

RESEARCH

CANCER

Immuno-oncology agents for cancer therapy

SOPHIE CARTER & DAVID E THURSTON

Abstract: Until recently, cancer therapy comprised of four main types of treatment: surgery, radiotherapy, chemotherapy and targeted therapy. Over the past decade, immuno-oncology (IO) has emerged as a novel and important approach to cancer treatment through the stimulation of the body's own immune system to kill cancer cells. This newly recognised method of treating cancer is rapidly developing, with many accelerated approvals by the US Food and Drug Administration and European Medicines Agency in 2019.

Several therapeutic classes have emerged within IO, and are the focus of this review article. In particular, the immune checkpoint inhibitors have had remarkable success across multiple malignancies, and are the most well-established therapeutic class of IO agents to date. Biomarker testing for the programmed death-ligand 1 (PD-L1) checkpoint target has been developed and is now obligatory before treatment with pembrolizumab (Keytruda, Merck) when used for non-small-cell lung carcinoma, gastric cancer, head and neck squamous cell carcinoma and cervical cancer, as well as before treatment with atezolizumab (Tecentriq, Roche) when used for urothelial carcinoma. However, ambiguity remains as to the relevance of PD-L1 expression for checkpoint inhibition therapy for other tumour types. More

recently, combining IO agents with conventional therapies has been evaluated with some significant improvements in patient outcomes.

While IO agents are rapidly changing the standard of care for those with cancer, there are still many challenges to overcome in terms of managing their toxicities and ensuring that healthcare systems, such as the NHS, can afford the high cost of these therapies. The IO pipeline also includes chimeric antigen receptor T-cell therapies and cancer vaccines, both of which show great promise for the future but have their own unique toxicity and cost-effectiveness issues.

Keywords: biomarkers; cancer; immune checkpoint inhibitors; immuno-oncology; oncology.

Original submitted: 13 April 2019;

Revised submitted: 30 June 2019;

Accepted for publication: 10 October 2019;

Updated: 20 April 2020

Published online: 7 May 2020;

doi: 10.1211/PJ.2020.20207825

Introduction

Cancer incidence rates have steadily increased over the past 20 years, while mortality rates have shown a considerable decline¹. Although significant variation in survival rates is still observed across cancer types (i.e. there are more 200 distinct diseases recognised), for most types, survival is improving owing to earlier diagnosis and improved treatments^{2,3}.

Treatment has undergone a slow evolution from its start in the 1800s, with the sequential development of four main recognised modes of treatment. The first was surgery, which was made possible after the discovery of general anaesthetics in the late 1800s⁴. This was a revolutionary development because it was the first time the disease could be completely eradicated as long as the tumour was small and well-defined.

The second development was radiotherapy, established at the end of the 19th century, which utilises X-rays and/or G-rays to damage the DNA within tumour cells, thus blocking essential biochemical processes and leading to cell death⁴.

The third development, chemotherapy, was discovered in the 1940s, during World War II, when it was observed that individuals exposed to mustard gas suffered myelosuppression⁴. Clinicians speculated that patients with proliferative diseases (e.g. leukaemia) might benefit from treatment with agents of this type that kill highly proliferating cells. Crucially, introduction of the first

chemotherapy agents (analogues of nitrogen mustard gas) meant that cancers which were more complex or had metastasised, and could not be successfully treated by surgery or radiotherapy, could now be addressed. Furthermore, chemotherapy agents have since been developed that work at different stages of the cell cycle, and can be used in combination to prevent the development of resistance.

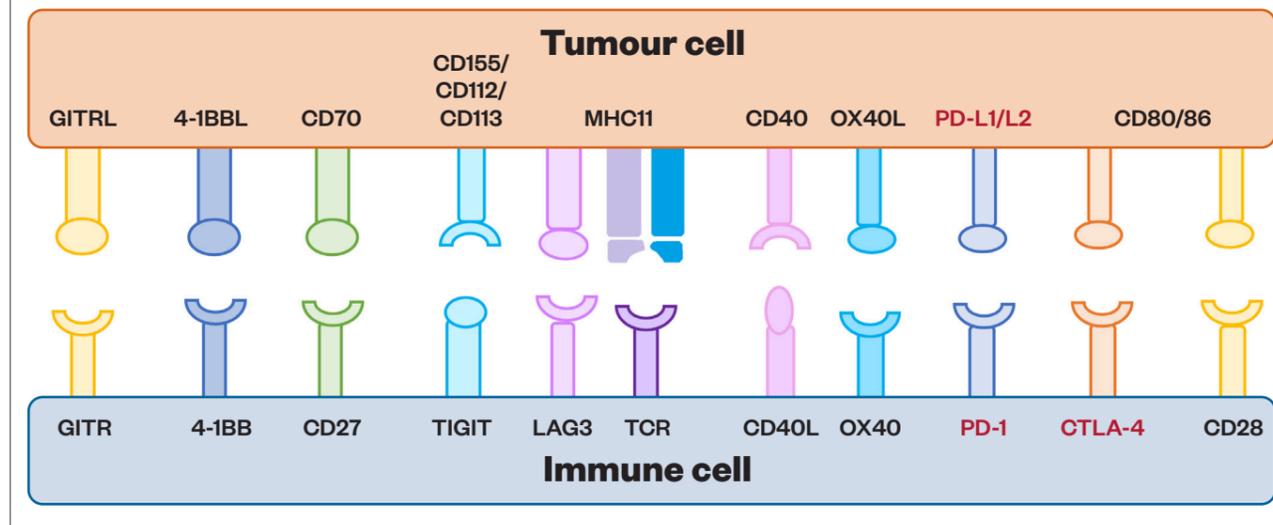
The fourth development was targeted cancer therapies (also known as precision therapies). This was established with the discovery of imatinib (Glivec; Novartis) in the late 1990s – a small-molecule kinase inhibitor targeted to the mutant BCR-ABL protein present in the tumour cells of patients with chronic myeloid leukaemia (CML), but not in their healthy cells⁵.

This concept of using modern structural biology and drug discovery methods to produce small molecules, proteins, antibodies and even cellular therapies designed to target unique biomarkers associated with tumour cells, but not healthy cells, is now considered to be the 'gold standard' approach for discovering new cancer treatments. Currently, four major treatment modes – surgery, radiotherapy, chemotherapy and targeted agents – are frequently used in combination to ensure that all cancer cells are eradicated from the body. During the past decade, the first immuno-oncology (IO) treatments (e.g. checkpoint inhibitors) have emerged, which work by harnessing the body's own

FIGURE 1

Some of the main ligands and receptors present on the surface of tumour and immune cells that are targets for approved and emerging immuno-oncology therapies

These targets are exploited by tumours to evade the immune system, and so represent an opportunity for immuno-oncology (IO) intervention. Ligands and receptors are represented as ball and cup structures, respectively, with approved therapies highlighted in red and emerging therapies highlighted in black. Other emerging IO targets are not located on the surface of cells, and so cannot be represented in this diagram (e.g. indoleamine 2,3-dioxygenase, which is an intracellular enzyme)



immune system to kill tumour cells⁶. They are presently showing great promise in the clinic, and are the main focus of this review.

Immune checkpoint proteins are found on the surface of T-cells and act as regulators of the immune system. They are crucial for self-tolerance, and prevent the immune system from attacking the body's own cells indiscriminately, thus allowing a distinction to be made between 'self' and 'non-self'⁷. Immune checkpoints also play a vital role in preventing uncontrolled immune responses by modulating the duration and amplitude of a physiological immune response, thus preventing collateral damage, which is why the term 'off-switch' is sometimes used to describe their role. It is known that tumours adopt certain immune checkpoint pathways as a mechanism to evade an immune response towards them⁷. For example, some tumour cell types express these proteins on their surface to disguise themselves as 'self', allowing them to go unnoticed by the immune system and promoting tumour progression⁸.

PD-1 (programmed death 1) is an example of an inhibitory checkpoint receptor protein found on the surface of T-cells that normally acts as an 'off-switch' after interaction with the PD-1 ligand (PD-L1), a protein expressed on the surface of normal cells. However, PD-L1 is expressed by many types of tumour cells and upregulated in some, thus activating the 'off-switch' and protecting the malignant cells from an immune attack^{9,10}. Immune checkpoint inhibitors (ICPIs), such as the anti-PD-1/PD-L1 agents, prevent the interaction between PD-L1 on tumour cells and PD-1 on T-cells, allowing the immune system to launch an anti-tumour response.

Many observers believe that, over the next decade, IO agents could become the fifth acknowledged cancer treatment modality^{11,13}. Some of the main ligands and receptors present on the surface of tumour and immune cells that are targets for approved and emerging IO therapies are summarised in Figure 1.

History of immuno-oncology

It has long been known, but is now increasingly appreciated, that tumour cells can be recognised and disabled by the immune system. Some tumours show evidence of spontaneous regression early in their development, suggesting that the immune

system may be capable of recognising and eliminating early-stage tumour cells¹⁴.

Observation of spontaneous remissions in patients led to the foundation of the area of IO. A spontaneous remission is defined as a reduction in severity of, or disappearance of, the signs and symptoms of a disease, without any apparent cause and in the absence of treatment. This is most often noted in patients who have recently had acute infections, especially when this results in fever which appears to stimulate the immune system. It is now recognised that, in some cases, the immune system is capable of completely eliminating a tumour. Spontaneous remissions have been observed in most cancer types, but most frequently in advanced melanoma, renal cell carcinoma (RCC) and urothelial carcinomas, although the phenomenon has also been reported in breast cancer, neuroblastomas, some sarcomas and embryonal cancers¹⁵.

William Coley was the first to investigate the potential for IO, and successfully treated malignancies based on immune stimulation in the 1890s¹⁶. After discovering that cancer patients who contracted post-surgical infections seemed to improve faster than those who did not, he investigated the use of bacteria to stimulate and enhance the body's natural immune response to fight cancer. Through these studies, he later developed Coley's toxin, which was based on attenuated bacteria and is thought to be the first known IO therapy¹⁷.

A later development involved the Bacillus Calmette-Guerin (BCG) vaccine, originally produced in the early 1900s for use against tuberculosis (TB), and first used therapeutically for TB in the 1920s. However, its role in cancer therapy dates back to 1929 when a reduced incidence of cancer among patients with TB was observed at autopsy¹⁸. Experiments revealed that BCG produced a profound stimulation of the mononuclear phagocyte system (also known as the reticuloendothelial system), which was recognised as an important defense against cancer. Furthermore, it was observed that neonates who had been immunised with BCG had a significantly lower incidence of leukaemia later in their lives¹⁶.

This background and basic understanding of IO sparked an interest in the use of BCG for other types of malignancies, in

particular bladder cancer. Early investigations demonstrated responses in patients with melanoma metastatic to the bladder when treated with intravesical BCG. In light of this success, work in animal models led to publication of the results of the first successful clinical trial of intravesical BCG in patients with recurrent bladder cancer¹⁶.

It is now understood that intravesicularly administered BCG attaches to bladder tumours and urothelial cells via specific fibronectin and integrin receptors. Following internalisation by macropinocytosis, the mononuclear phagocyte system is stimulated by the BCG, inducing a local inflammatory response characterised by the infiltration of granulocytes, macrophages and lymphocytes. Important elements of the humoral immune response to BCG include the interleukins (ILs) IL-1, IL-2, IL-6, IL-8, IL-10, IL-12, tumour necrosis factor alpha (TNF- α) and interferon gamma (INF- γ)¹⁹. More recently, studies have shown that BCG contains high levels of CpG oligodeoxynucleotide motifs that are known to induce the TNF-related apoptosis-inducing ligand (TRAIL) through IFN production¹⁶. Intravesical BCG is still indicated for the treatment and prevention of recurrence of some types of non-invasive bladder cancers^{20,21}.

Classification of immuno-oncology agents

The categorisation of IO agents is challenging and there is significant crossover and ambiguity with emerging agents. The classification devised and utilised throughout this review is represented in Tables 1–4. For example, ICPis (see Table 1) are sometimes classified separately to monoclonal antibodies (mAbs; see Table 2), yet the ICPis are, themselves, monoclonal antibodies. The Cancer Research Institute takes two broad approaches to classification based on treatment type or cancer type²². Few observers employ the three very broad categories that have emerged over the years: non-specific cytokines, cancer vaccines and mAbs²³.

Another approach is to classify IO agents from a mechanistic perspective as ‘active’ or ‘passive’. However, this is perhaps too simplistic, as it does not properly reflect the many possible complex drug–host–tumour interactions^{24,25}. In this review, passive naked mAbs, such as the ICPis (see Table 1) and those directed at other external and internal cellular targets (see Table 2), are grouped adjacently, while conjugated mAbs (i.e. antibody–drug conjugates and immunotoxins; see Table 3) and active therapies (see Table 4) are classified separately.

The mAbs form the largest and best-characterised group of passive IO agents. Within this broad group are the ICPis (see Table 1), which constitute the most promising emerging area at present. Several active immunotherapies are licensed as IO agents (see Table 4), and these fall into four groups: immunomodulatory agents, cancer vaccines, oncolytic viruses and CAR-T cell therapy. The latter is a newly emerging therapy that is generating significant interest. It involves the collection of T-cells from cancer patients followed by their *ex vivo* modification and re-administration to the same patient. Currently there are only two approved CAR-T cell therapies (Yescarta, Kite Pharma; and Kymriah, Novartis), although many more are in the pipeline^{26,27}.

Pharmacogenomic and precision medicine approaches to immuno-oncology

Drug discovery and development in the IO area is moving rapidly toward a pharmacogenomic approach, where biomarkers are identified in biopsy material from tumours so that predictions can be made about which therapies would be the most efficacious for a given patient^{28,29}.

A recent retrospective study in a large number of pancreatic cancer patients (n=1,856) highlighted the significant effect a precision medicine approach can have on survival, particularly in cancer types with poor outcomes¹⁷². In this study, it was shown that

patients with actionable mutations (including some associated with checkpoint inhibitors) who received matching targeted therapies had longer overall survival times by up to 1.07 years compared to those receiving only unmatched therapies, respectively.

For the main families of IO agents, the anti-PD-1/PD-L1 and anti-cytotoxic T-lymphocyte-associated protein-4 (anti-CTLA-4) agents, the clinical data relating to target expression and response to therapy is complex. For example, there are reports of responses to treatment irrespective of PD-L1 expression. There is also ambiguity around the thresholds used to define ‘positive’ and ‘negative’ biomarker expression values. For example, for PD-L1, ‘weak positive’ is defined as 1–49% expression and ‘strong positive’ as greater than 50% expression³⁰. These broad definitions suggest that PD-L1 is not a clear dichotomous biomarker, and there is a need to find new biomarkers for IO treatments with increased specificity and reproducibility. The PD-1/PD-L1 biomarker assays available, the response rates in PD-L-positive and PD-L-negative patients, and emergent biomarkers are briefly addressed below.

PD-1/PD-L1 biomarker assays

The PD-L1 ligand, which is expressed on the surface of some tumour cell types, is a vital molecular target for around half of all ICPis approved to date. Binding of this ligand to PD-1 receptors, expressed on the surface of T-cells, blocks their inhibitory activity toward tumour cells. PD-L1 is also expressed by various normal cells but is up-regulated in tumour cells and tumour-infiltrating immune cells, thus protecting them from an immune response³¹. Therefore, testing patients for tumour cell PD-L1 expression may lead to better clinical outcomes if they are selected for treatment with anti-PD-L1 agents. Early clinical studies investigating PD-L1 expression and the subsequent response of patients to the anti-PD-L1 agent nivolumab (Opdivo, Bristol-Myers Squibb) demonstrated the potential benefit of pharmacogenomic testing; in PD-L1-positive patients the objective response rate was 36%, while in PD-L1-negative patients there were no responses³². However, later reports from other clinical trials (e.g. NCT01642004, NCT01668784 and NCT02008227) showed that positive responses with prolonged overall survival can occur (compared with current standards of care) in PD-L1-negative patients^{33–36}. Therefore, based on the results of meta-analyses of clinical trial data, it is evident that PD-L1 expression status alone is insufficient to determine whether patients should be offered PD-1 or PD-L1 therapy³³.

Nivolumab (anti-PD-1) and atezolizumab (anti-PD-L1) were both approved without PD-L1 testing, while pembrolizumab (anti-PD-L1) is approved for first-line treatment of non-small-cell lung cancer (NSCLC) only after testing patients for PD-L1 expression. An immunohistochemistry (IHC) test is used to determine biomarker expression, with a threshold set for first-line clinical use of the agent. PD-L1 expression must be greater than 50% using the Dako 22C3 IHC assay, whereas for second-line treatment only greater than 1% expression is required. However, another study has shown that patients with 5% or more positivity do not have a benefit over standard chemotherapy²⁸. Complementary PD-L1 tests have been approved by the US Food and Drug Administration (FDA), but are not mandatory other than for pembrolizumab (which is a companion test; see Table 5)³⁷. There are currently four other companion PD-L1 assays in development for PD-1/PD-L1 inhibitors²⁸.

It has been postulated that PD-L1 blockade can re-activate rare tumour-reactive T-cells. This can result in cytokine secretion leading to induction of multiple positive feedback loops and enhanced antigen presentation, increasing the visibility of tumour cells to T-cells³³. Furthermore, the PD-L1 pathway can protect tumours from cytotoxic T-cells, disrupting the cancer immunity cycle by preventing the priming and activation of cytotoxic T-cells, and by up-regulation of PD-L1 on dendritic cells,

thus resulting in deactivation of cytotoxic T-cells³⁸. Therefore, it may be more important to establish whether the PD-1/PD-L1 pathway is active in the tumour rather than focusing solely on expression of the PD-L1 ligand³³.

Despite the ambiguity surrounding PD-L1 as a biomarker, there are currently both companion (i.e. mandatory prior to commencing treatment; currently only approved for pembrolizumab) and complementary (i.e. intended to provide an aid to clinical decision making, but not a prerequisite to prescribing) tests approved for use prior to anti-PD-1/PD-L1 therapy (see Table 5).

A pilot project called ‘Blueprint’ has been launched through a collaboration between pharmaceutical companies, the FDA and several oncology organisations in an attempt to clarify some of the concerns relating to PD-L1 IHC assays (e.g. the cut-off values for PD-L1 positivity, the interchangeability of different assays and data reproducibility). Initial results suggest that assays may vary in performance, and that there is potential for false-positive or negative results with assays of this type. In particular, the Blueprint project compared the analytical performance of the four validated assays and found that three (i.e. the 22C3, 28-8 and SP263 assays) produced similar outcomes based on a tumour proportion score, although immune cell staining was poor²⁸. One harmonisation study revealed that the scoring of tumour cells was reproducible, yet staining patterns were not similar in all situations, and the scoring of immune cells gave low concordance³⁹. Multiple studies have suggested that the 22C3 and 28-8 tests may be used interchangeably, while SP142 and SP263 may not²⁸.

Response rates in PD-L1-positive and PD-L1-negative patients

There are multiple reports of PD-L1-negative patients responding to anti-PD-L1 IO agents, and immunostaining of tumour biopsies has revealed a possible explanation for this^{40,41}. There can be significant heterogeneity of expression of PD-L1 across an individual biopsy, with areas of no or very low PD-L1 expression but others with very dense expression⁴². Therefore, a patient may be categorised as PD-L1-negative if the area analysed from a biopsy shows no staining, whereas other regions of the tumour missed during the biopsy may have dense PD-L1 expression. Thus, it may not be possible to conclude from a single biopsy whether a patient is definitively PD-L1-negative or PD-L1-positive. While a higher level of PD-L1 expression has been associated with more favourable response rates to anti-PD-1/PD-L1 agents in some studies, positive responses have also been observed in some PD-L1-negative patients. Therefore, PD-L1 does not appear to offer binary discrimination of responsiveness⁴³.

Another possible contributory factor is that PD-L1 is not a static biomarker, but is dynamic with the degree of expression dependant on many biological processes. For example, there are genetic-based mechanisms that lead to constitutive PD-L1 expression, although expression can also be induced by the presence of T-cells⁴⁴. Therefore, a tumour may be PD-L1-negative at a given point in time because there is no T-cell infiltrate, but this situation may be reversed owing to an immune response that itself may be stimulated by treatment with IO agents.

Finally, biomarker heterogeneity of expression can arise owing to a variety of other factors, including: the stage of disease; prior treatments (e.g. type of chemoradiotherapy); tumour mutation status (e.g. PD-L1 expression in NSCLC is regulated by several oncogenic drivers, such as estimated glomerular filtration rate and anaplastic lymphoma kinase, that can alter expression levels); and concomitant medication use (e.g. corticosteroids)⁴⁵.

Emergent biomarkers

There are too many emergent biomarkers in the IO area to describe in detail in this review; however, some examples are outlined below.

Tumour mutational burden (TMB) is a measure of the number of mutations within a tumour genome, and a high TMB has been shown to be associated with a favourable outcome for ICPis. For example, many tumours that respond to anti-PD-1 agents (e.g. melanoma, NSCLC and bladder cancer) have a high mutational load²⁸. Some studies have attempted to correlate mutational load in NSCLC and melanoma with a response to ICPis, but the results have been unable to prove that a high mutational load alone enhances the response to therapy, therefore, its clinical utility is presently unclear⁴⁶.

Although clinically validated biomarkers for predicting response to pembrolizumab include PD-L1 expression (in specific tumour types) and high microsatellite instability (MSI-H; independent of tumour type), several emergent IO-related biomarkers associated with improved overall response rate (ORR) and progression-free survival (PFS) for ICPis are being studied. These include T-cell-inflamed gene expression profile (GEP), TMB and mutated mismatch repair (MMR) genes. PD-L1 and GEP are both inflammatory biomarkers associated with a T-cell-inflamed tumour microenvironment, whereas MSI-H and TMB are indirect measures of tumour antigenicity derived from somatic tumour mutations. In a 2018 study of more than 300 advanced solid tumour and melanoma samples from across 22 cancer types from four KEYNOTE clinical trials, Cristescu *et al.* assessed the potential for TMB and T-cell-inflamed GEP to jointly predict clinical response to pembrolizumab, with patients stratified into four biomarker-defined clinical groups of: GEP low/TMB low; GEP low/TMB high; GEP high/TMB low; and GEP high/TMB high²⁸. The analysis showed that TMB and inflammatory biomarkers (i.e. T-cell-inflamed GEP and PD-L1 expression) can jointly stratify human cancers into groups with different clinical responses to pembrolizumab monotherapy, and that TMB and inflammatory biomarkers independently predict response and may be associated with neoantigenicity (the formation of new antigens not previously seen by the immune system) and T-cell activation, respectively.

Overall, these studies found that longer PFS was observed for patients with high TMB and GEP values with a modest correlation between the two, although TMB and GEP could also predict response independently. However, determining TMB in tissue samples has several limitations, including heterogeneous sample characteristics and a dependence on the timing of the assay. Furthermore, assays used to evaluate TMB have not been standardised, and the definition of ‘high’ TMB varies significantly across different studies⁴⁷. Most clinical studies carried out to date have been based on a variety of techniques making it difficult to compare available data and collate sufficient evidence to support its clinical use⁴⁸.

Finally, it has long been established that loss of function mutations in the MMR pathway are associated with favourable responses towards PD-1 blockade therapy, hence the interest in using MMR as a biomarker to predict responses. In an expansion of a proof-of-concept study reported in 2017 investigating disease progression in patients with MMR deficiencies across 12 different tumour types, all patients had been previously treated with pembrolizumab for anything up to 2 years⁴⁹. Positive results were noted across all tumour types, with 77% of patients attaining disease control for at least 12 weeks, including 18 who had complete responses. Therefore, MMR deficiency is now considered a viable biomarker for patient selection for treatment with pembrolizumab⁴⁹.

CD45RA is an example of an emerging biomarker for anti-CTLA-4 agents. Its baseline expression level in T-cells has been found to correlate with clinical response to anti-CTLA-4 agents. Patients with higher numbers of CD45RA+ cells compared to CD45RA+ cells in both CD4 and CD8 T-cell compartments responded more effectively to anti-CTLA-4 treatment. The

TABLE 1
Approved immune checkpoint inhibitors as of April 2020

Continued from page S4

Immuno-oncology agent	Type	Target	Mechanism of action	Indications (with approval dates)
Ipilimumab (Yervoy, Bristol-Myers Squibb)	Human (IgG1)	CTLA-4	CTLA-4 plays an important role in the regulation of T-cell activity. Inhibition of CTLA-4 blocks T-cell inhibitory signals induced by the CTLA-4 pathway, therefore releasing the brake on inhibition. This results in proliferation and activation of T-effector cells which can mobilise and mount an immune response against tumour cells. Selective depletion of T-regulatory cells at the tumour site leads to an increase in intra-tumoural T-effector/T-regulatory ratio, thus driving tumour cell death.	<ul style="list-style-type: none"> ● Late stage melanoma (second-line; March 2011 (US Food and Drug Administration [FDA] and European Medicines Agency [EMA])) ● Unresectable or metastatic melanoma (age 12 years and over; July 2017) ● Adjuvant treatment of melanoma (October 2015) ● Intermediate and poor risk advanced renal cell carcinoma (in combination with nivolumab; 2018 [FDA]) ● Colorectal Cancer (relapsed or refractory microsatellite instability high/mismatch repair deficient in combination with nivolumab; 2018 [FDA])
Tremelimumab	Fully humanised (IgG2)	CTLA-4	As above for ipilimumab	<ul style="list-style-type: none"> ● Malignant mesothelioma (2015 [FDA] — orphan drug)
Nivolumab (Opdivo, Bristol-Myers Squibb)	Human (IgG4)	PD-1	Binds to PD-1 receptor preventing interaction with PD-L1/2 antigens on APCs/ other cells in the tumour microenvironment. PD-1 is a negative regulator of T-cell activity, thus when inhibited by anti-PD-1 agents potentiates T-cell proliferation and cytokine secretion.	<ul style="list-style-type: none"> ● Adjuvant in melanoma, with lymph node involvement or metastatic disease after re-section (June 2015 [EMA], 2014 [FDA]) ● Non-small cell lung cancer (NSCLC) / metastatic disease (2015 [FDA]) ● Metastatic renal cell carcinoma (RCC; 2015 [FDA]) ● Classical Hodgkin lymphoma (2016 [FDA]) ● Metastatic/recurrent squamous cell carcinoma of the head and neck (SSCHN; 2017 [FDA]) ● MSI-H/dMMR metastatic CRC (2017 [FDA]) ● Hepatocellular carcinoma (2017 [FDA]) ● SCLC (2018 [FDA]) ● RCC (2017 [FDA] — first line in combination with ipilimumab)
Cemiplimab (Libtayo, Regeneron and Sanofi)	Human (IgG4)	PD-1	As above for nivolumab	<ul style="list-style-type: none"> ● Metastatic cutaneous squamous cell carcinoma (2018 [FDA])
Pembrolizumab (Keytruda, Merck & Co Inc.)	Human (IgG4)	PD-1	As above for nivolumab	<ul style="list-style-type: none"> ● Advanced melanoma / adjuvant treatment of melanoma (in adults with lymph node involvement who have undergone complete resection. Approved by the EMA in October 2018 for adjuvant melanoma therapy; 2014 [FDA], 2015 [EMA]) ● Metastatic NSCLC (2015 [FDA], 2018 first line in October 2018 in combination with paclitaxel or nab-paclitaxel, expanded monotherapy 2019) ● Recurrent SSCHN (2016 [FDA]) ● Classical Hodgkin lymphoma (2017 [FDA]) ● Metastatic urothelial carcinoma (2017 [FDA]) ● MSI-H or dMMR unresectable or metastatic solid tumours (2017 [FDA]) ● Metastatic gastric or gastroesophageal junction (GEJ) adenocarcinoma (PD-L1+; 2018 [FDA]) ● Recurrent or metastatic cervical cancer (second-line, PD-L1+; 2018 [FDA]) ● Primary mediastinal large B-cell lymphoma (PMBCL; relapsed after 2 or more prior lines of therapy; 2018 [FDA]) ● Hepatocellular carcinoma (2018 [FDA]) ● Merkel cell carcinoma (2018 [FDA]) ● cervical cancer (2018 [FDA]) ● Stage III NSCLC (first-line, PD-L1+, not amenable to surgery or chemo-radiation; 2019 [FDA]) ● Advanced RCC (2019 [FDA] — first line in combination with Inlyta) ● Metastatic SCLC (2019 [FDA]) ● Recurrent locally advanced or metastatic squamous cell carcinoma of the oesophagus (2019 [FDA]) ● Endometrial carcinoma (2019 [FDA] — in combination with lenvima) ● BCG-unresponsive, high-risk, non-muscle invasive bladder cancer (2020 [FDA])
Avelumab (Bavencio, Merck kGaA)	Human (IgG1)	PD-L1	Binds to the PD-L1 antigen preventing interaction with PD-1/CD80 receptors, thus removing the suppressive effects of PD-L1 on CD8+ T cells and allowing a cytotoxic T-cell response to prevail. An induction of NK cell-mediated direct tumour cell lysis via the ADCC mechanism can also occur.	<ul style="list-style-type: none"> ● Merkel cell carcinoma (MCC; 2017 [FDA and EMA]) ● Urothelial carcinoma (if disease progression during or following Platinum-based chemotherapy; 2017 [FDA and EMA]) ● Advanced RCC (in combination with chemotherapy; May 2019 [FDA])
Durvalumab (Imfinzi, Astra Zeneca — Med Immune)	Human (IgG1)	PD-L1	As above for avelumab	<ul style="list-style-type: none"> ● Locally advanced (Stage III) NSCLC (non-progressive while on chemotherapy; 2017 [EMA], 2018 [FDA]) ● Metastatic urothelial carcinoma (progression during or following Platinum-based chemotherapy; 2017 [FDA])
Atezolizumab (Tecentriq, Roche/Genentech Ltd)	Human (IgG1)	PD-L1	Atezolizumab binds directly to PD-L1 and produces dual blockade of PD-1 and CD80, thus releasing PD-L1/PD-1-mediated inhibition of T-cell activity. The PD-L2/ PD-1 interaction remains.	<ul style="list-style-type: none"> ● Urothelial carcinoma or Metastatic NSCLC (if disease progression during or following Platinum-based chemotherapy; 2016 [FDA], 2017 [EMA]) ● NSCLC (First line approval in December 2018 in combination with bevacizumab (Avastin), carboplatin and paclitaxel; 2018 [FDA]) ● NSCLC in combination with chemotherapy for initial treatment (2019 [FDA]) ● Extensive stage small cell lung cancer (first-line, in combination with chemotherapy; 2019 [FDA]) ● Unresectable, metastatic triple-negative breast cancer (first-line, PD-L1+; 2019 [FDA])

Sources: Cancer Research Institute²², Ann Onco²⁷, US Food and Drug Administration³³, European Medicines Agency³⁴, DrugBank³⁵, Biochemistry³⁶, Oncologist³⁷, Biochemical Pharmacology³⁸, Journal of Pharmaceutical Sciences³⁹,

Journal of Antibiotics⁴⁰, Tetrahedron Letters⁴¹, Bristol Myers Squibb⁴², Cancer Research Institute⁴³, BMJ⁴⁴, European Society for Medical Oncology^{45/47/51}, The Melanoma Letter⁴⁶, P T⁴⁸, Pharmaceutical Technology⁴⁹, Bristol Myers Squibb⁵⁰

TABLE 2

Approved immuno-oncology agents directed against external and internal targets as of April 2020

Continued from page S6

Immuno-oncology agent	Type	Target	Mechanism of action	Indication
Alemtuzumab (Lemtrada; Campath, Genzyme)	Humanised (IgG1)	CD52	Selectively binds to CD52 which is expressed at high levels on T- and B-lymphocytes. Following treatment with Alemtuzumab, rapid and long-lasting depletion of CD52-bearing B and T cells occurs. The mechanism is not fully understood.	<ul style="list-style-type: none"> ● B-cell chronic lymphocytic leukaemia (B-CLL; 2001 [accelerated approval] 2007 [regular approval] — FDA)
Rituximab (Rituxan; Mabthera, Genentech)	Chimeric (IgG1)	CD20	Binds specifically to the Type I CD20 antigen on pre-B and mature B lymphocytes which is expressed on >90% of the B lymphocytes of B-cell non-Hodgkin lymphoma patients. Binding results in lysis and death of the B-lymphocytes.	<ul style="list-style-type: none"> ● CD20 positive non-Hodgkin lymphoma (November 1997 [FDA], 1998 [EMA]) ● Chronic lymphocytic leukaemia (CLL; February 2010 [FDA]) ● First line maintenance use in follicular lymphoma (also approved for Wegener's granulomatosis and microscopic polyangiitis in 2011 [FDA], and for pemphigus vulgaris in 2018 [FDA]; January 2011 [FDA])
Tositumomab (Bexxar, GlaxoSmithKline)	Murine (IgG2a)	CD20	Binds specifically to the Type I CD20 antigen on pre-B and mature B lymphocytes which is expressed on >90% of the B lymphocytes of B-cell non-Hodgkin lymphoma patients. Binding results in lysis and death of the B-lymphocytes.	<ul style="list-style-type: none"> ● Non-Hodgkin lymphoma (2003 [FDA] — not EMA approved)
Obinutuzumab (Gazyva/ Gazyvaro, Roche)	Humanised (IgG1)	CD20	Binds specifically to the Type I CD20 antigen on pre-B and mature B lymphocytes which is expressed on >90% of the B lymphocytes of B-cell non-Hodgkin lymphoma patients. Binding results in lysis and death of the B-lymphocytes. Obinutuzumab binds to Type II CD20, activating intracellular death signalling pathways and inducing Antibody-Dependent Cellular Cytotoxicity (ADCC).	<ul style="list-style-type: none"> ● Previously untreated CLL (2014 [EMA], 2013 [FDA]) ● Follicular lymphoma (Orphan medicine 2015 — [EMA], 2016 [FDA]) ● Treatment of naive CLL in combination with Imbruvica (2019 [FDA])
Ofatumumab (Arzerra, Novartis)	Human (IgG1)	CD20	Binds specifically to the Type I CD20 antigen on pre-B and mature B lymphocytes which is expressed on >90% of the B lymphocytes of B-cell non-Hodgkin lymphoma patients. Binding results in lysis and death of the B-lymphocytes.	<ul style="list-style-type: none"> ● CLL (2009 [FDA], 2010 [EMA]) ● Extended treatment for recurrent or progressive CLL (2016 [FDA])
Ibritumomab tiuxetan (Zevalin, Biogen Idec)	Murine (IgG1) — Yttrium (90Y) conjugated	CD20	Binds specifically to the Type I CD20 antigen on pre-B and mature B lymphocytes which is expressed on >90% of the B lymphocytes of B-cell non-Hodgkin lymphoma patients. Binding results in lysis and death of the B-lymphocytes. The Fab domain binds to CD20 allowing the associated 90Y isotope to kill B-cells through radiation.	<ul style="list-style-type: none"> ● Relapsed or refractory, low-grade or follicular B-cell NHL (also indicated for previously untreated follicular NHL in patients who achieve a partial or complete response to first line chemotherapy; 2002 [FDA] — first line treatment in 2009 [FDA], 2004 [EMA])
Dinutuximab (Unituxin, United Therapeutic Europe)	Chimeric (IgG1)	GD2	Binds to GD2 thus inducing ADCC/ Complement-Dependent Cytotoxicity (CDC) mechanisms and causing apoptosis and inhibition of proliferation.	<ul style="list-style-type: none"> ● Paediatric high-risk neuroblastoma (2015 [FDA and EMA] [As of April 2017, Unituxin's marketing authorisation (MA) was withdrawn in the EU (initiated by MA holder — United Therapeutics) owing to an inability to supply sufficient quantities to meet current global demand.]
Blinatumomab (Blincyto, Amgen)	Murine (IgG1)	CD19/ CD3	As a bispecific antibody, it binds to both CD19 on B-cells and CD3 on T-cells thus bringing them into close proximity. This up-regulates cellular adhesion molecules, the production of cytolytic proteins, the release of cytokines, and the proliferation of T-Cells.	<ul style="list-style-type: none"> ● Acute lymphocytic leukaemia (ALL; orphan medicine 2009, conditional MA 2015, full MA 2018 [EMA], 2014 [FDA]) ● Paediatric Philadelphia chromosome-negative relapsed or refractory ALL (2016 [FDA] — full approval in 2017) ● Minimal residual disease ALL (March 2018 [FDA])
Daratumumab (Darzalex, Janssen-Cilag)	Human (IgG1)	CD38	Binds to CD38, inducing broad spectrum apoptosis by Fc-mediated cross-linking, CDC, ADCC and immune-mediated tumour cell lysis.	<ul style="list-style-type: none"> ● Multiple myeloma (previously treated; orphan medicine 2013, conditional MA 2016, full MA 2017 [EMA], 2015 [FDA]) ● Newly diagnosed multiple myeloma (transplant ineligible) (May 2018 [FDA]) ● Newly diagnosed multiple myeloma (transplant ineligible) in combination with lenalidomide and dexamethasone (2019)
Isatuximab-irfc (Sarclisa, Sanofi)	Chimeric (IgG1)	CD38	Binds to CD38, inducing broad spectrum apoptosis by Fc-mediated cross-linking, CDC, ADCC and immune-mediated tumour cell lysis.	<ul style="list-style-type: none"> ● Relapsed and refractory multiple myeloma in patients who have received at least two prior therapies including lenalidomide and a proteasome inhibitor (March 2020, FDA), in combination with pomalidomide and dexamethasone (March 2020, EMA).
Elotuzumab (Empliciti, Bristol-Myers Squibb)	Humanised (IgG1)	SLAMF7	Direct activation of NK cells via the SLAMF7 pathway. Mediation of cell death via ADCC.	<ul style="list-style-type: none"> ● Multiple myeloma (previously received one to three prior medications; 2015 [FDA], 2016 [EMA])

TABLE 2 (CONTINUED FROM OVERLEAF)

Approved immuno-oncology agents directed against external and internal targets as of April 2020

Continued from page S8

Immuno-oncology agent	Type	Target	Mechanism of action	Indication
Cetuximab (Erbix, Bristol-Myers Squibb)	Chimeric (IgG1)	EGFR	Competitively binds to EGFR, inhibiting the binding of EGF and TGF- α and preventing activation of EGFR. The binding of anti-EGFR drugs to EGFR on the cell surface induces receptor internalisation and degradation. EGFR is over-expressed by many human cancers, namely colorectal cancer. Activation of EGFR results in cell proliferation, differentiation, migration, adhesion and inhibition of apoptosis. Binding of ligands to EGFR plays a role in angiogenesis and metastasis of tumour cells.	<ul style="list-style-type: none"> Advanced CRC (patients who's cells express EGFR and contain wild-type versions of RAS; 2004 [FDA and EMA]) Late-stage SSCHN (2011 [FDA], 2006 [EMA])
Panitumumab (Vectibix, Amgen)	Human (IgG2)	EGFR	Competitively binds to EGFR, inhibiting the binding of EGF and TGF- α and preventing activation of EGFR. The binding of anti-EGFR drugs to EGFR on the cell surface induces receptor internalisation and degradation. EGFR is over-expressed by many human cancers, namely colorectal cancer. Activation of EGFR results in cell proliferation, differentiation, migration, adhesion and inhibition of apoptosis. Binding of ligands to EGFR plays a role in angiogenesis and metastasis of tumour cells.	<ul style="list-style-type: none"> Metastatic CRC (Conditional MA 2007, full MA 2015 [EMA], 2006 [FDA]) Wild-type RAS metastatic CRC (2017 [FDA])
Necitumumab (Portrazza, Eli Lilly)	Human (IgG1)	EGFR	Competitively binds to EGFR, inhibiting the binding of EGF and TGF- α and preventing activation of EGFR. The binding of anti-EGFR drugs to EGFR on the cell surface induces receptor internalisation and degradation. EGFR is over-expressed by many human cancers, namely colorectal cancer. Activation of EGFR results in cell proliferation, differentiation, migration, adhesion and inhibition of apoptosis. Binding of ligands to EGFR plays a role in angiogenesis and metastasis of tumour cells.	<ul style="list-style-type: none"> Advanced NSCLC (EGFR positive; 2015 [FDA], 2016 [EMA])
Catumaxomab (Removab, Viventia, Fresenius Biotech)	Humanised	Ep CAM	Binds to CD3 on T-cells, EpCAM on tumour cells and Fc- α on macrophages and NK cells, causing accumulation of immune cells around EpCAM-positive tumour cells. Catumaxomab stimulates bound NK cells to release cytotoxic mediators. These can phagocytose and process tumour cells so that their antigens are presented on MHC II leading to the activation of T-Helper/Killer cells. When antibodies reach higher concentrations, they can stimulate ODC.	<ul style="list-style-type: none"> Malignant ascites (2009 [EMA] [Removab was withdrawn from the United States in 2013 and the EU in June 2017 at the request of the MA holder])
Trastuzumab (Herceptin, Roche)	Humanised (IgG1)	HER2	Binds to the HER-2 proto-oncogene found on 20-30% of breast cancer cells, thus leading to cell death through the ADCC and CDC mechanisms.	<ul style="list-style-type: none"> HER-2(+) breast cancer (1998 [FDA]) HER-2(+) metastatic gastric cancer (in combination with other anticancer agents; 2010 [EMA], 2011 and 2017 [FDA] The FDA approved Ogivri [trastuzumab-dkst, Mylan], a biosimilar for the treatment of HER2 over-expressing breast or metastatic gastric cancer, in 2017)
Pertuzumab (Perjeta, Genentech)	Humanised (IgG1)	HER2	Binds to the HER-2 proto-oncogene found on 20-30% of breast cancer cells, thus leading to cell death through the ADCC and CDC mechanisms.	<ul style="list-style-type: none"> HER-2 positive breast cancer (2013 [EMA], 2012 [FDA])
Olaratumab (Lartruvo, Eli Lilly)	Human (IgG1)	PDGF-R α	Binds to PDGF-R α and blocks ligand-induced tumour cell proliferation.	<ul style="list-style-type: none"> Advanced soft tissue sarcoma (orphan medicine 2015 [EMA], 2016 [FDA])
Bevacizumab (Avastin, Genentech)	Humanised (IgG1)	VEGF	Binds to VEGF-A, an isoform of VEGF, preventing its interaction with VEGF receptors (VEGFRs) such as Flt-1 and KDR on the surface of endothelial cells, thus inhibiting angiogenesis and reducing tumour growth.	<ul style="list-style-type: none"> Metastatic CRC (2004 [FDA], 2005 [EMA]) NSCLC (combined with chemotherapy/biologic; 2006 [FDA], 2006 [EMA]) Advanced HER-2(-) breast cancer (with paclitaxel; 2008 [FDA], 2010 [EMA]) Glioblastoma (progressed following prior therapy; 2009 [FDA]) Renal cell carcinoma (2009 [FDA], 2007 [EMA]) Metastatic cervical cancer (2014 [FDA]) Advanced ovarian cancer following surgery in combination with chemotherapy. (2018 [FDA], 2011 [EMA])
Ramucirumab (Cyramza, Eli Lilly)	Human (IgG1)	VEGF R2	Binds to VEGF Receptor 2 (VEGFR) on the surface of endothelial cells, thus preventing the binding of ligands such as VEGF-A, -C and -D, and reducing proliferation, permeability and migration.	<ul style="list-style-type: none"> Gastric cancer (2014 [FDA], 2014 [EMA]) Aggressive NSCLC (2014 [FDA], 2015 [EMA]) Second-line in combination with folinic acid, 5-FU and Irinotecan in metastatic CRC Hepatocellular carcinoma (2019 [FDA])
Imiquimod (Aldara; Zyclara, Valeant Pharma International)	Small molecule	TLR7	Binds to the TLR7 receptor found on macrophage and dendritic cells, stimulating an innate and acquired immune response leading to infiltration of inflammatory cytokines (IL-6, IL-8 and TNF), and stimulation of apoptosis in tumour cells.	<ul style="list-style-type: none"> Basal cell carcinomas (small and slow growing; 1998 [EMA — Aldara])
Tocilizumab — also known as atlizumab (Actemra, Chugai/Hoffmann-La Roche/Genentech)	Humanized (IgG1)	IL-R6	Inhibits the binding of IL-6 to the interleukin-6 receptor (IL-6R), thus reducing pro-inflammatory cytokine-based responses by competing for both the soluble and membrane-bound forms of IL-6R.	<ul style="list-style-type: none"> To moderate cytokine release syndrome induced by CAR-T therapy [2017, FDA]

Sources: US Food and Drug Administration¹⁹³, DrugBank¹⁹⁵, Biochemistry¹⁹⁶, Biochemical Pharmacology¹⁹⁸, Journal of Pharmaceutical Sciences¹⁹⁹, Journal of Antibiotics²⁰⁰, Tetrahedron Letters²⁰¹, Bristol Myers Squibb²⁰²,

Cancer Research Institute²⁰³, BMJ²⁰⁴, European Society for Medical Oncology²⁰⁵, American Association for Cancer Research²⁰⁶, Expert Rev Clin Immunol²⁰⁷

TABLE 3
Approved antibody-drug conjugates and immunotoxins as of April 2020

Antibody-drug conjugates	Approval date	Indication	Target	Payload	Linker type	Linker composition
Moxetumomab pasudotox-tdfk (Lumoxiti, AstraZeneca)	September 2018	Hairy cell Leukaemia	CD22	Pseudomonas exotoxin A (bacterial toxin)	Cleavable	Disulphide-bonded using engineered cysteine residues
Brentuximab vedotin (Adcetris)	August 2011 (FDA) October 2012 (EMA)	Hodgkin lymphoma, anaplastic large cell lymphoma First line of stage III or IV classical Hodgkin lymphoma in combination with chemotherapy (as of March 2018)	CD30	Auristatin (tubulin inhibitor)	Cleavable	Valine-citrulline
Trastuzumab emtansine (Kadcyla, Roche)	2013 (FDA & EMA)	HER2-positive metastatic breast cancer	HER2	DM1 (tubulin Inhibitor)	Cleavable	Valine-citrulline
Inotuzumab ozogamicin (Besponsa, Pfizer)	2017 (FDA & EMA)	Relapsed or refractory B-cell precursor Acute Lymphoblastic Leukaemia (ALL)	CD22	Calicheamicin (DNA cleaving agent)	pH and Redox Sensitive	AcBut Disulphide
Gemtuzumab ozogamicin (Mylotarg, Pfizer)	September 2017 (FDA) April 2018 (EMA)	Acute myeloid leukaemia	CD33	Calicheamicin (DNA cleaving agent)	pH and Redox Sensitive	AcBut Disulphide
Tagraxofusp-erz (Elzonris, Stemline)	December 2018 (FDA)	Blastic plasmacytoid dendritic cell neoplasm (BPDCN)	CD123	Diphtheria toxin	Fusion	N/A
Polatuzumab vedotin-piiq (Polivy, Roche)	June 2019 (FDA)	Relapsed of refractory diffuse large B-cell lymphoma	CD79b	Auristatin	Cleavable	Valine-citrulline
Enfortumab vedotin-ejfg (Padcev, Astellas and Seattle Genetics)	December 2019 (FDA)	Locally advanced or metastatic urothelial cancer	Nectin-4	Monomethyl auristatin E (tubulin Inhibitor)	Cleavable	Valine-citrulline
Trastuzumab deruxtecan (Enhertu, Daiichi Sankyo/AstraZeneca)	December 2019 (FDA)	Unresectable or metastatic HER2-positive breast cancer after previous HER2 treatments	HER2	DXd (topoisomerase I inhibitor)	Cleavable	Glycine-glycine-phenylalanine-glycine
Sacituzumab govitecan-hziy (Trodelvy)	April 2020 (FDA)	Metastatic triple-negative breast cancer (mTNBC)	Trop-2	SN-38 (topoisomerase inhibitor)	Cleavable	PEG-lysine-PAB (CL2A)

Sources: *Journal of Experimental & Clinical Cancer Research*⁵³, *J Exp Clin Cancer Res*⁵⁴, *Clin Cancer Res*⁵⁵, European Medicines Agency^{56,59,60,62}, US Food and Drug Administration^{57,58,61,63}

CD45RA- biomarker is associated with induction of central and effector memory T-cells, and so these results suggest that the CD45RA status of baseline memory CD4 and CD8 T-cells and CD8 effector memory T-cells may be used to predict response to anti-CTLA-4 treatment⁵⁰. Another study looking at clinical response in melanoma patients treated with ipilimumab found that patients with normal baseline levels of CD45RO+ CD8 T-cells responded more frequently to treatment, and a significantly longer overall survival (OS) was also observed in normal baseline CD45RO+ patients⁵¹.

As IO agents are associated with potentially life-threatening toxicities, some observers have suggested that it would be preferable for the focus of biomarker research to shift toward attempting to predict toxicity rather than therapeutic response, thus identifying patients who may better tolerate treatment and gain overall benefit. This approach may be particularly important for combination therapies, which are generally associated with a greater frequency of high-grade toxicities.

Increasing the responsiveness of tumours to the immune system

Tumour immunogenicity varies significantly between cancers of the same type in different individuals, and between different malignancies. Some cancers, such as pancreatic ductal adenocarcinoma are considered to be non-immunogenic (i.e. lacking the ability to induce an immune response)⁵². Common features of non-immunogenic tumours include a lack of tumour-infiltrating lymphocytes (TILs) and a lower response to immunotherapy^{52,53}. An elevated neutrophil/lymphocyte ratio (from baseline) has been correlated with poor patient outcomes following immunotherapy across multiple cancer types⁵⁴.

A promising related treatment strategy has emerged based on categorising tumours as 'hot' or 'cold' from an immunological perspective. For example, if the tumour microenvironment contains antigen-specific CD8 TILs it is regarded as 'hot', because the extent of infiltration of lymphocytes correlates with the degree of inflammation, and this has the potential to act as a biomarker to determine whether a tumour will respond to IO therapy. The aim is to transform 'cold' tumours into 'hot' tumours, thus increasing their responsiveness to IO agents and to prevent these tumours from 'cooling off' and becoming unresponsive to therapy.

Another proposed method to increase the immunogenicity of tumours is to administer an oncolytic virus to promote a strong anti-viral immune response⁵⁵. The resulting cytokine production (e.g. type-1 interferons) can directly promote the expression of PD-L1, while chemokines (e.g. CCL3 and CCL4) can attract PD-1/CTLA-4-expressing immune cells. This increased expression of cell-surface targets and infiltration of immune cells can facilitate the binding of ICPis and facilitate their effects. A small phase Ib trial has demonstrated that intralesional injection of herpes simplex virus (i.e. talimogene laherparepvec) in combination with systemic anti-PD-1 treatment results in a 62% ORR (and 33% complete response rate) in patients with metastatic melanoma. This treatment strategy is accompanied by an increased TIL presence, which has been interpreted as an alteration of the tumour microenvironment from immunologically 'cold' to inflamed and tumour responsive (i.e. 'warm')⁵⁶.

Although chemotherapy and radiotherapy are generally regarded as immunosuppressive, it is now accepted that they can work synergistically with IO-based therapies to achieve additive clinical benefit⁵⁷. The mechanism is thought to involve induction of immunogenic cell death by chemotherapy that causes the release of damage-associated molecular patterns – host biomolecules with the ability to initiate an inflammatory immune response that can increase the responsiveness to IO agents.

Radiation-induced cancer cell damage can expose tumour-specific antigens, thus making them visible to the immune system

and leading to promotion of the priming and activation of T-cells⁵⁸. Radiotherapy can also modulate the tumour microenvironment to facilitate the infiltration of immune cells, and can activate innate and adaptive immune responses through the stimulation of STING (stimulator of interferon genes), a pathway that plays a critical role in anticancer immunity.

Evidence of efficacy

IO agents focus on the tumour microenvironment, thus allowing the immune system to produce efficient antitumour responses via negative regulatory pathways such as PD-1/PD-L1 and CTLA-4⁵⁹. The ICPis (see Table 1) have consistently provided outstanding clinical outcomes across many tumour types (e.g. NSCLC and advanced RCC), leading to many accelerated approvals from the FDA and European Medicines Agency (EMA), regardless of PD-L1 and CTLA-4 expression status. Approvals and clinical guidance are based upon three main outcome measures: OS, PFS and ORR.

The KEYNOTE-407 trial found statistically significant improvements in OS, PFS and ORR for patients receiving pembrolizumab plus chemotherapy, compared with randomised placebo plus chemotherapy, in patients with NSCLC, regardless of histologic subtype or PD-L1 expression. The investigators concluded that there was a high level of activity compared with chemotherapy alone for PD-L1-negative patients^{60,61}. Significant PFS and OS were also observed across differing PD-L1 expression levels in patients with NSCLC who had received durvalumab (Imfinzi, AstraZeneca) following chemoradiation treatment⁶². Durvalumab has also shown significant activity in late-stage NSCLC; study results showed that it significantly increased the OS rate at 24 months to 66.3% compared with 55.6% in the placebo groups, and PFS was also improved by more than 30% when compared with placebo (i.e. 17.2 months vs. 5.6 months, respectively). This result was pivotal because it was the first time that an IO treatment had improved survival in patients with late-stage NSCLC⁶³. In addition, the findings of a phase III randomised trial evaluating the use of an IO-biologic combination therapy, compared with a biologic monotherapy in patients with advanced RCC, revealed PFS and ORR benefits in patients irrespective of PD-L1 expression⁴⁰. In addition, a randomised controlled study revealed that nivolumab was associated with higher ORRs than chemotherapy in patients with ipilimumab-resistant metastatic melanoma. The ORR for nivolumab was 40.0% – significantly higher than that for dacarbazine at 13.9%, and complete response rates were 7.6% and 1.0%, respectively. The subgroup analyses found that nivolumab-treated patients had improved OS when compared with chemotherapy, regardless of PD-L1 status⁶⁴. While these studies implied that determining PD-L1 expression prior to treatment was of little importance, other reports suggested improved outcomes for PD-L1-positive patients⁶⁵. Researchers who studied the effects of adding nab-paclitaxel to atezolizumab therapy in triple-negative breast cancer patients emphasised the importance of determining PD-L1 expression status to inform treatment choices, with their results suggesting that most of the benefit, but not all, was realised in the PD-L1-positive subgroup⁶⁶.

Although the current approvals are for combinations with existing therapies, the likely growth of further approvals of both single and combination therapies in the near future could alter the standard of care across many tumour types in the next decade and beyond. The PD-1/PD-L1 pathway is now considered to be one of the most promising areas of IO, and is the backbone of IO research and development⁶⁷. For example, all six approved anti-PD-1/PD-L1 agents received approval within a three-year period (see Table 1), and clinical trial data have demonstrated activity across many different tumour types with prolonged and durable responses. Although PD-1/PD-L1 modulation is considered to

TABLE 4

Approved immuno-oncology therapies with an 'active' mechanism of action as of April 2020

Continued from page S12

Therapy type	Immuno-oncology agent	Type	Target	Mechanism of action	Indication
Chimeric antigen receptor (CAR) T-cell therapy	Tisagenlecleucel (Kymriah, Novartis AG)	N/A	CD19	A patient's own T-cells are harvested and genetically engineered (i.e. re-programmed) with a transgene encoding a CAR which can identify and eliminate CD19-expressing cells.	<ul style="list-style-type: none"> ● Patients aged up to 25 years with B-cell ALL that is refractory or in second or later stage relapse (2017 [FDA], orphan medicine 2014 [EMA]) ● Large B-Cell Lymphoma (2018 [FDA], orphan medicine 2016 [EMA])
	Axicabtagene ciloleucel (Yescarta, Gilead)	N/A	CD19	Engineered T-cells bind to CD19-expressing tumour cells and normal B-cells. CD28 and CD3 co-stimulatory domains activate downstream signalling cascades, thus leading to T-cell proliferation and activation. This results in apoptosis and necrosis of CD19-expressing tumour cells.	<ul style="list-style-type: none"> ● Diffuse large B-cell Lymphoma (2014 [FDA], orphan medicine) ● Primary mediastinal large B-Cell Lymphoma (2015 [FDA], orphan medicine) ● B-cell ALL (2017[FDA])
Other immune regulators	Aldesleukin (Proleukin, Novartis AG)	Lymphokine	IL2R	Aldesleukin binds to IL-2 on immune cell receptors creating an activated receptor complex that results in the growth and differentiation of T-cells.	<ul style="list-style-type: none"> ● Metastatic Renal Cell Carcinoma (1992 [FDA]) ● Metastatic Melanoma (1998 [FDA])
	Interferon Alfa-2a/-2b (Roferon A/Intron A, Sumitomo Dainippon Pharma/Cadila Health care / Merck & Co Ltd)	Protein	IFNAR1 IFNAR2	Complex and not fully understood mode of action; up-regulation of MHC Class I proteins which leads to enhanced activation of CD8+ T cells.	<ul style="list-style-type: none"> ● Advanced Malignant Melanoma (1996 [FDA — intronA]) ● AIDS associated Kaposi's sarcoma (1988 [FDA — intronA and RoferonA]) ● Hairy cell leukaemia (1985/1989 [FDA — intronA/ RoferonA]) ● CML (1995 [FDA — RoferonA])
	Pexidaratinib (Turalio)	Small molecule	CSF-1R pathway	Small molecule tyrosine kinase inhibitor that targets CSF-1R, KIT and FLT3, thereby inhibiting the proliferation of cell lines dependant on CSF-1R.	<ul style="list-style-type: none"> ● Tenosynovial giant cell tumour (2019 [FDA])
Vaccines	BCG live (TheraCys; TICE; ImmuCyst, Shire Plc)	Attenuated	FAP	BCG attaches to FAP expressed by tumour cells on the bladder wall and is internalised into macrophages through macropinocytosis. This induces a local inflammatory response.	<ul style="list-style-type: none"> ● Urothelial Carcinoma (1990 [FDA])
	Sipuleucel-T (Provenge, Dendreon)	Cell transplant therapy	PSA	The precise mechanism is unknown but is thought to act via APCs to stimulate a T-cell immune response targeted against PAP, an antigen highly expressed in many prostate cancers.	<ul style="list-style-type: none"> ● Prostate cancer (metastatic/hormone resistant; 2010 [FDA] 2013 [EMA] [Withdrawn in the EU in May 2015 at the request of the Marketing Authorisation holder for commercial reasons])
Oncolytic virus therapy	H101 (Oncorine, Shanghai Sunway Biotech Co Ltd)	Serotype 5 human adenovirus	Virally- induced marker of cellular stress (e.g. MIC-A/B)	The virus can target, infect and kill tumour cells. The exact mechanism is not fully understood but is thought to involve a dual mechanism of selective killing of tumour cells and induction of systemic anti-tumour immunity.	<ul style="list-style-type: none"> ● Head and neck cancer (2005 [FDA and SFDA]) ● Oesophageal cancer (2005 [FDA])
	Talimogene laherparepvec (T-Vec; Imlygic, Amgen Inc.)	Herpes Simplex Virus 1	Surface nectins	Modified HSV1 (deletion of ICP43.5 and ICP47) with the ability to preferentially replicate in and lyse cancer cells, causing the release of tumour-derived antigens.	<ul style="list-style-type: none"> ● Melanoma (injectable, but non-resectable lesions in the skin and lymph nodes; 2015 [FDA] 2016 [EMA])

Sources: US Food and Drug Administration¹⁵³, DrugBank¹⁵⁵, Biochemistry¹⁵⁶, Biochemical Pharmacology¹⁵⁸, Journal of Pharmaceutical Sciences¹⁵⁹, Journal of Antibiotics¹⁶⁰, Tetrahedron Letters¹⁶¹, Bristol Myers Squibb¹⁶²,Cancer Research Institute¹⁶³, BMJ¹⁶⁴, European Society for Medical Oncology¹⁶⁵, Cancer Immunol Immunother¹⁶⁶, Oncologist¹⁶⁵, J Int J Cancer¹⁶⁶, P T¹⁶⁷, Cancer Sci¹⁶⁸, Front Oncol¹⁶⁹, Clin Cancer Res¹⁷⁰

TABLE 5
Immunohistochemistry assays to measure PD-L1 expression

Therapeutic agent	Companion antibody clone (antibody host species)	Instrument and detection systems	FDA/EMA status*	Definition of positive test	Indication	PD-L1 status required prior to starting treatment
Nivolumab (Opdivo, Bristol-Myers Squibb)	28-8 — Dako (Rabbit)	EnVision Flex on AutostainerLink 48	Complementary/CE Mark	≥5% Membranous staining of tumour cells (minimum 100 cells evaluated)	Second line NSCLC	No
Pembrolizumab (Keytruda, Merck)	22C3 — Dako (Mouse IgG1)	EnVision Flex on AutostainerLink 48	Companion/CE Mark	≥1% Membranous staining of tumour cells or immune cells that are intercalating or at the tumour surface. The FDA indication in NSCLC for pembrolizumab requires a proportion score of ≥50%, as does the EMA approval for HNSCC.	First and Second line NSCLC (≥50% first line, ≥1% 2L), Gastric (≥1%), HNSCC (≥50%) and cervical cancer (≥1%).	Yes
Atezolizumab (Tecentriq, Genentech)	SP142 — Ventana (Rabbit)	OptiView detection and amplification on Benchmark ULTRA	Complementary (approved by the FDA as a complementary diagnostic tool for atezolizumab treatment in patients with metastatic NSCLC whose disease has progressed during or following platinum-containing chemotherapy, as well as for urothelial carcinoma)/CE Mark	Each specimen is assigned a score based on tumour cell and immune cell PD-L1 ● TC3/IC3 PD-L1 ≥50% ● TC2/IC2 PD-L1 5-49% ● TC1/IC1 PD-L1 1-4% ● TC0/IC0 PD-L1 <1% Scoring algorithm is based on either the percentage of PD-L1-expressing tumour cells or immune cells of any intensity; PD-L1 expression in ≥50% tumour cells or ≥10% of immune cells may be associated with enhanced OS.	Second line NSCLC	No
Durvalumab (Imfinzi, AstraZeneca)	SP263 — Ventana (Rabbit)	OptiView detection and amplification on Benchmark ULTRA	Complementary/CE mark	≥25% Membranous staining of tumour cells	Locally advanced NSCLC (CE mark only) /UC	No

Sources: Science²⁸, Ther Adv Med Onco⁶⁹

be a relatively established area of IO, there are ongoing efforts to discover novel agents of this class to treat new indications.

Importantly, five-year follow-up data from the phase 3 KEYNOTE-006 trial reported in 2019 have confirmed that pembrolizumab is superior to ipilimumab for the treatment of melanoma in patients who have had no more than one prior systemic therapy⁶⁸. The median OS was 32.7 months for pembrolizumab versus 15.9 months for ipilimumab.

There are currently only two anti-CTLA-4 agents (ipilimumab and tremelimumab) approved for use in both melanoma and mesothelioma. Although this narrow spectrum of indications

compared with the anti-PD-1/PD-L1 agents suggests this might be an area for further development, there have been multiple reports of high-grade toxicities⁶⁹. Despite this, CTLA-4 blockade is associated with durable and consistent survival benefits in some patients⁷⁰. Therefore, researchers are keen to find ways to manage or overcome these toxicities so that new indications and combination therapies can be explored.

Current research

As of September 2017, 58% of all clinical trials evaluating IO therapies were combination trials, 82% of which involved either

TABLE 6
Immune-related adverse events associated with immune checkpoint inhibitors treatment (including incidence and onset of presentation)

Toxicity	Incidence	Onset after initiation of treatment
Skin	<ul style="list-style-type: none"> ● Amongst the most frequent ● Almost ¼ of patients experience rash — >G3 rashes are rare (<3%) ● 25-35% of patients experience pruritis — severity greater with combination therapy 	2-3 weeks
Endocrine	<ul style="list-style-type: none"> ● Hyper and hypothyroidism have been reported; the latter is more common ● Incidence varies from 5%-10%, up to 20% observed (depending on dose and mono/combination therapy) ● Rarely higher than Grade 2 	6-7 weeks
Hepatotoxicity	<ul style="list-style-type: none"> ● Occurs in up to 10% of patients — 1-2% is Grade 3 with ICPI monotherapy ● Occurs in up to 30% of patients with combination therapy — of which 15% is Grade 3 	6-14 weeks
Gastrointestinal	<ul style="list-style-type: none"> ● Most common associated irAE — 27-54% of patients treated experience diarrhoea and 8-22% experience colitis (when treated with anti-CTLA-4 monotherapy) ● Often most frequent/severe of irAEs associated with ICPI therapy as compared to other toxicities ● Incidence much less for anti-PD-1/PDL1 treatments 	5-10 weeks
Respiratory	<ul style="list-style-type: none"> ● Pneumonitis is 1.5-2.0-times more frequent with anti-PD-1 therapy compared to anti-CTLA-4 monotherapy ● Combination therapy — up to 3 times more likely to experience irAE (Grade 3) 	8-14 weeks

Sources: Ann Oncol⁶⁹, Oncologist⁷¹

another IO agent, a targeted therapy and/or a cytotoxic agent, while around 16% of combination trials involved PD-L1 antagonists and 20% CTLA-4 inhibitors⁷⁰. However, as of September 2019, there were 1,469 more active clinical trials evaluating PD-1/PD-L1 mAbs alone or in combination with other agents, with 76% of these active trials investigating combination therapies⁷¹.

NSCLC, melanoma and non-Hodgkin's lymphoma have been at the forefront of IO research since its infancy, although, in recent years, interest in other malignancies such as renal, pancreatic and advanced (metastatic) cancer have significantly increased⁷². However, since 2014 the average number of planned enrolments has declined from an average of 429 to 129 patients per trial, reflecting the shift in focus from major tumour types (e.g. melanoma and breast cancer) to rarer cancers with a significantly smaller eligible population⁷¹.

Current clinical research efforts are focussed largely on combining recently approved IO agents with either another IO agent or an existing treatment (i.e. chemotherapy or radiotherapy). Data from 2018 show that there are more than 1,700 clinical trials worldwide assessing combinations of anti-PD-1/PD-L1 agents with other cancer therapies, including anti-CTLA-4 agents (n=339), chemotherapy (n=283) and radiotherapy (n=114)⁷². This shift from monotherapies to combination therapies within clinical trials has resulted in 14 approvals of combination therapies by the FDA, with the three most common being PD-1/PD-L1 inhibitors in combination with chemotherapy, CTLA-4 inhibitors and vascular endothelial growth factor (VEGF)-targeted therapies (as of September 2019)⁷¹.

T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibition motif domains (TIGIT) is an immune receptor present on the surface of some T-cells and natural killer (NK) cells. Similar to PD-1, it is an inhibitory checkpoint that is upregulated in multiple cancer types (e.g. melanoma, colon and renal cancer) and also plays a role in the activation and maturation of T-cells and NK cells⁷³. The associated ligand, poliovirus receptor (PVR), is highly expressed on the surface of dendritic, endothelial and some tumour cells. TIGIT plays a vital

role in suppressing the antitumour immune response within the tumour microenvironment. Therefore, blockade of binding to the ligand PVR may suppress its immunosuppressive signalling and allow the co-receptor CD-226 pathway to resume its T-cell activating functions⁷⁴. The NCT02794571 and NCT02913313 trials are investigating TIGIT-blocking antibodies, both as monotherapy or as part of a combination with the PD-1/PD-L1 blocking antibodies atezolizumab and nivolumab, respectively⁷⁵. The trial NCT03119428 that was evaluating the safety and tolerability of OMP-31M32 as a single agent or in combination with nivolumab was terminated in 2019⁷⁶.

OX40, a member of the TNF receptor family, is primarily expressed on CD8 T-cells, NK cells and neutrophils. Its ligand, OX40L, is expressed by B-cells and macrophages, and binding of OX40 to its ligand modulates T-cell activation and effector function. Studies in pre-clinical models have demonstrated that anti-OX40 antibodies can increase antitumour immunity and improve tumour-free survival⁷⁷. Currently, multiple OX40-targeted antibodies are being evaluated in several phase I/II clinical trials, either as a monotherapy or in combination with other IO agents⁷⁴⁻⁷⁹.

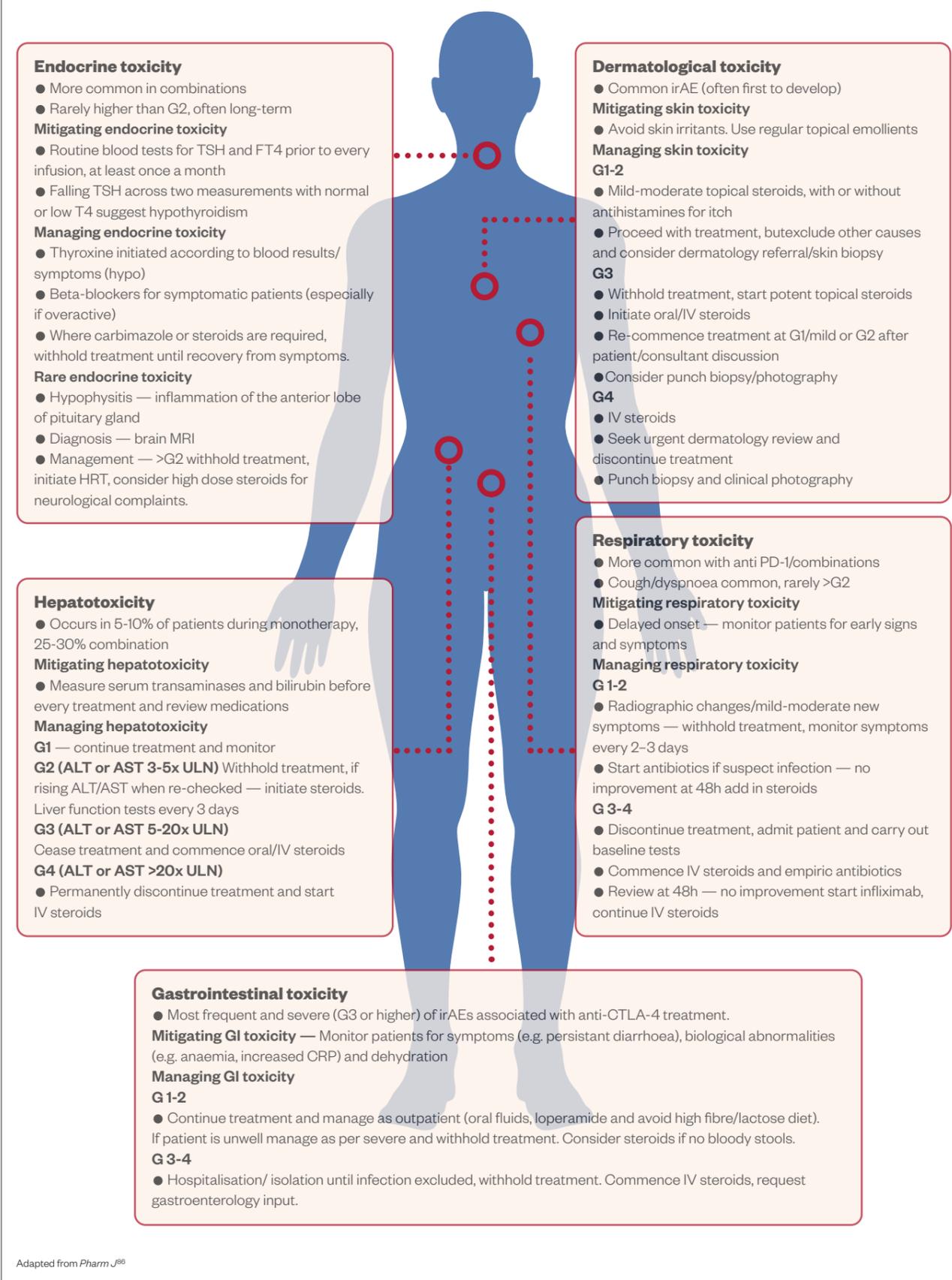
Recent evidence suggests that activation of the STING pathway, a major innate immune pathway, is involved in the generation of spontaneous antitumour T-cell responses. STING activation within antigen-presenting cells in the tumour microenvironment leads to production of IFN β and spontaneous generation of anti-tumour CD8 T-cell responses. In addition, it has been observed that a deficiency in this pathway increases susceptibility to tumour progression. Therefore, the deliberate activation of the STING pathway has been identified as a major research area for the future⁸⁰.

Indoleamine-pyrrole 2,3-dioxygenase (IDO) is a heme-containing enzyme encoded by the IDO1 gene. With other related enzymes it catalyzes the first and rate-limiting step in the kynurenine pathway (i.e. the oxygen-dependent oxidation of L-tryptophan to N-formylkynurenine). It has been implicated in immune modulation by limiting T-cell function

FIGURE 2

The most common immune-related adverse events associated with the use of immune checkpoint inhibitors

Abx: Antibiotics; CRP: C-reactive protein; G: grade; irAE: immune-related adverse event

Adapted from Pharm J⁸⁶

and engaging mechanisms of immune tolerance. Previous studies have suggested that IDO becomes activated during tumour development, helping malignant cells escape eradication by the immune system. Furthermore, IDO expression is closely linked to both CTLA-4 and PD-1/PD-L1 expression via several complex pathways. For example, the IDO enzyme, which is an intracellular target, can be induced by the interaction of PD-1 with PD-L1 on the surface of mast cells. It is expressed by various tumour types and, in many, high IDO expression correlates with poor survival and prognosis⁸¹. Pre-clinical and early phase clinical trials have shown that a combination of CTLA-4 blockade with IDO inhibition can provide more effective antitumour immunity, making IDO a potential novel target for IO therapy. Current IDO inhibitors are small-molecule rather than antibody-based, and examples include indoximod, epacadostat and navoximod, which have been studied both alone and in combination.

Another experimental IO target is the glucocorticoid-induced tumour necrosis factor receptor (GITR), a surface receptor molecule involved in inhibiting the suppressive activity of T-regulatory cells and extending the survival of T-effector cells. Thus, GITR has the capacity to promote effector T-cell functions and impede T-regulator suppression. The anti-GITR antibody TRX518 was developed to target GITR and bind in an agonistic fashion. This agent reached phase I clinical trials in 2010, with safety reports published in 2019, and led to combination studies with anti-PD-1 agents in patients with advanced refractory tumours⁸². Another study investigated the use of the anti-GITR antibody MK-4166, both as a monotherapy and in combination with pembrolizumab⁸². Overall the results showed that mild immune-related adverse effects (irAEs) were common, occurring in more than 20% of patients after treatment with MK-4166 in combination with pembrolizumab, with only one dose-limiting toxicity of bladder perforation in a urothelial patient-reported. An ORR of 69% was achieved in ICPI-naïve melanoma patients, which included four complete and five partial responses. However, although these results were promising, the sample size was small (n=13).

Lymphocyte activation gene 3 (LAG3), also known as CD223, is a cell surface protein expressed on activated CD8+ T-cells and other immune cells, which enhances the regulatory T-cell activity and negatively regulates cellular proliferation and activation and T-cell homeostasis. It specifically inhibits CD8+ effector T-cell functions and can enhance the suppressive activity of T-regulators. Multiple models have demonstrated that blockade of LAG3 with mAbs can augment T-cell function, although the mechanism(s) by which this occurs are poorly understood⁸³. LAG3 is often co-expressed with other inhibitory proteins, especially PD-1. Several pre-clinical studies have suggested the potentially greater therapeutic benefit of dual blockade of these receptors (LAG3 and PD-1) compared with a single agent blockade. The dual blockade approach has demonstrated promising survival benefits and durable response rates in early phase I clinical trials in small subgroups of patients with specific cancer types (e.g. RCC), although detailed knowledge of the biology of LAG3 is presently lacking⁸³.

There is also significant ongoing research in the pre-clinical area, with a notable increase in efforts to identify and evaluate new IO drug targets. For example, in 2017, 165 targets were being evaluated, while in 2018 this increased by around 45% to 240 targets⁷².

Toxicity

Owing to their mechanism of action, IO agents are associated with a unique but variable spectrum of toxicities, known as irAEs^{84,85}. While the toxicities can vary greatly depending on the patient, the risk of serious clinical problems developing limits the use of IO agents to specialist clinicians with the experience

to deal with irAEs should they arise. The most serious concern is potential supra-physiologic stimulation of the immune system leading to a potentially life-threatening uncontrolled and rapid production of pro-inflammatory cytokines (a so-called 'cytokine storm')⁸⁵. Since there are several potential irAEs that can present following initiation of IO therapy, it is important to have clear and robust guidelines of when to refer to other medical specialists who are able to provide input to managing individual patients. For this to work in practice, it is essential that good relationships are developed with other specialties, perhaps most importantly gastroenterology, endocrinology and dermatology. The European Society for Medical Oncology (ESMO) clinical practice guidelines, 'Management of toxicities from immunotherapy', contain comprehensive guidance for the use of IOs in the clinic (see Figure 2).

For the ICPIs, the frequency of irAEs with anti-PD-1/PD-L1 agents appears to be lower than that for anti-CTLA-4 therapies, with the most frequently observed irAEs being mild fatigue, rash, pruritis and gastrointestinal disturbances. The occurrence of grade 3 or grade 4 toxicities with CTLA-4 inhibitors appears to be 20-30% compared with 10-15% for anti-PD-1/L1 agents, with the most serious being dysimmune colitis and interstitial pneumonitis. Delayed hepatic, gastrointestinal and endocrine toxicities can also occur with IO agents and, as with ipilimumab, might only present after the final dose has been administered (see Figure 5)⁸⁶. Therefore, patient follow-ups are of paramount importance and, for ipilimumab, liver function tests are required prior to each dose, with elevated levels of liver enzymes or bilirubin usually prompting withholding of treatment.

Combination treatment using two IO agents (i.e. nivolumab and ipilimumab) is currently approved for advanced melanoma, colorectal and kidney cancer^{87,88,89}. In one study, administration of anti-PD-1 and anti-CTLA-4 agents in combination resulted in 95% of patients experiencing irAEs, 55% of which were grade 3 or higher⁹⁰. Higher toxicity rates are also observed when combining conventional chemotherapy with anti-CTLA-4 agents, such as ipilimumab (58%) compared with chemotherapy alone (42%)⁹⁰.

A meta-analysis, published in 2017, has revealed that ICPI therapy is associated with a risk of death from a variety of irAEs⁹¹. Although this study concluded that clinical specialists should be aware of these potentially serious complications, in reality, the risk of fatal irAEs is low and should not prevent the use of IO agents that can be potentially curative for some patients. For example, in one study, 3,545 patients treated with ICPIs were reviewed and the rate of fatal irAEs found to be 0.59% (i.e. seven cases with ipilimumab, nine with anti-PD-1 agents and five with a combination)⁹². In another study, a meta-analysis of ICPI trials involving 19,217 patients demonstrated that the overall toxicity rate ranged from 0.36% to 1.23%. Other researchers identified a total of 613 irAE-related fatalities from screening the World Health Organization Vigilyze pharmacovigilance database for fatal toxicity events associated with ICPIs⁹². They concluded that fatal irAEs may not have been recorded as consistently as for conventional AEs, but instead noted as 'complications' of irAEs (e.g. sepsis following colon perforation)⁹².

The results of clinical trials with combinations of ICPIs have revealed an increased incidence of irAEs, and the occurrence of grade 3 or grade 4 toxicities is significantly higher with IO combination therapies compared with monotherapy⁹³. There is currently only one ICPI combination approved for clinical use — nivolumab and ipilimumab — for the treatment of metastatic melanoma and previously untreated advanced RCC⁹⁴. While the concept of IO combination therapies is still in its infancy, in the first six months of 2017, 403 combination trials were underway, a dramatic increase from 2013 when only 20 combination clinical trials were active⁹⁵.

As a general rule, where irAEs of any grade present in a patient, the initial management is to monitor, which is often quickly followed by withholding treatment and commencing corticosteroids. GI toxicity is the most frequently reported irAE of any grade associated with anti-CTLA4 therapy and is managed with a combination of fluids and anti-diarrhoeal agents, and sometimes intravenous steroids if symptoms are severe (see Figure 4)⁹⁰. However, in 2018, two cancer patients who developed colitis caused by immunotherapy and failed to respond to these supportive therapies based on current guidelines, were successfully treated with faecal microbiota transplants. While this study was based on a small number of patients, it suggested the potential for this innovative approach of using faecal microbiota to reduce adverse drug reactions (ADRs)⁹⁶.

Predicting the efficacy and safety of immune checkpoint inhibitors based on immune-related adverse effects

A recent study, published in 2019, has shown that patients who experience irAEs during anti-PD-1 monotherapy have a higher chance of achieving an objective response compared with those who do not experience any irAEs⁹⁷. This provides an opportunity to predict the likely efficacy of treatment, potentially allowing more informed decisions about whether treatment should be continued in certain patients. The study involved 106 patients who were treated with either nivolumab or pembrolizumab over a two-year period; the most common irAEs were thyroid dysfunction and nephritis. The ORR for the cohort was 41.5% (n=44), but these patients represented 82.5% (n=40) of those experiencing irAEs of any grade, compared with 16.6% (n=66) who did not experience any irAEs. Furthermore, patients who experienced irAEs had significantly improved PFS than those who did not (i.e. 10 months vs. 3 months) as well as an improvement in OS (i.e. 32 months vs. 22 months), although the latter was not deemed significant on multivariate analysis⁹⁷.

In another study, clinical benefit associated with irAEs was observed in NSCLC patients receiving anti-PD-1 therapy who were shown to have autoimmune antibodies detectable prior to treatment. Investigators concluded that these autoimmune markers may help to determine the risk-benefit ratio for individual patients, allowing therapeutic benefit to be maximised while minimising irAEs⁹⁸. Based on analysis of patient records, 48% of the 137 patients were identified as having experienced irAEs, and for those whose blood samples testing positive for autoimmune factors (i.e. rheumatoid factor, antinuclear or antithyroid antibodies), significantly higher ORRs (i.e. 41% vs. 18%) and disease control rates (i.e. 81% vs. 54%) were achieved compared with those who had tested negative. PFS was also significantly improved by median values of 6.5 months versus 3.5 months. This effect appeared to be driven primarily by patients testing positive for rheumatoid factor compared with those who tested negative, with PFS values of 10.1 months and 3.7 months, respectively⁹⁸. A related study, published in 2019, found that patients with pre-existing antibodies were significantly more likely to experience any-grade irAEs with rates of occurrence of 60% compared with 32% in patients who tested negatively for autoimmune antibodies⁹⁹.

A more recent study in glioblastoma demonstrated a relationship between specific genetic alterations and immune expression signatures, and a tumour's clonal evolution during treatment with anti-PD-1 therapy¹⁰⁰. For example, certain non-response mutations have been identified in the *PTEN* gene, while some response-linked alterations have been identified in components of the MAP kinase pathway. This is a significant finding for glioblastoma therapy because PD-1 inhibitors have not provided a survival benefit for these patients to date. Initial clinical results suggest that a subgroup of patients may benefit from anti-PD-1 treatment (e.g. median survival in responders with ICPi therapy was 14 months vs. 10 months in non-responders).

The results of a prospective study published in early 2020 have suggested that it may be possible to predict the risk of thyroid dysfunction (i.e. destructive thyroiditis and hypothyroidism) in patients undergoing PD-1 inhibitor therapy¹⁰¹. The study involved baseline measurements of anti-thyroid antibodies for 209 patients with re-measurement every 6 weeks for a total of 24 weeks after initiation of therapy. Thyroid dysfunction occurred in 34.1% of the 44 patients testing positive for anti-thyroid antibodies before treatment, versus 2.4% for the 165 patients testing negative at baseline. The results also support the 6–7 week presentation timeline for thyroid dysfunction as indicated in Table 6, as no new cases occurred after 24 weeks post-treatment.

Further research in this area may lead to methods to predict which patients are most likely to benefit from IO therapy across a wide range of tumour types.

Combination therapies

Patients who respond to IO monotherapy often have impressive and durable clinical responses without the side effects observed with traditional cytotoxic therapies. However, fewer than 25–50% of patients treated with ICPis fall into this population of responders. This relatively poor ORR has led to significant interest in combining ICPis with additional treatment modalities, including other IO agents, with the aim of improving response rates and durability of response. This is evident from the recent growth in combination clinical trials. For example, in the three-year period from 2014 to 2017, there was a 705% increase in the number of combination trials, but a 42% decline in enrolment sizes for individual trials, in part reflecting more targeted clinical trials. Combination therapy is attractive because it offers a means to target several mechanisms of tumour cell killing simultaneously in order to minimise tumour growth and the development of resistance^{95,102}.

To date, the combination of ipilimumab with nivolumab to simultaneously target CTLA-4 and PD-1, respectively, is the only approved checkpoint inhibitor combination. It was approved in 2015 by the FDA and 2016 by the EMA for use in advanced melanoma in adults. In 2018, the FDA also approved this combination for patients with intermediate- or poor-risk, previously untreated advanced RCC¹⁰³. While combining two ICPis is potentially associated with a higher toxicity burden, other clinical trials are now investigating whether this combination might be useful in other cancer types. Furthermore, there is a growing interest in the use of PD-1/PD-L1 inhibitors in combination with CAR-T cell therapy, given the ability of engineered T-cells to create an inflamed tumour microenvironment.

The rationale for combining IO agents and chemotherapy is that the efficacies may be additive, but toxicity profiles should not overlap, potentially enhancing patient tolerability and maintaining safety. This synergy between a long-established approach to cancer treatment and a rapidly developing novel type of treatment has led to multiple approvals of chemotherapy/IO combinations, particularly for NSCLC. For example, pembrolizumab in combination with pemetrexed and platinum chemotherapy is now the first-line treatment for non-squamous NSCLC, irrespective of PD-L1 expression¹⁰⁴. Another example is the combination of atezolizumab, bevacizumab, carboplatin and paclitaxel, which is now recommended as an option for metastatic non-squamous NSCLC¹⁰⁵. This approach is particularly interesting, as it combines 'conventional' IO (i.e. targeting PD-L1) with intra-tumoural T-cell infiltration via blockade of VEGF, alongside traditional chemotherapy. However, these highly-targeted approaches can lead to more restrictive criteria for their recommendation.

Interestingly, some targeted therapies such as the BRAF inhibitors are associated with some degree of immuno-modulation,

and it has been postulated that their combination with IO agents may provide a synergistic effect^{54,106}. In support of this, a pre-clinical study in mice has found that the kinase inhibitor, dasatinib, significantly enhances the response to immunotherapies through its ability to inhibit the effects of the *DDR2* gene, which normally helps tumours to invade healthy tissue¹⁰⁷. It has been shown that depletion of *DDR2* can lead to increased sensitivity of cancer cells to anti-PD-1 therapy. In the future, this may lead to a combination study with dasatinib and an anti-PD-1/anti-PD-L1 therapy in diseases such as bladder, breast and colon cancer.

While radiotherapy is immunosuppressive, it promotes the release and expression of tumour neo-antigens (i.e. antigens encoded for by tumour-specific mutated genes), which results in changes to the tumour microenvironment and enhanced T-cell activity¹⁰⁸. In addition, radiotherapy up-regulates PD-1 and PD-L1 expression. Both of these effects support the rationale for combining anti-PD-1/PD-L1 and radiotherapy. In particular, it is thought that ICPis should synergise with radiation-induced T-cell activity, and evaluations of this approach suggest that a clinically significant tumour response can be obtained without increased risk of toxicity compared to monotherapy¹⁰⁹.

Although an active area of research, the clinical use of CAR-T therapies is limited at present, and the number of clinical trials is low in comparison with other types of IO agents. For example, in 2017, there were 291 CAR-T studies reported worldwide as progressing, with 162 at the clinical stage, whereas there were 1,502 studies at the clinical stage investigating PD-1/PD-L1 agents²⁷. As of mid-2018, there were 439 CAR-T combination clinical trials underway globally, of which 422 focussing on different B-cell haematological malignancies^{110,111}.

Current challenges

The two most important challenges for IO therapies are the inability to accurately predict patient response and managing toxicities. However, the lack of information on relevant biomarkers and the high cost of research, development and treatment are also significant concerns¹². Some observers also argue that future research should be directed towards reducing toxicity as a means to improve overall clinical benefit.

Unpredictability of clinical efficacy

Newly developed agents tend to have unpredictable efficacies. There are several possible reasons for these differences in clinical responses, including the presence of different gene mutations and varying degrees of activity of specific signalling pathways in individual patients. The overall aim is to produce consistently effective agents in most patients across the majority of cancer types. Developments appear to be moving in this direction with the recent expansion of indications. For example, in 2018, the EMA expanded the marketing authorisation for pembrolizumab by adopting a new indication for the adjuvant treatment of stage III melanoma¹¹³.

It has been suggested that the longstanding use of chemotherapy as first-line treatment for the majority of cancer types may be impeding the development and use of IO agents that are not yet widely approved for first-line use. At present, they are administered to patients who are immunocompromised owing to prior chemotherapy, so the restoration of antitumour immune function under these conditions is challenging. Therefore, it has been postulated that greater efficacies might be achieved if IO agents are utilised earlier in the treatment plan in order to utilise the full capability of the immune system¹¹².

Another challenge is that IO agents should ideally be directed against tumour-specific antigens solely expressed by tumour cells in order to minimise off-target effects. There would be significant clinical and economic benefits if accurate predictive

biomarkers could be identified and developed, as only patients who are likely to have the greatest response would be treated. However, as seen with PD-L1 expression assays, at present there is a lack of reliability in using IO-related biomarkers to direct treatment.

Another emerging challenge is the management and/or prediction of drug-drug interactions. A study reported in 2020 looking into the efficacy of atezolizumab in NSCLC patients receiving proton pump inhibitors (PPIs) and antibiotics found that patients who received PPIs or antibiotics had a poorer OS than those who did not¹¹⁴. This analysis included data from patients participating in the POPLAR and OAK trials who received either ≥2nd line atezolizumab therapy (n=757) or docetaxel therapy (n=755). Overall, 22.3% (n=169) of atezolizumab patients and 26.8% (n=202) of docetaxel patients received antibiotics, while 30.9% (n=234) and 34.4% (n=260), respectively, received PPIs 30 days before or after starting atezolizumab or docetaxel. No significant correlation between OS and the use of antibiotics/PPIs was found for the docetaxel-treated patients. However, in contrast, patients treated with atezolizumab who had also received antibiotics had an OS of 8.5 months, compared to 14.1 months for those who had not; and in PPI-treated patients, the OS was 9.6 months compared to 14.5 months for those who had not received a PPI. Overall, these results suggest that immune checkpoint efficacy can be significantly affected by some routinely prescribed drugs.

Cost of immuno-oncology therapies

There are significant cost implications associated with IO-based therapies. For example, the one-year global cost of treating NSCLC with selected ICPis has been estimated at over US\$80 billion¹². The estimated cost per patient per year for a variety of IO agents is over £100,000, which places significant pressure on healthcare systems¹⁵. Costs for implementing these newer targeted therapies have escalated dramatically, and the duration of treatment has also lengthened because many cancer types are increasingly being treated as chronic rather than acute diseases. In the UK, the National Institute for Health and Care Excellence (NICE) is the organisation responsible for determining whether new treatments are cost-effective for the NHS. The cost of a new therapy is evaluated for its clinical effectiveness using a standardised measurement known as a quality-adjusted life year (QALY). In order to be deemed cost-effective for the NHS, a therapy should cost no more than £20,000–30,000 per QALY gained, or £50,000 for end-of-life therapies. New IO agents are increasingly exceeding these thresholds, resulting in rejection by NICE and reduced access for patients¹¹⁶.

The Institute for Clinical and Economic Review, a US-based nonprofit organisation providing comprehensive clinical and cost-effectiveness analyses of treatments, tests, and procedures, has studied the cost-effectiveness of the three leading immunotherapies (i.e. atezolizumab, nivolumab and pembrolizumab) and concluded that each therapy would need to be discounted by 31%–68% to reach the QALY threshold¹¹⁷. Taking this into account, NICE has stated that nivolumab cannot be recommended for routine use in the NHS with estimated QALYs of £58,791 and £78,869 versus paclitaxel and docetaxel, respectively, for treatment for urothelial cancer after cisplatin chemotherapy. NICE has also recommended that use of these agents should not be supported by the Cancer Drugs Fund (a 'back-up' government-sponsored fund allowing patients to obtain expensive cancer treatments through the NHS) because they do not have the potential to be cost effective¹¹⁸.

Although the cost of IO agents tends to exceed QALY thresholds, consideration of the cost-effectiveness of a drug or technology is not the sole basis for decision making; clinical effectiveness and multiple patient factors are typically assessed

in parallel^{119,120}. Often, when a new treatment strategy is evaluated, it is more clinically effective than many existing treatments, but is significantly more expensive. In this case, further economic evaluation is carried out, for example establishing the magnitude of the incremental cost-effectiveness ratio, for which an upper threshold set by NICE may not be exceeded. A decision can then be made as to whether the increase in cost is associated with an enhancement in clinical effectiveness that represents value for money¹²⁰. Currently, there are several indications for IO agents recommended by NICE based on both cost- and clinical-effectiveness (e.g. melanoma, UC, RCC, NSCLC, lymphoma and breast cancer^{121–129}).

Many pharmaceutical industry analysts have suggested that, moving forward, there should be a greater emphasis on the value and affordability of novel IO agents, rather than on generating larger numbers of potential candidates of similar therapeutic activity. There is no easy solution to this problem as it is difficult to curtail the enthusiasm of the biotechnology sector; however, it is evident that a longer-term more-sustainable research and development strategy for novel IO therapies is required.

Precision medicine approaches have the potential to reduce the costs and risks associated with drug discovery and development, particularly for the clinical trials that are typically the most expensive stage of the process. The cost-saving comes from stratifying patients into smaller subsets and identifying groups that are more likely to respond, thus reducing the sizes of clinical trials and substantially reducing costs. Identifying those who are more likely to respond is also more beneficial for patients. For example, an analysis of 676 phase IIIb–IV clinical trials of NSCLC over a 14-year period found that the use of a biomarker resulted in a 26% reduction in risk-adjusted drug development costs¹³⁰.

Another option to reduce costs would be to modify treatment pathways to utilise IO agents earlier in a patient's cancer journey, thus potentially reducing costs from treating severe ADRs often associated with conventional chemotherapy and radiotherapy, and the subsequent hospitalisation that many patients require.

Future of immunotherapy

This area appears to be moving away from the development of agents selective for a given cancer type^{27,115}. IO agents are now rarely approved for one particular type of cancer; instead, there is a focus on the pathways involved and the expression of specific biomarkers in tumours, regardless of their origin or location (i.e. 'tissue agnostic' therapies)¹³¹. This pan-cancer approach is evident with the first tumour-agnostic approval of Keytruda by the FDA, in 2017, for patients with unresectable or metastatic solid tumours based on their MSI-high and dMMR status, as opposed to the location or origin of the tumour. Merck, the company which developed Keytruda, is now seeking a second pan-cancer indication against the TMB biomarker, aiming to widen patient access still further¹⁷³. There has been a similar trend towards a tumour-agnostic approach in the small-molecule oncology area; for example, in the past two years, the kinase inhibitors larotrectinib and entrectinib have been granted accelerated approval by the FDA for use in patients with any solid tumour-type that has the NTRK fusion mutation¹⁷⁴.

To date, two comprehensive studies of the global IO landscape have been conducted^{27,115}. Over a one-year period, between September 2017 and August 2018, it was established that the global IO pipeline had increased by 67%, with cell therapy showing the most significant increase of 113% in the number of active agents, followed by other immunomodulatory (e.g. aldesleukin and interferons; 79%) and T-cell-targeted immunomodulatory therapies (76%).

Importantly, the number of IO targets also increased by 50% from September 2017 to August 2018, suggesting that there

could be significant broadening of the IO landscape in the future. Both reviews concluded that, of the many IO agents in clinical development, a large percentage are concentrated on only a few targets (e.g. PD-1, PD-L1 and CTLA4)^{27,115}. In addition to the five antibodies already granted FDA and EMA approval, the UK-based Cancer Research Institute has identified 164 agents in development targeting either PD-1 or PD-L1, with 50 of these at the clinical stage. This suggests that there is significant duplication in product development, and raises concerns as to whether the current approach of focusing on a small number of biomarker targets is stifling further innovation. It is noteworthy that the number of agents being developed against non-tumour-specific antigens actually decreased during the same period, consistent with the suggestion that IO is becoming too focused on a few specific targets. However, there is growing interest and enthusiasm for the IO area in both the pharmaceutical industry and academia. In addition, clinical data suggest that IO agents have significant potential for the future and may lead to several breakthrough treatments that could improve the standard of care in many different cancer types.

Conclusion

IO is a fundamentally different approach to cancer therapy and is redefining the way that both solid and haematological tumours are treated. However, this new treatment paradigm is still in its infancy, and there is a long way to go in optimising the use of these novel therapies, minimising their toxicities and learning how to integrate them into the current standard of care. Furthermore, given their high cost, there are challenges ahead in incorporating them into healthcare systems in an economically sustainable manner, while increasing availability for patients.

ICPis have been the focus of the recent revolution in IO, with two main antibodies (i.e. pembrolizumab and ipilimumab) receiving multiple approvals for PD-1/PD-L1 and CTLA-4 blockade, respectively. Owing to their success, there has been significant interest in combining IO agents with conventional therapies. However, despite their promising efficacy in the clinic, the ICPis produce significant toxicities in some patients. These adverse effects are frequent, but different from those seen with conventional cancer therapies. Therefore, clinical research is beginning to focus on managing and predicting these toxicities, and monitoring long-term outcomes. This should lead to guidelines on how to manage these new therapies and should encourage clinicians to use them as early as possible in treatment pathways.

While the pipeline of ICPis is ever-expanding, the introduction of cancer vaccines and CAR-T cell therapies is also rapidly growing. In particular, there is a strong emphasis on developing new IO agents that can modulate T-cell activity through signalling pathways (e.g. VEGF-A, LAG-3 and IDO-1), with a view to increasing understanding of how modulation of these pathways can restore the body's natural ability to fight cancer.

The investigation of new targets and pathways in the IO area is vital to developing new therapies; however, it is important to note that combinations of presently approved IO agents with existing chemotherapeutic or biological agents are also generating significant interest. For example, a study evaluating a combination of an IO agent with an antibody-drug conjugate has reported encouraging results¹³².

Disclaimer

The treatment strategies described in this review are for educational purposes only and should not be used to guide the treatment of patients. Readers are referred to National Institute for Health and Care Excellence or Scottish Intercollegiate Guidelines Network guidance in the UK, and relevant medical texts and specialist journals, for information regarding prescribing and treatment regimens.

Key points

- Immuno-oncology (IO) is emerging as a novel approach to cancer treatment through the stimulation of the body's own immune system.
- Immune checkpoint inhibitors (ICPis) have had remarkable success across multiple malignancies, and are the most well-established IO agents to date, with several approvals.
- Biomarker testing for the programmed death-ligand 1 checkpoint target is obligatory before treating some tumour types with ICPis (e.g. pembrolizumab and atezolizumab).
- Combining IO agents with conventional therapies has provided significant improvements in patient outcomes in some cases.
- The two main challenges for IO agents are managing their toxicities and affording the high cost of these novel therapies.

About the authors

Sophie Carter is a preregistration pharmacist, The Green Pharmacy, Colchester, Essex. **David E Thurston** is professor of drug discovery at the School of Cancer and Pharmaceutical Sciences, Faculty of Life Sciences and Medicine, King's College London. **Correspondence to:** david.thurston@kol.ac.uk

Peer-reviewed article

This article has been peer reviewed by relevant subject experts prior to acceptance for publication. The reviewers declared no relevant affiliations or financial involvement with any organisation or entity with a financial interest in or in financial conflict with the subject matter or materials discussed in this article.

References

- King A & Broggio J. Cancer registration statistics, England: 2016. 2018. <https://www.ons.gov.uk/releases/cancerregistrationstatisticsengland2016> (accessed May 2020)
- King A. Index of cancer survival for clinical commissioning groups in England: adults diagnosed 2000 to 2015 and followed up to 2016. 2017. Available at: <https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/conditionsanddiseases/bulletins/indexofcancersurvivalforclinicalcommissioninggroupsinengland/adultsdiagnosed2000to2015andfollowedupto2016> (accessed May 2020)
- Cancer Research UK. Cancer survival for common cancers. 2014. Available at: <https://www.cancerresearchuk.org/health-professional/cancer-statistics/survival/common-cancers-compared#heading=Three> (accessed May 2020)
- American Cancer Society. The history of cancer. 2014. Available at: <https://www.cancer.org/cancer/cancer-basics/history-of-cancer/cancer-treatment-surgery.html> (accessed May 2020)
- Iqbal N & Iqbal N. Imatinib: a breakthrough of targeted therapy in cancer. *Chemother Res Pract* 2014;357027. doi: 10.1155/2014/357027
- Decker WK da Silva RF, Sanabria MH *et al*. Cancer immunotherapy: historical perspective of a clinical revolution and emerging preclinical animal models. *Front Immunol* 2017;2(8):829. doi: 10.3389/fimmu.2017.00829
- Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 2012;12(4):252–264. doi: 10.1038/nrc3239
- American Cancer Society. Immune checkpoint inhibitors to treat cancer. 2019. Available at: <https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types/immunotherapy/immune-checkpoint-inhibitors.html> (accessed May 2020)

- Mittendorf EA, Phillips AV, Meric-Bernstam F *et al*. PD-L1 expression in triple-negative breast cancer. *Cancer Immunol Res* 2014;2(4):361–370. doi: 10.1158/2326-6066.CIR-13-0127
- Meyers DE, Bryan PM, Banerji S & Morris DG. Targeting the PD-1/PD-L1 axis for the treatment of non-small-cell lung cancer. *Curr Oncol* 2018;25(4):e324–e334. doi: 10.3747/co.25.3976
- Margo J. Immunotherapy: the next frontier in cancer treatment in financial review. 2017. Available at: <https://www.afr.com/life-and-luxury/health-and-wellness/immunotherapy-the-next-frontier-in-cancer-treatment-20171219-h076ct> (accessed May 2020)
- Baudino TA. Targeted cancer therapy: the next generation of cancer treatment. *Curr Drug Discov Technol* 2015;12(1):3–20. doi: 10.2174/1570163812666150602144310
- O'Reilly A. ESMO e-learning: next generation drugs in kidney cancer. 2018. Available at: <https://oncologypro.esmo.org/Education-Library/ESMO-E-Learning-and-V-Learning/Next-Generation-Drugs-in-Kidney-Cancer> (accessed May 2020)
- Bristol Myers Squibb. Understanding the Science Behind Immuno-Oncology. 2019. Available at: <https://www.immunooncologyhcp.com/servlet/servlet.FileDownload?file=00PYO00017k114UAA> (accessed May 2020)
- Jessey T. Immunity over inability: the spontaneous regression of cancer. *J Nat Sci Biol Med* 2011;2(1):43–9. doi: 10.4103/0976-9668.82318
- Gandhi NM, Morales A & Lamm DL. Bacillus Calmette-Guerin immunotherapy for genitourinary cancer. *BJU Int* 2013;112(3):287–299. doi: 10.1111/j.1464-410X.2012.11754.x
- Cancer Research UK. What is Coley's toxins treatment for cancer? 2012. Available at: <https://www.cancerresearchuk.org/about-cancer/cancer-in-general/treatment/complementary-alternative-therapies/individual-therapies/coleys-toxins-cancer-treatment> (accessed May 2020)
- Fuge O, Vasdev N, Allchorne P & Green JS. Immunotherapy for bladder cancer. *Res Rep Urol* 2015;7:65–79. doi: 10.2147/RRU.S63447
- Redelman-Sidi G, Glickman MS & Bochner BH. The mechanism of action of BCG therapy for bladder cancer – a current perspective. *Nat Rev Urol* 2014;11(3):153–162. doi: 10.1038/nrurol.2014.15
- Macmillan. BCG treatment for non-invasive bladder cancer. 2016. Available at: <https://www.macmillan.org.uk/information-and-support/bladder-cancer/non-invasive-bladder-cancer/treating/bcg-treatment/bcg-treatment-non-invasive-bladder.html#3977> (accessed May 2020)
- National Institute for Health and Care Excellence. Managing non-muscle-invasive bladder cancer. 2019. Available at: <https://pathways.nice.org.uk/pathways/bladder-cancer/managing-non-muscle-invasive-bladder-cancer> (accessed May 2020)
- Cancer Research Institute. Immunotherapy treatment types. 2019. Available at: <https://www.cancerresearch.org/immunotherapy/treatment-types> (accessed May 2020)
- Geynisman DM, Chien CR, Smieliauskas F *et al*. Economic evaluation of therapeutic cancer vaccines and immunotherapy: a systematic review. *Hum Vaccin Immunother* 2014;10(11):3415–3424. doi: 10.4161/hv.29407
- Zhang H & Chen J. Current status and future directions of cancer immunotherapy. *J Cancer* 2018;9(10):1773–1781. doi: 10.7150/jca.24577
- Galluzzi L, Vacchelli E, Bravo-San Pedro JM *et al*. Classification of current anticancer immunotherapies. *Oncotarget* 2014 Dec;5(24):12472–12508. doi: 10.18632/oncotarget.2998
- McKee S. EU approves first CAR-T therapies. 2018. Available at: http://www.pharmatimes.com/news/eu_approves_first_car-t_therapies_1250347 (accessed May 2020)
- Tang J, Shalabi A & Hubbard-Lucey VM. Comprehensive analysis of the clinical immuno-oncology landscape. *Ann Oncol* 2018;29(1):84–91. doi: 10.1093/annonc/mdx755
- Cristescu R, Mogg R, Ayers M *et al*. Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. *Science* 2018; 362(6411):eaar3593. doi: 10.1126/science.aar3593
- Teixidó C, Vilarinho N, Reyes R & Reguart N. PD-L1 expression testing in non-small cell lung cancer. *Ther Adv Med Oncol* 2018;10:1758835918763493. doi: 10.1177/1758835918763493
- Sorensen SF, Zhou W, Dolled-Filhart M *et al*. PD-L1 expression and survival among patients with advanced non-small cell lung cancer treated with

- chemotherapy. *Transl Oncol* 2016;9(1):64–69. doi: 10.1016/j.tranon.2016.01.003
31. Chen D, Irving B & Hodi FS. Molecular pathways: next-generation immunotherapy – inhibiting programmed death–ligand 1 and programmed death-1. *Clin Cancer Res*. 2012;18(24):6580–6587. doi: 10.1158/1078-0432.CCR-12-1362
 32. Topalian SL, Hodi FS, Brahmer JR *et al*. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* 2012;366(26):2443–2454. doi: 10.1056/NEJMoal200690
 33. Shen X & Zhao B. Efficacy of PD-1 or PD-L1 inhibitors and PD-L1 expression status in cancer: meta-analysis. *BMJ* 2018;362. doi: 10.1136/bmj.k3529
 34. US National Library of Medicine. Study of BMS-936558 (nivolumab) compared to docetaxel in previously treated advanced or metastatic squamous cell non-small cell lung cancer (NSCLC; CheckMate O17). 2012. Available at: <https://clinicaltrials.gov/ct2/show/NCT01642004> (accessed May 2020)
 35. US National Library of Medicine. Study of nivolumab (BMS-936558) vs. everolimus in pre-treated advanced or metastatic clear-cell renal cell carcinoma (CheckMate 025). 2012. Available at: <https://clinicaltrials.gov/ct2/show/results/NCT01668784> (accessed May 2020)
 36. US National Library of Medicine. A study of atezolizumab compared with docetaxel in participants with locally advanced or metastatic non-small cell lung cancer who have failed platinum-containing therapy (OAK). 2013. Available at: <https://clinicaltrials.gov/ct2/show/NCT02008227> (accessed May 2020)
 37. Dempke WCM, Fenichel K, & Dale SP. Programmed cell death ligand-1 (PD-L1) as a biomarker for non-small cell lung cancer (NSCLC) treatment – are we barking up the wrong tree? *Transl Lung Cancer Res* 2018;7(Suppl 3):S275–S279. doi: 10.21037/tlcr.2018.04.18
 38. Robainas M, Otano R, Bueno S & Ait-Oudhia S. Understanding the role of PD-L1/PD1 pathway blockade and autophagy in cancer therapy. *Onco Targets Ther* 2017;10:1803–1807. doi: 10.2147/OTT.S132508
 39. Scheel AH, Dietel M, Heukamp LC *et al*. Harmonized PD-L1 immunohistochemistry for pulmonary squamous-cell and adenocarcinomas. *Mod Pathol* 2016;29(10):1165–1172. doi: 10.1038/modpathol.2016.117
 40. Nanda S. Avelumab plus axitinib ‘new first-line standard of care’ for advanced RCC. 2018. Available at: <https://oncologypro.esmo.org/Oncology-News/Daily-News/Avelumab-Plus-Axitinib-New-First-Line-Standard-Of-Care-For-Advanced-RCC> (accessed May 2020)
 41. Williams, L. Pembrolizumab achieves survival improvements across PD-L1 status in KEYNOTE-407 trial. 2018. Available at: <https://oncologypro.esmo.org/Oncology-News/Daily-News/Pembrolizumab-Achieves-Survival-Improvements-Across-PD-L1-Status-In-KEYNOTE-407-Trial> (accessed May 2020)
 42. McLaughlin J, Han G, Schalper KA *et al*. Quantitative assessment of the heterogeneity of PD-L1 expression in non-small-cell lung cancer. *JAMA Oncol* 2016;2(1):46–54. doi: 10.1001/jamaoncol.2015.3638
 43. Arkenau HT. PD-L1 in cancer: ESMO biomarker factsheet. 2017. Available at: <https://oncologypro.esmo.org/Education-Library/Factsheets-on-Biomarkers/PD-L1-in-Cancer> (accessed May 2020)
 44. Ribas A & Hu-Lieskovan S. What does PD-L1 positive or negative mean? *J Exp Med* 2016;213(13): 2835–2840. doi: 10.1084/jem.20161462
 45. Bassanelli M, Sioletic S, Martini M *et al*. Heterogeneity of PD-L1 expression and relationship with biology of NSCLC. *Anticancer Res*. 2018;38(7): 3789–3796. doi: 10.21873/anticancer.12662
 46. Allen EMV, Miao D, Schilling B *et al*. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science*. 2015;9:207–211. doi: 10.1126/science.aad0095
 47. Hendriks LE, Rouleau E & Besse B. Clinical utility of tumor mutational burden in patients with non-small cell lung cancer treated with immunotherapy. *Transl Lung Cancer Res* 2018;7(6):647–660. doi: 10.21037/tlcr.2018.09.22
 48. Büttner R, Longshore JW, López-Ríos F *et al*. Implementing TMB measurement in clinical practice: considerations and requirements. *ESMO Open* 2019;4(1):442. doi: 10.1136/esmoopen-2018-000442
 49. Le DT *et al*. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* 2017;357(6349):409–413. doi: 10.1126/science.aan6733
 50. Subrahmanyam PB, Dong Z, Gusenleitner D *et al*. Distinct predictive biomarker candidates for response to anti-CTLA-4 and anti-PD-1 immunotherapy in melanoma patients. *J Immunother Cancer* 2018;6(1):18. doi: 10.1186/s40425-018-0328-8
 51. Tietze JK, Angelova D, Heppt MV *et al*. The proportion of circulating CD45RO+CD8+ memory T cells is correlated with clinical response in melanoma patients treated with ipilimumab. *Eur J Cancer* 2017;75:268–279. doi: 10.1016/j.ejca.2016.12.031
 52. Lutz ER, Wu AA, Bigelow E *et al*. Immunotherapy converts non-immunogenic pancreatic tumours into immunogenic foci of immune regulation. *Cancer Immunol Res* 2014;2(7):616–631. doi: 10.1158/2326-6066.CCR-14-0027
 53. Blank CU, Haanen JB, Ribas A & Schumacher TN. The “cancer immunogram”. *Science* 2016;658–660. doi: 10.1126/science.aaf2834
 54. Cassidy MR, Wolchok RE, Zheng J *et al*. Neutrophil to lymphocyte ratio is associated with outcome during ipilimumab treatment. *EBioMedicine* 2017;18:56–61. doi: 10.1016/j.ebiom.2017.03.029
 55. Gujar S, Pol JG & Kroemer G. Heating it up: oncolytic viruses make tumours hot and suitable for checkpoint blockade immunotherapies. *Oncoimmunology* 2018;7(8):e1442169. doi: 10.1080/2162402X.2018.1442169
 56. Haanen JBAG. Converting cold into hot tumours by combining immunotherapies. *Cell* 2017;170(6):1055–1056. doi: 10.1016/j.cell.2017.08.031
 57. Kershaw MH, Devaud C, John LB *et al*. Enhancing immunotherapy using chemotherapy and radiation to modify the tumor microenvironment. *Oncoimmunology* 201;2(9):e25962. doi: 10.4161/onci.25962
 58. Wang Y, Deng W, Li N *et al*. Combining immunotherapy and radiotherapy for cancer treatment: current challenges and future directions. *Front Pharmacol* 2018;9:185. doi: 10.3389/fphar.2018.00185
 59. Alsaab HO, Sau S, Alzhrani R *et al*. PD-1 and PD-L1 checkpoint signaling inhibition for cancer immunotherapy: mechanism, combinations, and clinical outcome. *Front Pharmacol* 2017;8:561. doi: 10.3389/fphar.2017.00561
 60. Paz-Ares L, Luft A, Vicente D *et al*. Pembrolizumab plus chemotherapy for squamous non-small-cell lung cancer. *N Engl J Med* 2018;379(21):2040–2051. doi: 10.1056/NEJMoal810865
 61. European Society for Medical Oncology. FDA approves pembrolizumab in combination with chemotherapy for first-line treatment of metastatic squamous NSCLC. 2018. Available at: <https://www.esmo.org/oncology-news/FDA-Approves-Pembrolizumab-in-Combination-with-Chemotherapy-for-First-Line-Treatment-of-Metastatic-Squamous-NSCLC> (accessed May 2020)
 62. Antonia SJ, Villegas A, Daniel D *et al*. Overall survival with durvalumab after chemoradiotherapy in stage III NSCLC. *N Engl J Med* 2018;379(24):2342–2350. doi: 10.1056/NEJMoal809697
 63. Antonia A, Villegas A, Vicente D *et al*. Overall survival with durvalumab after chemoradiotherapy in stage III NSCLC. *N Engl J Med* 2018;379(24):2342–2350. doi: 10.1056/NEJMoal809697
 64. Robert C, Long GV, Brady B *et al*. Nivolumab in previously untreated melanoma without BRAF mutation. *N Engl J Med* 2015;372(4):320–330. doi: 10.1056/NEJMoal412082
 65. Reck M, Rodríguez-Abreu D, Robinson AG *et al*. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. *N Engl J Med* 2016;375(19):1823–1833. doi: 10.1056/NEJMoal606774
 66. Schmid P, Adams S, Rugo HS *et al*. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. *N Engl J Med* 2018;379(22):2108–2121. doi: 10.1056/NEJMoal809615
 67. Kaufman H, Szol M, Hamid O *et al*. Immuno-oncology: perspectives on current therapies & future developments, in reach. 2019. Available at: <https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=2&cad=rja&uact=8&ved=2ahUKEwjM7NTM5tPoAhXGivwKHXliBgcQFjABegQIBB&url=https%3A%2F%2Freachmd.com%2Fprograms%2Fmedical-industry-feature%2Fimmuno-oncology-perspectives-current-therapies-future-developments%2F9502%2Ftranscript%2F16996%2F&sg=AOvVawIm6duTPEyfgWXSsnNmaanTu> (accessed May 2020)
 68. Robet CM, Ribas A, Schachter J *et al*. Pembrolizumab versus ipilimumab in advanced melanoma (KEYNOTE-006): post-hoc 5-year results from an open-label, multicentre, randomised, controlled, phase 3 study. *Lancet* 2019;20(9):1239–1251. doi: 10.1016/S1470-2045(19)30388-2
 69. Berezina MA & Meighan-Mantha R. Immuno-oncology combination trials now easily accessible in Trialrove. 2017. Available at: [trials-now-easily-accessible \(accessed May 2020\)](https://pharmaintelligence.informa.com/resources/product-content/immuno-oncology-combination-

</div>
<div data-bbox=)

70. Buchbinder EI & Desai A. CTLA-4 and PD-1 pathways: similarities, differences, and implications of their inhibition. *Am J Clin Oncol* 2016;39(1):98–106. doi: 10.1097/COC.0000000000000239
71. Yu JX, Hodge JP, Oliva C *et al*. Trends in clinical development for PD-1/PD-L1 inhibitors. *Nature Reviews: Drug Discovery* 2019. Available at: <https://www.nature.com/articles/d41573-019-00182-w> (accessed May 2020)
72. Tang J & Yu A. PD-1/PD-L1 landscape. 2019. Available at: <https://www.cancerresearch.org/scientists/clinical-accelerator/landscape-of-immuno-oncology-drug-development/pd-1-pd-1-landscape> (accessed May 2020)
73. Solomon B & Garrido-Laguna I. TIGIT: a novel immunotherapy target moving from bench to bedside. *Cancer Immunol Immunother* 2018;67(11):1659–1667. doi: 10.1007/s00262-018-2246-5
74. Roche. Investigational pathways: explore TIGIT. 2018. Available at: <https://www.researchcancerimmunotherapy.com/investigational-pathways/explore-tigit> (accessed May 2020)
75. Stamm H, Wellbrock J & Fiedler W. Interaction of PVR/PVRL2 with TIGIT/DNAM-1 as a novel immune checkpoint axis and therapeutic target in cancer. *Mamm Genome* 2018;29(11–12):694–702. doi: 10.1007/s00335-018-9770-7
76. US National Library of Medicine. A study of OMP-313M32 in subjects with locally advanced or metastatic solid tumors. 2019. Available at: <https://clinicaltrials.gov/ct2/show/NCT03119428> (accessed May 2020)
77. Bourre L. OX40 agonists: boosting cancer immunotherapy. 2017. Available at: <https://blog.crownbio.com/ox40-ox40l-immune-co-stimulators> (accessed May 2020)
78. US National Library of Medicine. A phase I study to evaluate MEDI6383 alone and in combination with MEDI4736 in adult subjects with select advanced solid tumours. 2017. Available at: <https://clinicaltrials.gov/ct2/show/NCT02221960> (accessed May 2020)
79. US National Library of Medicine. A phase Ib/2 safety and tolerability study of MEDI6469 in combination with therapeutic immune agents or monoclonal antibodies (MEDI6469). 2017. Available at: <https://clinicaltrials.gov/ct2/show/NCT02205333> (accessed May 2020)
80. Corrales L, McWhirter SM, Dubensky Jr TW *et al*. The host STING pathway at the interface of cancer and immunity. *J Clin Invest* 2016;126(7):2404–2411. doi: 10.1172/JCI86892
81. Liu M, Wang X, Wang L *et al*. Targeting the IDO1 pathway in cancer: from bench to bedside. *J Hematol Oncol* 2018;1(1):100. doi: 10.1186/s13045-018-0644-y
82. European Society for Medical Oncology. GITR is an attractive target for immunotherapy: targeting alternative immune pathways. 2019. Available at: <https://www.esmo.org/Oncology-News/GITR-Is-an-Attractive-Target-for-Immunotherapy> (accessed May 2020)
83. Puhr HC & Ilhan-Mutlu A. New emerging targets in cancer immunotherapy: the role of LAG3. *ESMO Open* 2019;4:e000482. doi: 10.1136/esmoopen-2018-000482
84. European Society for Medical Oncology. Recognising and controlling the adverse effects of CTLA-4 and PD-1 blocking agents: managing the side effects of novel cancer immunotherapies. 2014. Available at: <https://www.esmo.org/Oncology-News/Managing-the-Side-Effects-of-Novel-Cancer-Immunotherapeutics> (accessed May 2020)
85. Ventola CL. Cancer immunotherapy, part 2: efficacy, safety, and other clinical considerations. *P T* 2017;42(7):452–463. PMID: 28674473
86. Evans B & Evans S. Immune checkpoint inhibitors in cancer: pharmacology and toxicities. *Pharm J* 2018; 300(7913). doi: 10.1211/PJ.2018.20204831
87. Wolchok JD. How is immunotherapy for melanoma changing the outlook for patients? 2018. Available at: <https://www.cancerresearch.org/immunotherapy/cancer-types/melanoma> (accessed May 2020)
88. National Cancer Institute. Combination of immunotherapy drugs approved for metastatic colorectal cancer. 2018. Available at: <https://www.cancer.gov/news-events/cancer-currents-blog/2018/fda-ipilimumab-nivolumab-colorectal-dna-repair> (accessed May 2020)
89. Brodsky AN. Checkpoint immunotherapy combination approved for first-line treatment of advanced kidney cancer. 2018. Available at: <https://www.cancerresearch.org/blog/april-2018/fda-approval-ccc-combination-checkpoint-therapy> (accessed May 2020)

90. Haanen J, Carbonnel F, Robert C *et al*. Management of toxicities from immunotherapy: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2017;28(suppl 4):iv119–iv142. Available at: <https://www.esmo.org/guidelines/supportive-and-palliative-care/toxicities-from-immunotherapy> (accessed May 2020)
91. Wang PF, Chen Y, Song SY *et al*. Immune-related adverse events associated with anti-PD-1/PD-L1 treatment for malignancies: a meta-analysis. *Front Pharmacol* 2017;8:730. doi: 10.3389/fphar.2017.00730
92. Wang DY, Salem J-E, Cohen JV *et al*. Fatal toxic events have been characterised for patients using treatments targeting CTLA-4, PD-1 or PD-L1. *JAMA Oncol* 2018;4(12):1721–1728. doi: 10.1001/jamaoncol.2018.3923
93. Larkin J, Chiarion-Sileni V, Gonzalez R *et al*. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N Engl J Med*. 2015;373(1):23–34. doi: 10.1056/NEJMoal504030
94. National Institute for Health and Care Excellence. Nivolumab with ipilimumab for untreated advanced renal cell carcinoma. Technology Appraisal guideline [TA581]. 2019. Available at: <https://www.nice.org.uk/guidance/TA581/chapter/1-Recommendations> (accessed May 2020)
95. Schmidt C. The benefits of immunotherapy combinations. *Nature Outlook* 2017;552(7685):67–69. doi: 10.1038/d41586-017-08702-7
96. Wang Y, Wiesnoski DH, Helmink BA *et al*. Fecal microbiota transplantation for refractory immune checkpoint inhibitor-associated colitis. *Nat Med* 2018;24(12):1804–1808. doi: 10.1038/s41591-018-0238-9
97. Williams L. PD-1 inhibitor efficacy predicted by immune-related adverse events. 2019. Available at: <https://oncologypro.esmo.org/Oncology-News/Daily-News/PD-1-Inhibitor-Efficacy-Predicted-By-Immune-Related-Adverse-Events> (accessed May 2020)
98. Nanda S. Autoimmune markers linked to immunotherapy efficacy, adverse events. 2019. Available at: <https://oncologypro.esmo.org/Oncology-News/Daily-News/Autoimmune-Markers-Linked-To-Immunotherapy-Efficacy-Adverse-Events> (accessed May 2020)
99. Toi Y, Sugawara S, Sugisaka J *et al*. Profiling preexisting antibodies in patients treated with anti-PD-1 therapy for advanced non-small cell lung cancer. *JAMA Oncol* 2019;5(3):376–383. doi: 10.1001/jamaoncol.2018.5860
100. Zhao J, Chen AX, Gartrell AD *et al*. Immune and genomic correlates of response to anti-PD-1 immunotherapy in glioblastoma. *Nat Med* 2019;25(3):462–469. doi: 10.1038/s41591-019-0349-y
101. Okada N, Iwama S, Okuji T *et al*. Anti-thyroid antibodies and thyroid echo pattern at baseline as risk factors for thyroid dysfunction induced by anti-programmed cell death-1 antibodies: a prospective study. *Br J Cancer* 2020;122(6):771–777. doi: 10.1038/s41416-020-0736-7
102. Association of Community Cancer Centers. Immuno-oncology Institute. 2020. Available at: <https://acc-iclio.org/resources/o-combination-therapy> (accessed May 2020)
103. Food and Drug Administration. FDA approves nivolumab plus ipilimumab combination for intermediate or poor-risk advanced renal cell carcinoma. 2018. Available at: <https://www.fda.gov/drugs/informationondrugs/approveddrugs/ucm604685.htm> (accessed May 2020)
104. National Institute for Health and Care Excellence. Lung cancer: diagnosis and management. NICE pathways. 2019. Available at: <https://www.nice.org.uk/guidance/TA584/chapter/1-Recommendations> (accessed May 2020)
105. National Institute for Health and Care Excellence. Atezolizumab in combination for treating metastatic non-squamous non-small-cell lung cancer. Technology appraisal guidance [TA584]. 2019. Available at: <https://www.nice.org.uk/guidance/ta584/chapter/2-Information-about-atezolizumab-plus-bevacizumab-carboplatin-and-paclitaxel> (accessed May 2020)
106. Cooper ZA, Reuben A, Amaria RN *et al*. Evidence of synergy with combined BRAF-targeted therapy and immune checkpoint blockade for metastatic melanoma. *Oncol Immunology* 2014;3(9). doi: 10.4161/21624011.2014.954956
107. Tu MM, Lee FYF, Jones RT *et al*. Targeting DDR2 enhances tumor response to anti-PD-1 immunotherapy. *Sci Adv* 2019;5(2):eaav2437. doi: 10.1126/sciadv.aav2437
108. Mueller K. Understanding neoantigens: a critical target for improving immunotherapy. 2017. Available at: <https://www.curemelanoma.org/blog/article/neoantigens-what-are-they-and-why-do-they-have-researchers-excited> (accessed May 2020)

109. Thangamathesvaran L, Shah R, Verma R *et al*. Immune checkpoint inhibitors and radiotherapy: concept and review of current literature. *Annals of Transl Med* 2018;6(8):155. doi: 10.21037/atm.2018.03.09
110. Cell and gene therapy tracker: global CAR T-cell trials. 2018. Available at: <https://www.the-scientist.com/infographics/cell-and-gene-therapy-tracker-64450> (accessed May 2020)
111. Zhang J, Medeiros LJ & Young KH. Cancer immunotherapy in diffuse large B-cell lymphoma. *Front Oncol* 2018;8:351. doi: 10.3389/fonc.2018.00351
112. Ventola CL. Cancer immunotherapy, part 3: challenges and future trends. *P T* 2017;42:514–521. PMID: 28781505
113. European Medicines Agency. Keytruda. 2018. Available at: <https://www.ema.europa.eu/en/medicines/human/EPAR/keytruda> (accessed May 2020)
114. Chalabi M, Cardona A, Nagarkar DR *et al*. Efficacy of chemotherapy and atezolizumab in patients with non-small cell lung cancer receiving antibiotics and proton pump inhibitors: pooled *post-hoc* analyses of the OAK and POPLAR trials. *Ann Oncol* 2020;31(4):525–531. doi: 10.1016/j.annonc.2020.01.006
115. Tang J, Pearce L, O'Donnell-Tormey J *et al*. Trends in the global immuno-oncology landscape. *Nat Rev Drug Discov* 2018;17(11):783–784. doi: 10.1038/nrd.2018.167
116. Cancer Research UK. Health economics: the cancer drugs cost conundrum. 2018. Available at: <https://www.cancerresearchuk.org/funding-for-researchers/research-features/2016-08-10-health-economics-the-cancer-drugs-cost-conundrum> (accessed May 2020)
117. Journal of Clinical Pathways. ICER report outlines TKI and PD-1 immunotherapy cost effectiveness, policy implications. 2016. Available at: <https://www.journalofclinicalpathways.com/news/icer-report-outlines-tki-and-pd-1-immunotherapy-cost-effectiveness-policy-implications> (accessed May 2020)
118. National Institute for Health and Care Excellence. Nivolumab for treating locally advanced unresectable or metastatic urothelial cancer after platinum-containing chemotherapy. Technology appraisal guidance [TA530]. 2018. Available at: <https://www.nice.org.uk/guidance/ta530> (accessed May 2020)
119. Ogden J. QALYs and their role in the NICE decision-making process. 2017. Available at: <https://onlinelibrary.wiley.com/doi/pdf/10.1002/psb.1562> (accessed May 2020)
120. National Institute for Health and Care Excellence. Assessing cost effectiveness. Process and methods [PMG6]. 2019. Available at: <https://www.nice.org.uk/process/pmg6/chapter/assessing-cost-effectiveness> (accessed May 2020)
121. National Institute for Health and Care Excellence. Nivolumab for adjuvant treatment of completely resected melanoma with lymph node involvement or metastatic disease. Technology appraisal guidance [TA558]. 2019. Available at: <https://www.nice.org.uk/guidance/TA558> (accessed May 2020)
122. National Institute for Health and Care Excellence. Pembrolizumab for treating locally advanced or metastatic urothelial carcinoma after platinum-containing chemotherapy. Technology appraisal guidance [TA519]. 2019. Available at: <https://www.nice.org.uk/guidance/TA519/chapter/1-Recommendations> (accessed May 2020)
123. National Institute for Health and Care Excellence. Nivolumab with ipilimumab for untreated advanced renal cell carcinoma. Technology appraisal guidance [TA581]. 2019. Available at: <https://www.nice.org.uk/guidance/ta581/chapter/3-Committee-discussion#-cancer-drugs-fund> (accessed May 2020)
124. National Institute for Health and Care Excellence. Atezolizumab for treating locally advanced or metastatic non-small cell lung cancer after chemotherapy. Technology appraisal guidance [TA520]. 2018. Available at: <https://www.nice.org.uk/guidance/ta520> (accessed May 2020)
125. National Institute for Health and Care Excellence. Nivolumab for previously treated non-squamous non-small-cell lung cancer. Technology appraisal guidance [TA484]. 2019. Available at: <https://www.nice.org.uk/guidance/ta484/chapter/4-Committee-discussion#-overall-conclusions> (accessed May 2020)
126. National Institute for Health and Care Excellence. NICE approval for nivolumab provides new treatment for advanced blood cancer. 2017. Available at: <https://www.nice.org.uk/news/article/nice-approval-for-nivolumab-provides-new-treatment-for-advanced-blood-cancer> (accessed May 2020)
127. National Institute for Health and Care Excellence. Brentuximab vedotin for treating CD30-positive cutaneous T-cell lymphoma. Technology appraisal guidance [TA577]. 2019. Available at: <https://www.nice.org.uk/guidance/ta577/chapter/3-Committee-discussion#-cost-effectiveness-results> (accessed May 2020)
128. National Institute for Health and Care Excellence. Advanced breast cancer – everything NICE says in an interactive flow chart: hormone receptor-positive and HER2-positive disease. NICE pathway. 2019. Available at: <https://pathways.nice.org.uk/pathways/advanced-breast-cancer#path-view%3A/pathways/advanced-breast-cancer/managing-advanced-breast-cancer.xml&content-view-node%3Anodes-hrpos-and-her2pos> (accessed May 2020)
129. National Institute for Health and Care Excellence. Advanced breast cancer – everything NICE says in an interactive flowchart: triple negative disease (hormone receptor-negative and HER2-negative). NICE pathway. 2019. Available at: <https://pathways.nice.org.uk/pathways/advanced-breast-cancer#path-view%3A/pathways/advanced-breast-cancer/managing-advanced-breast-cancer.xml&content-view-node%3Anodes-triple-negative-disease> (accessed May 2020)
130. Krzyszczyk P, Acevedo A, Davidoff EJ *et al*. The growing role of precision and personalized medicine for cancer treatment. *Technology (Singap World Sci)* 2018;6(3–4):79–100. doi: 10.1142/S2339547818300020
131. Flaherty KT, Le DT & Lemery S. Tissue-agnostic drug development. *Am Soc Clin Oncol Educ Book* 2017;37:222–230. doi: 10.1200/EDBK.173855
132. Bristol Myers Squibb. Seattle Genetics and Bristol-Myers Squibb highlight first data from phase 1/2 study evaluating ADCETRIS® (brentuximab vedotin) in combination with Opdivo (nivolumab) in relapsed or refractory hodgkin lymphoma at ASH annual meeting. 2016. Available at: <https://news.bms.com/press-release/bmy/seattle-genetics-and-bristol-myers-squibb-highlight-first-data-phase-12-study-eval> (accessed May 2020)
133. Food and Drug Administration. Novel drug approvals for 2018. Available at: <https://www.fda.gov/drugs/new-drugs-fda-cders-new-molecular-entities-and-new-therapeutic-biological-products/novel-drug-approvals-2018> (accessed May 2020)
134. European Medicines Agency. 2018. Available at: <https://www.ema.europa.eu> (accessed May 2020)
135. Wishart DS, Feunang YD, Guo AC *et al*. DrugBank 5.0: a major update to the DrugBank database for 2018. 2018. *Nucleic Acids Res* 2018;46(D1):D1074–D1082. doi: 10.1093/nar/gkx1037
136. Hartley JA, Berardini M, Ponti M *et al*. DNA cross-linking and sequence selectivity of aziridinylbenzoquinones – a unique reaction at 5'-GC-3' sequences with 2,5-diaziridinyl-1,4-benzoquinone upon reduction. *Biochemistry* 1991;30(50):11719–11724. doi: 10.1021/bi00114a016
137. Demko S, Summers J, Keegan P *et al*. FDA drug approval summary: alemtuzumab as single-agent treatment for B-cell chronic lymphocytic leukemia. *Oncologist* 2008;13:167–174. doi: 10.1634/theoncologist.2007-0218
138. Jackson C, Hartley JA, Jenkins TC *et al*. N-2,N-4,N-6-tri(hydroxymethyl)-N-2,N-4,N-6-trimethylmelamine (trimelamol) is an efficient DNA cross-linking agent *in vitro*. *Biochem Pharmacol* 1991;42(11):2091–2097. doi: 10.1016/0006-2952(91)90343-4
139. Jackson C, Crabb TA, Gibson M *et al*. Studies on the stability of trimelamol, a carbinolamine-containing antitumor drug. *J Pharm Sci* 1991;80(3):245–251. doi: 10.1002/jps.2600800311
140. Morris SJ, Thurston DE & Nevell TG. Evaluation of the electrophilicity of DNA-binding pyrrolo[2,1-c][1,4]benzodiazepines by HPLC. *J Antibiotics (Tokyo)* 1990;43(10):1286–1292. doi: 10.7164/antibiotics.43.1286
141. Bose DS & Thurston DE. Boron trifluoride promoted cleavage of benzyl carbamates. *Tetrahedron Letters* 1990;31(47):6903–6906. doi: 10.1016/S0040-4039(00)97203-4
142. Bristol Myers Squibb. Yervoy – US full prescribing information. 2018. Available at: https://packageinserts.bms.com/pi/pi_yervoy.pdf (accessed May 2020)
143. Cancer Research Institute. Timeline of progress. 2018. Available at: <https://www.cancerresearch.org/immunotherapy/timeline-of-progress> (accessed May 2020)

144. Imperial Cancer Research Fund General Practice Research Group. Effectiveness of a nicotine patch in helping people stop smoking: results of a randomised trial in general practice. *BMJ* 1993;306(6888):1304–1308. doi: 10.1136/bmj.306.6888.1304
145. European Society for Medical Oncology. OncologyPRO news 2018. 2018. Available at: <https://oncologypro.esmo.org> (accessed May 2020)
146. Postow MA & Wolchok J. Ipilimumab: developmental history, clinical considerations and future perspectives. *The Melanoma Letter* 2012;30:1–4. Available at: https://www.medscape.com/viewarticle/762282_5 (accessed May 2020)
147. FDA expands approval of Ipilimumab to include paediatric patients 12 years and older with unresectable or metastatic melanoma. 2017. Available at: <https://www.esmo.org/Oncology-News/FDA-Expands-Approval-of-Ipilimumab-to-Include-Paediatric-Patients-12-Years-and-Older-with-Unresectable-or-Metastatic-Melanoma> (accessed May 2020)
148. Alexander W. The checkpoint immunotherapy revolution. *P T* 2016;41(3):185–191. PMID: 26957887
149. Dharma R. AstraZeneca's tremelimumab receives FDA approval to treat mesothelioma. 2015. Available at: <https://www.pharmaceutical-technology.com/news/newsastrazenecas-tremelimumab-receives-fda-approval-to-treat-mesothelioma-4556286> (accessed May 2020)
150. Bristol Myers Squibb. Our company – history timeline FDA approves OPDIVO. 2014. Available at: https://packageinserts.bms.com/pi/pi_opdivo.pdf (accessed May 2020)
151. EMA recommends pembrolizumab for the adjuvant treatment of melanoma. 2018. Available at: https://www.esmo.org/Oncology-News/EMA-Recommendations-Pembrolizumab-for-the-Adjuvant-Treatment-of-Melanoma?hit-mail-snews&utm_campaign-Scientific&utm_source-hs_email&utm_medium-email&utm_content-67114737&hsenc-p2ANqtz-XbL6ziYc79Skp7-VI-HOTUDVKYA9cKH4VjpOfuiOPepOZBVOI2q_IkNTICC2rBBBZLjPgIb4h8p3iCty2sAUzFoniTUw&hsmi-67114737 (accessed May 2020)
152. Hu Y, Turner MJ, Shields J *et al*. Investigation of the mechanism of action of alemtuzumab in human CD52 transgenic mouse model. *Immunology* 2009;128(2):260–270. doi: 10.1111/j.1365-2567.2009.03115.x
153. Plieth J. Antibody-drug conjugate resurgence continues with Lumoxiti. 2018. Available at: <https://www.evaluate.com/vantage/articles/news/snippets/antibody-drug-conjugate-resurgence-continues-lumoxiti> (accessed May 2020)
154. Chalouni C & Doll S. Fate of antibody-drug conjugates in cancer cells. *J Exp Clin Cancer Res* 2018;6:37(1):20. doi: 10.1186/s13046-017-0667-1
155. Kreitman RJ & Pastan I. Antibody fusion proteins: anti-CD22 recombinant immunotoxin moxetumomab pasudotox. *Clin Cancer Res* 2011;17(20):6398–6405. doi: 10.1158/1078-0432.CCR-11-0487
156. European Medicines Agency. Adcetris: summary for the public. 2012. Available at: <https://www.ema.europa.eu/en/medicines/human/EPAR/adcetris> (accessed May 2020)
157. Food and Drug Administration. Adcetris drug approval package. 2011. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/125399_adcetris.toc.cfm (accessed May 2020)
158. Food and Drug Administration. Approval date(s) and history, letters, labels, reviews for BLA 125427. 2013. <https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=overview.process&varAppNo=125427> (accessed May 2020)

159. European Medicines Agency. Kadcyla: summary for the public. 2013. Available at: https://www.ema.europa.eu/documents/overview/kadcyla-epar-summary-public_en.pdf (accessed May 2020)
160. European Medicines Agency. Besponsa: summary for the public. 2017. Available at: https://www.ema.europa.eu/documents/overview/besponsa-epar-summary-public_en.pdf (accessed May 2020)
161. Food and Drug Administration. Approval date(s) and history, letters, labels, reviews for BLA 761040. 2017. Available at: <https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=overview.process&AppNo=761040> (accessed May 2020)
162. European Medicines Agency. Mylotarg: summary for the public. 2018. Available at: https://www.ema.europa.eu/documents/overview/mylotarg-epar-summary-public_en.pdf (accessed May 2020)
163. Food and Drug Administration. FDA approves gemtuzumab ozogamicin for CD33-positive AML. 2018. Available at: <https://www.fda.gov/drugs/informationondrugs/approveddrugs/ucm574518.htm> (accessed May 2020)
164. Alva A, Daneils GA, Wong MKK *et al*. Contemporary experience with high-dose interleukin-2 therapy and impact on survival in patients with metastatic melanoma and metastatic renal cell carcinoma. *Cancer Immunol Immunother* 2016;65(12):1533–1544. doi: 10.1007/s00262-016-1910-x
165. Wadler S & Schwartz EL. New advances in interferon therapy of cancer. *Oncologist* 1997;2:254–267. PMID: 10388057
166. Zhao W, Schorey JS, Bong-Mastek M *et al*. Role of a bacillus Calmette-Guérin fibronectin attachment protein in BCG-induced antitumor activity. *I Int J Cancer*. 2000;86(1):83–88. doi: 10.1002/(SICI)1097-0215(20000401)86:1-83::AID-IJCI3-3.0.CO;2-R
167. Anansi E & Ndefo UA. Sipuleucel-T (provenge) injection: the first immunotherapy agent (vaccine) for hormone-refractory prostate cancer. *P T* 2011;36(4):197–202. PMID: 2157277
168. Fukuhara H, Ino Y & Todo T. Oncolytic virus therapy: a new era of cancer treatment at dawn. *Cancer Sci* 2016;107(10):1373–1379. doi: 10.1111/cas.13027
169. Filley AC & Dey M. Immune system, friend or foe of oncolytic virotherapy? *Front Oncol* 2017;7:106. doi: 10.3389/fonc.2017.00106
170. Kohlhapp FJ & Kaufman HL. Molecular pathways: mechanism of action for talimogene laherparepvec, a new oncolytic virus immunotherapy. *Clini Cancer Res* 2016;22(5):1048–1054. doi: 10.1158/1078-0432.CCR-15-2667
171. González-Rodríguez E & Rodríguez-Abreu D. Immune checkpoint inhibitors: review and management of endocrine adverse events. *Oncologist* 2016;21(7):804–816. doi: 10.1634/theoncologist.2015-0509
172. Pishvaian MJ, Blais EM, Brody JR *et al*. Overall survival in patients with pancreatic cancer receiving matched therapies following molecular profiling: a retrospective analysis of the Know Your Tumor registry trial. *Lancet Oncol* 2020;21(4):508–518. doi: 10.1016/S1470-2045(20)30074-7
173. Ray T. Merck bid for second pan-cancer Keytruda indication would raise tumor mutation burden to CDx status. 2020. Available from: <https://www.genomeweb.com/molecular-diagnostics/merck-bid-second-pan-cancer-keytruda-indication-would-raise-tumor-mutation> (accessed May 2020)
174. Gray A. Going against type: the new class of cancer therapies targeting mutations rather than tissues. *Pharm J* 2020;304(7935):164–166. doi: 10.1211/PJ.2020.20207799
175. Kotch C, Barrett D & Teachey DT. Tocilizumab for the treatment of chimeric antigen receptor T cell-induced cytokine release syndrome. *Expert Rev Clin Immunol* 2019;15(8):813–822. doi: 10.1080/1744666X.2019.1629904