaptive immune system, resulting in enhanced anti-tumour responses. To-date immunological effects of chemotherapeutic agents, such as platinum-based drugs, are widely appreciated and the efficacy of therapeutic schemes combining chemotherapy with PD-1/PD-L1 blockade is under evaluation in more than 170 clinical trials in several cancer entities [30, 48].

Radiotherapy has also emerged as a valuable partner for immunotherapy since the description of immune-mediated inhibition of distant lesions following ionising radiation, a phenomenon known as abscopal effect [55]. This phenomenon relies on the amplification of immunostimulatory interferon-γ (INF-γ) -mediated responses that are orchestrated by tumour infiltrating dendritic cells (DC), a professional APC type, upon sensing of tumour DNA [56, 57].

As mentioned previously, responses to immunotherapy are mainly dictated by the pre-existing extent of anti-tumour responses; an additional aspect is the extent of TILs infiltration in the malignant tissue. Technological progress in the precise delivery of radiotherapy is allowing to further appreciate how inflammatory signals associated with various cell death pathways triggered by radiation can possibly convert the tumour into an *in situ* vaccine and promote the regression of metastases outside the field of irradiation, as defined by the abscopal effect [25].

Although the scientific community considers the combination of radiotherapy and PD-1/PD-L1 blockade promising, some negative results have been reported, suggesting that the specific therapeutic interventions, dosage regimens and trials design should be carefully evaluated [30].

5.2 New Indications

Recent studies have suggested that the efficacy of checkpoint inhibitors is not dictated by the specific tumour entity but by the high mutational load due to the presence of mutational defects in the DNA mismatch repair machinery, a condition that is known as micro satellite instability (MSI) [58]. This finding is not surprising considering the mechanism of action of checkpoint inhibitors. Indeed, the higher the mutational load, the higher the presentation of neo-antigens via MHC-I molecules, which would intrinsically result in improved recognition by the CD8+ T cells reinvigorated by checkpoint inhibition. Based on this observation, numerous clinical trials are currently investigating the use of checkpoint inhibitors in different cancer entities.

Conversely, a reduced efficacy is expected against tumour entities which do not express neoantigens or do not express MHC-I molecules – a known mechanism of evasion – as they could not be targeted by T cells despite substantive stimulation [30]. Intriguingly, there is preliminary clinical evidence that also tumour entities characterised by low mutational burden, such as breast cancer, could benefit from treatment with checkpoint inhibitors. As of exemplification, treatment of naïve patients affected by metastatic, triple-negative breast cancer (TNBC) with atezolizumab (anti-PD-L1) in combination with nab-paclitaxel has excitingly shown remarkable efficacy in a phase III trial [59]. Following on this observation, a considerable number of TNBC clinical trials based on targeted immunotherapy have been registered on *clinicaltrials.gov*.

On the other hand, the possibility to expand CAR T-cell therapy horizons to different tumour entities is limited by the ligand that constitutes the extracellular domain allowing targeting of target malignant cells. It is straightforward that targeting novel cancer entities requires significant R&D efforts, whereas the application of fully developed CAR T-cell agents to diverse malignancies of the same cell type could be easier. For example, putatively all B-cell malignancies can be targeted by CAR T employing CD19 as targeting ligand. Indeed, the success of CAR T cells in ALL and DLBCL triggered to the initiation of follow-up trials in these disease entities; clinical trials directed to chronic lymphocytic leukaemia, multiple myeloma and gastrointestinal cancers are also underway. However, as it was detailed in the previous sections, the intrinsic sophisticated complexity of the CAR T-cell technology results into fundamental challenges when aiming to extend its therapeutic indications.

It should be noticed that a new field of therapeutic indication is opening for checkpoint inhibitors, whose application was restricted by standard oncology care to advanced tumour stages, usually consisting of metastatic stage tumours. Remarkably, it is more and more appreciated that improved efficacy is associated with a low tumour burden upon treatment initiation [60]. Thus, treatment with immune-based agent after surgery, a clinical practise known as peri-operative use, is emerging as a promising treatment option.

A similar approach is known as neo-adjuvant therapy and is directed to prime systemic immunity towards tumour antigens (i.e. before primary treatment) aiming to promote long-term tumour surveillance after complete resection of the tumour. This application needs to take into account a correctly orchestrated treatment regimen to allow T cell priming by APCs when neoantigens would still be present [61].

However, it should be reported that several controversial observations were described regarding the application of adjuvant and neo-adjuvant therapy. The FDA approved adjuvant treatment with Ipilimumab for melanoma patients after tumour resection despite the high frequency of reported AEs [62–64]; contrariwise, the EMA approved nivolumab for the same indication, given the lower occurrence of reported AEs [65]. This concept is supported by recent translational findings from an early clinical study in patients with resectable melanoma: in a randomised phase Ib study, neoadjuvant treatment with nivolumab and ipilimumab induced a higher number of tumour-specific T-cell clones than adjuvant treatment [66]. These promising observations, further fuelled by the correlation between improved efficacy of neo-adjuvant therapy and the presence of MSI, are possibly at the basis of the current increase in trials comprising neo-adjuvant treatment with immune-based agents [30]. Despite the current landscape, the previous scepticism towards such treatments

should be carefully considered, stimulating investigators to wisely select patients who may benefit from neo-adjuvant treatment based on specific knowledge-based biomarkers, such as minimal residual disease (MRD) by circulating tumour DNA (ctDNA) [30].

5.3 Identification of Predictive and Companion Biomarkers

A major challenge in cancer immunotherapy is the ability to predict efficacy of a given treatment in different patients, given the intrinsic intra- and inter-tumour heterogeneity and variability.

A new frontier of cancer immunotherapy, aiming to maximise the efficacy of the treatment, is the identification of biomarkers. Clinical biomarkers may have diagnostic, predictive, prognostic, or pharmacogenomic value. They could allow a better stratification of patients, classify responders and non-responders, predict outcome and identify patients more likely to develop AEs. Clinically relevant biomarkers support medical decisions and promote a personalised application of immune-based therapeutic schemes, hopefully resulting into increased level of therapeutic successes and reduced side effects.

It should be noted that practical considerations accompany a sound biological rationale for the sake of a broad application of a given clinical biomarker, such as the applicability of the proposed analytical methodologies. This is one of the reasons underlying the fact that to date only few predictive biomarkers for cancer immunotherapy treatments have been robustly validated [47].

For example, the determination of PD-L1 expression by immunohistochemistry on tumour tissue biopsy was approved by the FDA as a diagnostic test to select patients eligible for treatment with therapeutic agents targeting PD-1/PD-L1 axis. However, potential limitations of this biomarker are the variable expression of PD-L1 in a single tumour and by the lack of harmonisation between available assays [31]. Additionally, the observation that PD-L1 expression does not categorise all patients who could potentially benefit from anti-PD-1/PD-L1 therapy calls for the identification of additional and more predictive biomarkers [67].

A parallel approach for predicting responses to checkpoint inhibitors blockade is the determination of MSI, especially by assessing a deficient mismatch repair (dMMR). As detailed in the previous sections, MSI and dMMR determine an increased tumour mutational burden (TMB), which in turn results into an increase in the number of neoantigens. The ultimate biological effect of this phenomenon is a substantive infiltration and activation of pre-existing tumour-specific CD4+ and CD8+ T cells, which render tumours susceptible to checkpoint inhibitors blockade [68]. This approach was approved as a biomarker test for pembrolizumab, in the context of the previously cited target-agnostic indication. However, it was also reported that MSI and dMMR do not always correlate with increased TMB. In contrast, considering that TMB can be observed in the absence of MSI and dMMR, for instance in carcinogen-induced tumours [68, 69], further investigations are needed to assess in which instances MSI and dMMR can be employed as predictive biomarkers.

The identification of reliable, precise companion diagnostic assets is thus an area of current focus both for already marketed and for future immune-based treatments.

A promising approach relies on assessing parameters that could be representative of the tumour's immunogenicity and of the underlying anti-tumour immunity. Accordingly, it was attempted to combine the aforementioned biomarkers to improve their predictivity. However, it was observed that a correlation between TMB and PD-L1 expression is absent [70]. Indeed, a combination of nivolumab and ipilimumab is superior to chemotherapy in patients with high TMB, irrespective of PD-L1 expression [71].

Additionally, correlative data have been generated by measuring changes in target immune cell populations, analysing inflammatory TNB-associated gene expression signatures indicating infiltration by specific immune cell subsets (e.g. myeloid-derived suppressor cells, Treg, effector T cells) and the activation of specific signalling pathways (e.g. INF-γ) [72]. Alternatively, the detection of neoantigens generated by gene fusions has been recently explored to predict responses in patients with low TMB [73]. A significant translational effort is to still to be performed to bring these approaches to the patients' bedside.

An inherent complexity of such approaches resides, in that a tumour biopsy needs to be performed. This implies logistic challenges when repeated biopsies need to be taken and analysed. Additionally, the search for predictive and prognostic biomarkers should not be limited to the tumour itself but should go beyond the malignant lesion. It is thus clear that the identification of soluble biomarkers in peripheral blood would be immensely advantageous and would increase patients' compliance. To this aim, several soluble biomarkers have been identified to predict positive clinical outcome in advanced melanoma patients receiving anti-CTLA-4 Ipilimumab, including C-reactive protein, lactate dehydrogenase, soluble CD25 and vascular endothelial growth factor (VEGF) [74].

PD-1 and PD-L1 are also detectable in peripheral blood in their soluble forms. However, recent studies have questioned the aptitude of soluble PD-1 and soluble PD-L1 as biomarkers for checkpoint blockade [75]. On the contrary, ctDNA is emerging as a suitable biomarker for TMB measurement, early response prediction, pseudo-progression versus disease progression and MRD assessment [30].

However, the identification of genomic mutations poses several technical challenges per se. Indeed, even for routine clinical testing it is necessary to apply sophisticated analytical techniques characterised by high sensitivity and by the possibility to test multiple genomic mutations simultaneously (multiplexing). High-throughput next-generation sequencing (NGS) technologies have thus overcome classic Sanger sequences for biomarker screening, especially considering that NGS technologies can be applied both to customised gene panels and to whole-exome, whole-genome or transcriptome panels [47].

Importantly, genotyping via customised gene panel suits to the challenges inherent to the clinical environment, which include limited availability of biopsy tissue, limitations regarding sample preparation and rapid timeframe required for therapeutic decisions [76]. Only recently, more sophisticated techniques such as whole-exome sequencing are emerging for clinical use. Their application is still limited due to their complexity and to the associated costs, but it is expected that they will become more widely used in the near future. Broad applicability of NGS techniques for biomarker discovery and routine analysis requires the clinical setting to acquire digital capability to handle, analyse and interpret a large quantity of complex genomic data [47].

However, it should be reported that current biomarker-driven trials are designed to allocate to targeted therapies patients whose tumours express the specific driver mutations. Therefore, only the excluded patients will receive immunotherapy. There is thus a need to design future biomarker-driven trials to include immune-based biomarkers [31].

An intriguing scenario suggests that immune-related AEs could also be considered biomarkers for tumour response [77]. Additional studies aimed at evaluating the independency and predictive power of AEs will be carefully monitored by the scientific and industrial community.

The identification of reliable predictive and prognostic biomarkers is expected to play a fundamental role also in guiding the selection of suitable combination approaches. Although predictive and companion biomarkers are considered to be crucial to guide optimisation of the cost and value of cancer immunotherapeutic agents [31], the development of companion diagnostic development lags behind therapeutics, creating scientific and regulatory complexity.

6 Challenges

6.1 Toxicity Management

The use of immune-based therapeutic agents, similarly to any other therapeutic intervention, is associated with AEs. However, a potential barrier to the application of cancer immunotherapy is the concern about its toxicity [25]. Most importantly, unanticipated AEs can dramatically impact not only on a drug product development but also on the valuation of the company itself, as it has occurred to the CAR T-cell-developer Juno Therapeutics [36].

The AEs associated to cancer immunotherapy belong to two main categories: immune-mediated side effects and positive interference with the tumour growth.

In some instances, reactivation of the immune system may sustain the proliferation of tumour cells and cancer stem cells via the production of growth factors. Such deleterious AEs may be prevented by careful *a priori* patient evaluation; importantly, a wise selection of appropriate combination regimens can potentially reduce these AEs in a substantial manner [25]. An additional concern is represented by the integration of the signals triggered by an immunotherapeutic intervention into the complex circuits of the immune system. Indeed, as it was mentioned in the previous sections, several mechanisms maintain a balance between immune activation and immune suppression, thus fine-tuning immune responses. Thus, it is expected that after treatment with ICIs T-cell activation will be gradually dampened by regulatory mechanisms in order to reach homeostasis. However, a sustained action of regulatory mechanisms could result in a temporary or permanent suppression of anti-tumour activity, even resulting in resistance to future activation [25].

Conversely, immune-mediated AEs derive from extensive T-cell activation and present similar characteristics to autoimmune symptoms, including colitis, autoimmune hepatitis, cytokine release, capillary leak syndromes, endocrine or neurological side effects. Immune-mediated AEs require immediate treatment with glucocorticoids to prevent permanent damage [43, 78]. Given that immune-mediated AEs have been considered as predictive biomarkers for response, there is a significant concern regarding the mitigation of immune-mediated effects, as this intervention could result in reduction in therapeutic efficacy. Therefore, there remains a need to investigate the impact of glucocorticoid treatment on the therapeutic efficacy of immune-based agent.

These observations will be fundamental to increase the confidence of patients and physicians dealing with immune-based agents, with a special regard to special patients' populations. Indeed, there is preliminary evidence that cancer immunotherapy could be effective and tolerated also in patients with pre-existing disorders affecting the immune system, such as autoimmune diseases and acquired immune-deficiency syndrome (AIDS) [43]. However, before extending indications to those populations, toxicity management protocols should be robustly validated.

Furthermore, compared to treatment with antibody-based immunotherapy, cellular approaches are still limited to specialised centres, putatively due to the concern that these therapies may present toxicity and may be difficult to manage

and costly. It is expected that the remarkable improvements regarding the safety and affordability of CAR T cells and TILs therapies will result in a wider applications [25].

Thus, it is expected that the broader application of cancer immunotherapy in preclinical and clinical settings will allow to decipher its short- and long-term interference with the physiology of the immune system, hopefully leading to improvements not only of the management of AEs but also on the treatment regimens themselves.

6.2 Tumour Heterogeneity and Resistance to Treatment

It is well established that continuous acquisition of aberrant genomic and subgenomic mutation is an hallmark of cancer [79]. Several models have been proposed to decipher how progressive mutations contribute to the development of a heterogeneous population of cells in a malignant lesion.

Considering that several anti-cancer agents are directed to specific targets expressed by malignant cells, tumour heterogeneity is *per se* a cause of therapeutic failures, because cells not expressing the target or expressing a mutated target will be resistant to treatment. The treatment itself, by applying a selective pressure on the tumour, may promote additional heterogeneity due to the exploitation of different cancer signalling networks by different resistant clones.

In general, mechanisms of acquired resistance either reactivate a cancer pathway, or involve secondary genomic mutations in the drug target, or activate alternative signalling pathways; in parallel, epigenetic and transcriptional changes can also play a role [43]. These mechanisms have been widely studied in the context of traditional anti-cancer drugs but a detailed understanding of how tumour heterogeneity and acquired resistance may impact on cancer immunotherapy is still to be achieved.

On the one hand, checkpoint inhibitors, by reactivating pre-existing anti-tumour responses, which are considered to be polyclonal, should be able to target effectively heterogeneous tumour lesions. In a similar manner, infusion of TILs is expected to be able to target heterogeneous cells populations. However, it should be noted that no prediction on efficacy can be made without assessing quantitatively and qualitatively the extent and breath of pre-existing anti-tumour response. Similar evaluations still appear to be inapplicable to clinical settings. Additionally, cancerous lesions may develop resistance to therapies aimed at reinvigorating the immune response by developing strategies to escape immune cell recognition.

On the other hand, approaches like CAR T-cell therapy and anti-cancer vaccines may be significantly affected by tumour heterogeneity and by the development of resistance, because the absence of the target will inevitably result in lack of efficacy.

Recently, the mechanism of resistance were investigated in an exploratory study performed on tissue biopsies from patients with advanced melanoma who became resistance to pembrolizumab treatment [80]. The findings of this study revealed that cancer cells developed resistance mechanisms responsible for evasion and resistance to T-cell-mediated immunity. Among these, mutations of β2-microglobulin (*B2M*), a component of the MHC-I, were able to affect the presentation and the recognition of tumour-antigens by CD8+ T cells, thus impairing cancer cell killing.

This approach clearly shows that the availability of tumour biopsies during the course of treatment could be fundamental to understand resistance mechanisms and apply second-line treatment regimen. However, due the inherent challenges associated with the acquisition of biopsies, the evaluation of less invasive biomarkers is a priority to the field.

6.3 Clinical Development, the Path to Regulatory Approval and Beyond

Cancer immunotherapy is a highly innovative field and comprises some of the so-called advanced therapy medicinal products (ATMPs); the definition of ATMP is particularly suited to adoptive immune cells therapy. Accordingly, cancer immunotherapy requires innovative approaches to trial design, risk-benefit assessments and market access. Hence, many challenges reside in how to balance rapid access to immune-based agents for cancer treatment and establishing new metrics for evaluation in clinical and regulatory settings.

Differently to chemical products or biologicals, ATMPs cannot be standardised and thus require other means of evaluation for product safety, efficacy and potency. Challenges relevant to cancer immunotherapy clinical development include the complexity in designing and interpreting of clinical trials [81, 82] and the selection of appropriate patient populations.

Complex manufacturing processes [81, 83–85] – which is a very common issue in protein production and formulation [86] – and the implementation of Good Manufacturing Practices (GMP) and stringent testing to personalised therapeutic agents, specifically for cell and gene products [87, 88], increase the complexity of the development for immune-based agents. In particular, the logistic complexity of autologous therapies (e.g. TILs and CAR T cells) requires the product to be process in a centralised GMP facility and returned to the treatment centre for infusion into patient; this aspect is expected to increase costs and negatively impact uptake by clinicians [36].

Over and above, heterogeneous regulatory national procedures at member-state level [84] and uncertain reimburse-

ment schemes, which are decisive to determine commercial success [36], are additional prominent challenges that developers of immune-base therapies have to face.

Of note, it has been recently estimated that in Europe 65% of ATMP developers are small- and medium-sized entrepreneurs (SME), while only 35% are large developers. It was also reported that ATMP developers – and especially SMEs – face difficulty with the regulatory requirements as they lack the expertise to address the country-specific requirements deriving, for instance, from different national interpretations of the EU regulation [89].

Since regulatory agencies appreciate the remarkable contribution that cancer immunotherapy is giving to the current therapeutic opportunities of patients affected by cancer, early-stage cooperation between all the parties involved is critical for success in cancer treatment development. Indeed, the regulatory landscape has been acting dynamically so as to promote rationalisation of the path to regulatory approval for immune-based agents. For example, a key initiative facilitating ATMP development was the adoption of European ATMP legislation (Regulation [EC] 1394/2007), which established the Committee for Advanced Therapies (CAT) within the EMA. The CAT is emerging as valuable partner in this field being responsible for assessing quality, safety and efficacy of advanced therapy products. Similarly, the EMA launched a scheme, called PRIME, to enhance support for the development of pharmaceuticals targeting unmet medical need, by promoting early dialogue between the parties and allow an optimised development plan and an acceleration on evaluation.

It is well-established that clinical development represents the most critical phase of a pharmaceutical product's lifecycle for several reasons, which include the challenge residing in the design and interpretation of clinical trials and in the associated costs, which may be considerable. Failure of clinical studies to prove efficacy of a given asset is an enormous risk, which could be mitigated as much as possible by careful preliminary evaluations.

In order to design a successful clinical roadmap, it is advisable to define as early as possible an integrated development roadmap, meaning that the planning of the regulatory process should be started at the earliest convenience and should be integrated with all other aspects of the development process.

The same applies to all other aspects of clinical development; indeed, the strategy for patients' selection should be defined early in development by choosing between an "individualized approach" (e.g. molecular phenotyping) or a subgroup analysis (e.g. expression of a given marker). Given that eligibility criteria based on molecular phenotypic result in an approach analogous to the one personalised medicine, the number of patients who can enrol to the trial and be eligible for treatment will be lower. This may result into poor predictive power of the trial and reduced revenues due to the low number of patients but can be counteracted by high levels of efficacy if the biomarker is highly predictive for efficacy.

This aspect corroborates the current need of predictive, reliable biomarkers to optimise the result of immune-based therapies. On the other hand, despite the trend for a more science-driven individualised approach, the current approach relies on precise patients' stratification, whereas the applicability of purely personalised approaches is still questioned. Additionally, with an increasing number of available biomarkers and assays thereof, independent validation will become a strict regulatory requirement.

It should be noted that in 2017, the FDA approved for the first time a treatment based on a biomarker (genome instability of the tumour) rather than an organ-specific tumour type, paving the way for further similar approvals worldwide.

The unique mechanism of action of immune-based agents creates a challenge for use of traditional efficacy endpoints used to assess clinical benefit of chemotherapy and other cytotoxic agents [90]. The choice of the study objectives and the timing of the assessment are critical, as effective immune response may need more time to develop, and pseudoprogression is often observed [91]. Although clinical benefit is often observed by analysing the tail end of the Kaplan–Meier survival curves, which is characteristic of immunotherapy and can be interpreted as cancer-free survival, it is necessary to avoid prolonged studies to pre-empt the arousal of confounding factors and to reduce costs. Thus, it is also advisable to include among the clinical study endpoints immune-related criteria and the assessment of the immune memory-mediated long-term disease-free survival [43, 92]. Another challenge involves the assessment of efficacy of anti-tumour therapies targeting specific alterations or pathways; due to tumour heterogeneity only a small cohort of patients will be eligible for such treatment, resulting in long-lasting, challenging clinical trials [47]. Therefore, in order to shorten time to market access for patients, it is needed to wisely design clinical trials and to sensibly outline endpoints for rapid assessment of clinical benefit.

An additional layer of complexity derives from the wide application of combinational therapy, where multiple agents are either combined in a sequential manner or co-administered, where even minor differences in the treatment regimen can dictate the trial's success or failure. In this context, assessment of efficacy might become difficult, especially when one agent is significantly more active than the other.

Given that cancer immunotherapy is considered from the regulatory standpoint as any other therapy, regulatory assessment is focused on establishing its risk-benefit profile. It is thus evident that minimisation of the risks associated to access to therapy is required to obtain regulatory approvals;

however, in a fast-evolving field like cancer immunotherapy risk perception and acceptance of uncertainty change as new therapeutic agents get approved. Furthermore, regulators in general require that the risks identified during the evaluation of a marketing authorisation (MA) should be minimised and/or further characterised via a post authorisation safety study (PASS).

In general, it is advisable to design clinical trials to allow collection of samples to perform *post hoc* analysis aimed at identifying biomarkers, comparing assays, and exploring mechanisms of resistance. This approach can drive further innovation which may result into future successful trials. An additional indication is the introduction of patients' questionnaires to evaluate the extent of minor AEs and the effect on the patients' and communities' quality of life; of note, this approach is compliant with the emerging patient-centric vision of cancer immunotherapy.

An additional challenge associated to cancer immunotherapy, which is characterised by a unique risk-benefit profile, is represented by the choice of information to include in product information brochures in order to facilitate both impartial evaluations from clinicians, patients and HTA.

Special considerations apply to cellular therapies, such as CAR T cells. In a scenario where each patient will receive a distinct therapeutic agent, given the autologous nature of the transplanted immune cells, significant challenges arise from the complex logistics for manufacturing and delivery of the product, including transport, import/export, and qualification of process changes where each batch correspond to a different patient. As cellular therapies are considered ATMPs, a risk-based approach is required to enable the control and management of the risks related to the product and manufacturing process, in which a potency assay reflecting the clinical mechanism of action is a crucial parameter. Thus, a strong emphasis on potency and quality is a prerequisite for cellular therapies approval. A comprehensive and detailed primary analysis of challenges encountered by ATMPs developers in Europe is reviewed elsewhere [89].

6.4 Intellectual Property

Immune-based therapeutic agents usually derive from a substantial innovative effort. Rewarding innovators by providing protection to novel inventions against competitors can be achieved by a smart approach to intellectual property (IP) rights.

Indeed, the understanding of the IP landscape in the field of cancer immunotherapy is crucial to define strategies aimed at securing market access and market position, protecting assets from being counterfeited, anticipating possible conflicts to either avoid or exploit them or produce income by royalty payments [39].

Patents and trade secrets appear as the most valuable type of IP rights in the current cancer immunotherapy landscape. Trade secrets are practices or processes by which a party can obtain an economic advantage over competitors, for example the production of an innovative product. Trade secrets, as long as they are not disclosed to the public, grant an unlimited exclusivity to the innovator. However, if the innovative product or process is, even inadvertently, disclosed to the public or if it is easily reverse-engineered, no formal regulation can impair competitors to reproduce the innovative asset. In this regard, it should be noted that it is compulsory to disclose to the public detailed information related to a pharmaceutical product, for example in the regulatory documents and dossier that are submitted in order to obtain a marketing authorisation or in investigator brochures. Hence, a trade secret would be inapplicable to this setting. Additionally, there are also ethical concerns regarding the use of trade secrets in pharmaceutical settings, where the non-disclosure of information could prevent scientific progress and technical development that could be advantageous for patients. Notwithstanding these aspects, trade secrets could be valuable to protect specific technical improvements related to the manufacture of a product, especially when the improvements themselves cannot be protected by a patent.

In contrast to trade secrets, patents grant the right to exclude third parties to make, use, sell, offer to sell, and import an invention for a limited period of time (usually 20 years) and in a limited territory, in exchange for the public disclosure of the invention. Patents can be seen as a mutual contract between an inventor and the public, where the public can benefit from the public disclosure of the invention, which can fuel further innovation, and the inventor can profit from the commercial exploitation of the invention, whose revenues can pay back previous R&D costs and be reinvested in developing additional innovative products.

However, not all inventions are patentable. Inventions need to be new, not obvious and to have an industrial applicability. Additionally, in some jurisdictions, such as in Europe, specific inventions, such as methods of treatments are excluded from patentability, in order to allow medical practitioners to perform such methods of treatments without risking infringement of a patent. Conversely, medical products *per se* are patentable in most jurisdictions.

Given the costs associated with R&D, the private sector will not undertake such investments without the existence of some significant commercial upside to counterbalance the considerable risks of failure. It is thus clear that a solid patent protection is mandatory to ensure exclusive rights on a product, allowing inventors to advance their research objectives and to achieve the commercial availability of a new pharmaceutical product [93].

As it was described in the introduction, the development of most of the current immunotherapeutic agents originates

from the discoveries performed by several academic research centres, where inventions often result from collaborations and scientific cross-fertilisation between different researchers. Moreover, the R&D track leading to some of the current ground-braking cancer immunotherapy agents was very circuitous, being characterised by collaboration between multiple companies and research centres. Thus, despite a few players dominating the field, the current landscape still appears dispersed, with multiple acquisitions, transfer of rights, licensing and collaborations agreements having occurred [35]. These factors have contributed to render the current patent landscape of immune-based agents intricate.

In particular, the field of immune checkpoint inhibitors appears to be especially convoluted [39].

For example, the IP rights involved in some of the agreements that were fundamental for the development of the anti-CTLA-4 antibodies ipilimumab from Bristol–Myers Squibb's (BMS) and tremelimumab from Pfizer have shaped their R&D, clinical and commercial route. In detail, the first patent portfolio covering anti-CTLA-4 antibodies originated at the University of California Berkeley from the work of Allison. CTLA-4-related patents were sublicensed to a company called Medarex, which generated the first human anti-CTLA-4 antibody, later called ipilimumab. Medarex also established a collaboration with Pfizer, who had a parallel anti-CTLA-4 program, which included the future tremelimumab; the agreement involved cross-licensing of relevant patents, wherein Medarex was eligible to obtain milestones and royalty payments for sales of any Pfizer anti-CTLA-4 antibodies based on the patents originating from Allison's work. Soon after, while Medarex became a subsidiary of BMS, Pfizer discontinued their program, which was later restarted when tremelimumab was in-licensed by AstraZeneca. It is clear that, if tremelimumab would have been able to reach the market before the patent term expiry of the Allison's portfolio, the sale of tremelimumab would be subject to royalty payment to BMS [39].

The patent landscape related to PD-1 is even more intricate, wherein seminal discoveries resulting in patents directed to PD-1 and PD-L1 were achieved in parallel by several researchers including Tasuku Honjo from Kyoto University, Gordon Freeman from Dana-Farber Cancer Institute, Arlene Sharpe from Harvard Medical School and Lieping Chen from Mayo Clinic. The key players in the field of anti-PD-1 antibodies are BMS and Merck. BMS by acquiring Medarex and collaborating with Ono Pharmaceuticals had access to Honjo's patent estate, which covers broad methods of treatment by administration of anti-PD-1 antibodies. A few months before the approval of ipilimumab, jointly developed by BMS and Ono, pembrolizumab, an anti-PD-1 antibody by Merck was approved by the FDA. This intertwined path resulted in litigation between the parties for patent infringement, as Merck's asset was falling in the broad claims of Honjo's patent estate; the lawsuit came to end in 2017 with Merck agreeing terms to settle the dispute. It should be noted that assuming that Merck's pembrolizumab meets expectations of becoming a "blockbuster" product, the upfront payment and royalties could be considerable [39].

In contrast, the field of anti-PD-L1 antibodies is considerably less conflict-prone. Indeed, despite several players having products in this space, including Genentech, AstraZeneca, MerckSerono and BMS, none of the patents covering those assets comprises broad claims which could interfere with third parties' activities. The reason for this narrow scope resides in the fact that the patents directed to a broad method of treatment by administration of anti-PD-L1 antibodies, which originated from the work of Freeman, were non-exclusively licensed to several parties, all of which thus have freedom to operate in this field [39].

The patent landscape of cellular immunotherapy differs substantially from the checkpoint inhibitor's one. Indeed, given the intrinsic personalised nature of cellular immunotherapy, patent protection is not generally directed to the pharmaceutical product *per se* but usually to constructs, vectors and associated methods that are necessary to obtain a cellular immune-based drug product, such as a CAR T-cell agent. The main IP actors in the CAR T-cell space have been the University of Pennsylvania and St. Jude's Children's Research Hospital, with substantial contributions from their commercial partners Novartis and Juno Therapeutics. These parties have been recently involved in a litigation over the above-described IP [36].

Based on these examples, it is clear how a strategically established patent portfolio is a prerequisite for success in the crowded space of immune-based therapeutic agents. *Ab initio* commitment and diligent planning are required to take advantage of a patent estate. IP can be exploited in a defensive manner, meaning as a tool to aim at market exclusivity by excluding competitors from the market or as an offensive tool, for instance to create revenues by out-licensing or royalties payment. Either ways, expert judgement and advice is needed during the whole life cycle of a product, in order to capture the value of inventions in strong patent claims and in wise negotiation of collaboration and licensing agreements.

Patents are also of primary importance in the protection of the latest innovations of the field. Accordingly, the commercial value of patents covering the use of specific predictive and prognostic biomarkers or kits for detecting the same is significant, given that, in several instances, the testing of a biomarker may be mandatory for the immune-based drug to be granted a MA or to be reimbursed [39]. It could be expected that also combination therapies would be the subject of a separate category patents in the field of cancer immunotherapy. Conversely, considering that patents directed to a new pharmaceutical product usually also

encompass claims directed to optional combinations with standard drugs, patents explicitly directed to combinations *per se* may be considered redundant, and are therefore quite rare. An exception to this trend is observed in patents covering assets developed by small biotech companies: in this case, a patent directed to a combination with a well-established drug product from a large pharmaceutical company may be a favourable factor in supporting a potential collaborative research and development agreement between the two companies [39].

A noteworthy challenge to the protection of established IP rights is represented by the fact that the exclusivity granted by a patent estate may be circumvented through special provisions granted by the World Trade Organization (WTO)'s agreement on intellectual property, known as the TRIPS (Trade-Related Aspects of Intellectual Property Rights) Agreement. In exceptional circumstances, a government may allow a third party to produce a patented product without the consent of the patent owner, upon compensation of the patent owner. This provision, known as compulsory licensing, has been introduced to international patent law to ensure access to innovative products in low-income countries, especially in emergency or extreme urgency. However, due to the high costs associated to cancer immunotherapy drug products, there is the possibility that also medium- and high-income countries might advocate compulsory licenses to grant patients access to innovative pharmaceutical products [94].

On the other hand, a crucial aspect that needs to be considered when analysing the impact of IP on cancer immunotherapy is the compensation that specific countries can put into practise to reward innovators for the development of innovative products, especially in the pharmaceutical field.

One example is represented by pilot programs aimed at implementing procedural methods to prioritise examinations of patent applications directed to cancer immunotherapy. It should be noted that the procedure needed to obtain a granted patent is usually long and expensive [93] and may both discourage inventors to file patent applications and delay market access of innovative products. The first and foremost illustration of such initiatives is the "Cancer Immunotherapy Pilot Program" from the United States Patent Office (USPTO). This initiative sets an expedite examination procedure, not requiring any added fees, for patent applications which meet stringent criteria and have at least one claim to a method of treating a cancer using immunotherapy [93]. Since the beginning of the program in 2016, as of January 2019 over 300 petitions requesting participation in the fast-track program have been filed and over 100 patents have been granted. This success has prompted the USPTO to extend the program until June 2020.

Another example is represented by the supplementary protection certificate (SPC) that is available in member-states of the European Union to extend the patent term related to a particular medicinal product. An SPC aims to compensate a patent owner for part of the patent term that was lost due to time needed to obtain a MA. An SPC can extend the term of a patent for up to 5 years, thus granting an additional time frame of exclusivity. Similarly, a request for patent term adjustment (PTA) is available in the United States to compensate for delays caused by the U.S. patent office during the prosecution of a U.S. patent application. Additionally, innovators can qualify for advantageous governmental incentives based on their patent estates. Such measures, usually known as patent box or innovation box, aim to incentivise R&D by applying a lower taxing regimen to patent revenues compared to other commercial revenues.

7 Conclusions

Cancer immunotherapy has progressed from its conceptual design to breakthrough clinical applications [95] and exciting further developments are supported by the pipeline of several pharmaceutical companies, which include new therapeutic paradigms such as personalised medicine [96, 97], combination therapy [98], novel delivery methods [99], biomaterials [98] and new diagnostic procedures [100].

Based on the trajectories of the last decades, wherein clinical translation of immunotherapy was characterised by lengthy translational timelines, false starts and by iterative cycles of scientific research [36], the industrial perspective on cancer immunotherapy is directed to maximise pipeline's value by applying a smart strategy not only to the early phases of drug discovery and preclinical development but also to clinical development and life cycle management.

Similar to the typical drug product development, the current cancer immunotherapy landscape is characterised by industry-driven development of assets directed to targets that have been extensively validated by the academia. It is expected that strategic partnerships and in-licensing of promising assets will become more and more frequent, with academia or smaller biotech companies providing validated assets at the interface between preclinical and phase I clinical trials and the pharmaceutical industries contributing with their expertise to scale up for late-phase development. The application of this scheme may substantially reduce the time to market and the risks associated to R&D, as the pharmaceutical industry will commit to clinical development only of the "best in class" assets, avoiding the risk of long, unsuccessful and expensive early-phase discovery.

Careful planning is required to maximise value and outcome, with the involvement of a multidisciplinary team of experts focussed on integrating all the mandatory stages of

drug development into a smart strategy. In line with this approach, there is a tendency to establish collaborations with regulators and players early in clinical development, following the motto "start with the end in mind". Another aspect of this value-oriented strategy is represented by extensive activities involving predictive and prognostic biomarkers. This appears to be a leading tendency in cancer immunotherapy, which may substantially maximise treatment value by reducing – or even abolishing – side effects, and by allowing drug administration to responders only – thus reducing the overall cost of the therapy.

Additionally, the development of cancer immunotherapy represents a milestone in the introduction of personalised medicine, not only to the field of cancer but also to the broader pharmaceutical landscape. Fighting cancer by means of invoking the immune system, whose resources pertain by definition to each individual, implies that allogenic therapies stimulating the immune system (such as immune checkpoint inhibitors and anti-cancer vaccines) may also be considered as a personalised approach. This concept, as it was detailed in the previous sections, presents several challenges and opportunities.

Allogenic therapies are more analogous to standard biopharmaceuticals and, due to significant cost reductions associated with scale in manufacture, quality control and release of a single batch that could be used to treat multiple patients [36], currently they are more appealable to the pharmaceutical industry. This is in contrast to autologous products, such as CAR-T cells and TILs, whose complex value chain still represents a barrier to their extensive application. Despite this additional layer of complexity, the recent approval of CAR T cell therapies is of fundamental importance not only because it paves the way for additional approvals but also because the logistics of the application of such therapies to "real-world" settings will be closely observed by several actors of the pharmaceutical arena, including companies focussed on regenerative medicine. Despite some concerns related to manufacturing, delivery models and cost-effectiveness of autologous therapies, the main focus of the private sector will be the development of more advanced methods for genetic manipulation of immune cells and their bioprocessing [36].

A parallel approach to maximise the value of current cancer immunotherapy is represented by the extensive translational and clinical efforts aimed to broaden the indications of already approved agents. Indeed, by exploiting the physiological polyclonal nature of immune responses, the reactivation of immune system through immunomodulators may result in anti-cancer activity towards a broad spectrum of tumour types. In a similar manner, exploiting the interconnected mechanisms of immune regulation by combining therapeutic agents targeting complementary pathways holds great promise for the treatment of tumours which acquire resistance to therapy. It is expected that this strategy will be fostered by collaboration agreements and joint development between key players that hold exclusive rights in respect to the therapeutic agents amenable for use in combination [35].

The overwhelming curative potential of cancer immunotherapy explains the current enthusiasm and the extensive investments in the field by the public and private sector. During the last decade, the first line of immune-based agents has emerged in clinical trials and in regulatory approvals, with remarkable benefit for patients. Nevertheless, many challenges still need to be overcome to make it universally available. Thus, the clinical community impatiently looks for a second generation of cancer immunotherapy which could be able to address the current challenges facing the field [30].

8 Future Perspectives

The challenges that cancer immunotherapy is facing at present also bring exciting opportunities for further technological innovations.

9 The Tumour Microenvironment

The present generation of immune-based agents acts by targeting immune cells or cancer cells as entities isolated from their context. Despite the remarkable efficacy of current cancer immunotherapy, the understanding of the mechanisms of immune-mediated tumour clearance within the tumour microenvironment (TME) is fundamental to establish a second generation of therapeutic options.

It is acknowledged that the TME, which consists of cancer cells, stroma, vascular elements and infiltrating immune cells, is a complex milieu characterised by an immunosuppressive nature [101]. Cancer cells have been shown to deliver immunosuppressive signals via exosomes and soluble factors, including cytokines, chemokines and inhibitory factors, which are unique to each individual tumour. The resulting level of immunosuppression is generally correlated to T-cell dysfunction and thus to tumour aggressiveness [25].

The success of immune checkpoint inhibitors suggests that interfering with TME-mediated immunosuppressive mechanisms is a valuable therapeutic strategy against cancer. Hence, a second-generation immune-based agents targeting immunosuppressive pathways within the TME is undergoing extensive investigation. In addition, analysis of the immunomodulatory and pro-/anti-inflammatory factors expressed by a tumour may guide the therapeutic intervention targeting the malignant lesion.

Besides CD8+ T cells, the target of current cancer immunotherapy, other immune cells may become primary targets of immune-based therapy, namely Treg and myeloid-derived suppressor cells (MDSC).

Tregs infiltrating the TME are highly immunosuppressive and contribute to impairment of CD8+ T-cells responses. The effect of current checkpoint inhibitors on tumour infiltrating Treg cells is still controversial, and further studies are needed to assess how anti-PD-1 and anti-CTLA-4 therapy impact on this T-cell subpopulation, considering that the maintenance of Treg cells is necessary to safeguard tissue homeostasis. Targeted therapies successful in depleting only tumour infiltrating Tregs may be of great clinical significance.

MDSCs have been shown to promote tumour progression by secretion of inducible nitric oxide synthase, reactive oxygen species (ROS), IL-23, TGF-β, and prostaglandin E2 (PGE2) [25]. Thus, therapies aimed either at depleting MDSC or at blocking their immunosuppressive secretome may represent an important component of novel anti-cancer therapeutic options. The strategies investigated so far include inhibition of IDO, the prevention of MDSC trafficking to the malignant lesion by blocking specific chemokines, targeting colony stimulating factor 1 receptor (CSF1R) on MDSC [102] and blocking IL-23 [103].

These considerations will become crucial also to design therapeutic strategies to enhance efficacy of novel CAR T-cell therapies in the context of solid tumours [30, 104].

10 Technical Developments

Considerable technical developments are expected to improve current therapeutic agents, especially in order to ameliorate their safety profile. Similarly, modifications to mAb scaffolds to fine-tune the drug's pharmacodynamic/pharmacokinetic profile, also CAR T-cells scaffolds are undergoing engineering processes. Improvement of CAR T cells safety profile could be obtained via modification of the CAR itself [105] or by molecular switches inducing programmed cell death [106, 107].

In parallel, the application of new molecular biology technologies, such as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), is expected to maximise the throughput and the accessibility of such personalised approaches.

An additional stream of development is represented by research focussing on improving the delivery of immune-based agents to tumours. Such improvements could maximise efficacy and reduce systemic toxicity, resulting in significant benefit for the patients.

11 The Digital Revolution

It was previously described that the identification of novel predictive and prognostic biomarkers is one of the main trends in cancer immunotherapy. It is estimated that non-invasive monitoring and omics-based tests and the broad concept of precision medicine will soon converge within cancer immunotherapy. However, the increasing number of patient-related data does not directly correlate with a more straightforward diagnosis or prognosis. Conversely, a new digital expertise needs to be established in the landscape of cancer immunotherapy to take full advantage of the wealth of data that will become available in the near future thanks to the broad application of NGS technologies for cancer biomarker screening.

In addition to the technical obstacles due to the data-rich technologies *per se*, the challenges related to the management of large datasets need to be carefully considered, starting from the design of appropriate digital architectures ensuring protection of sensitive information and the establishment of the ownership of data [108]. This last aspect is particularly crucial considering that large datasets are currently seen as a valuable basis for drug discovery and development and could represent crucial assets under evaluation in agreements between pharmaceutical companies. Conversely, the scientific community calls for maintaining publicly available databases to sustain research [43]. Preliminary efforts in this direction have been performed by The Cancer Genome Atlas and the International Cancer Genome Consortium [43, 47].

Most of these issues still need to be resolved through close collaboration between the public and the private sectors. In particular, because the legal aspects concerning patients' data storage and analysis, especially by means of machine learning and artificial intelligence (AI), are still unclear, the healthcare system is expected to either downgrade the enthusiasm regarding the application of data science to clinical practise or to proactively invest in the realisation of a legal and technical framework [108].

12 Integration of a Patient-Centric Model

In order to fully define the value of cancer immunotherapy, patient outcome perspective is emerging as a valuable source of data. Indeed, the definition of the value of immune-based therapies would be incomplete if the patients' perspective is not integrated to the evaluations performed by the other counterparts within the healthcare system.

A valuable example of the transition toward a patient-centric model consists of the incorporation of patient-reported outcomes (PROs) in clinical trials. PROs represent the report of the patients' health status performed by the patients themselves and it is widely recognised that PROs are usually accurate in revealing clinical benefit, AEs and changes in disease-related symptoms.

By increasing the engagement of patients in the course of the trial, compliance to the therapeutic scheme can be improved. In this context, it was also estimated that monitoring of clini-

cally relevant symptoms via PROs could improve quality of life and reduced emergency room visits, with an overall increase of the quality-adjusted 1-year survival rates among cancer patients [109]. The same report also suggest that PROs can also address health disparities of patients [31, 109].

An additional strategy to implement a patient-centric model is the engagement of patient advocacy organisations in the discussion regarding patients' and family's needs and regarding disease-specific issues [31].

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