



Review

Cancer immunotherapy: Opportunities and challenges in the rapidly evolving clinical landscape



Leisha A. Emens^{a,*}, Paolo A. Ascierto^b, Phillip K. Darcy^{c,d},
Sandra Demaria^e, Alexander M.M. Eggermont^f, William L. Redmond^g,
Barbara Seliger^h, Francesco M. Marincola^{i,**}

^a Johns Hopkins University School of Medicine, Department of Oncology, Graduate Program in Pathobiology, Baltimore, MD 21287, USA

^b Istituto Nazionale Tumori Fondazione G. Pascale, Melanoma, Cancer Immunotherapy and Innovative Therapy Unit, Napoli, Italy

^c Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia

^d Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville 3010, Australia

^e Weill Cornell Medical College, Department of Radiation Oncology, New York, NY 10065, USA

^f Cancer Institute Gustave-Roussy, 114 Rue Edouard Vaillant, Villejuif/Paris-Sud 94800, France

^g Robert W. Franz Cancer Research Center, Earle A. Chiles Research Institute, Providence Portland Medical Center, Portland, OR 97213, USA

^h Martin Luther University, Institute for Medical Immunology, Magdeburger Str. 2, 06112 Halle, Germany

ⁱ Research Branch, Sidra Medical and Research Centre, Doha, Qatar

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Abstract Cancer immunotherapy is now established as a powerful way to treat cancer. The recent clinical success of immune checkpoint blockade (antagonists of CTLA-4, PD-1 and PD-L1) highlights both the universal power of treating the immune system across tumour types and the unique features of cancer immunotherapy. Immune-related adverse events, atypical clinical response patterns, durable responses, and clear overall survival benefit distinguish cancer immunotherapy from cytotoxic cancer therapy. Combination immunotherapies that transform non-responders to responders are under rapid development. Current challenges facing the field include incorporating immunotherapy into adjuvant and neoadjuvant cancer therapy, refining dose, schedule and duration of treatment and developing novel surrogate endpoints that accurately capture overall survival benefit early in treatment. As the field rapidly evolves, we must prioritise the development of biomarkers to guide the use of

* Corresponding author: Johns Hopkins University School of Medicine, 1650 Orleans Street, Room 409, Baltimore, MD 21231-1000, USA. Fax: +1 (410) 614 8216.

** Corresponding author: Sidra Medical and Research Center, OPC, Po Box 26999, Al Luqta Street, Doha, Qatar. Fax: +974 70497777. E-mail addresses: emense@jhmi.edu (L.A. Emens), fmarincola@sidra.org (F.M. Marincola).

Programmed death ligand-1 (PD-L1)

immunotherapies in the most appropriate patients. Immunotherapy is already transforming cancer from a death sentence to a chronic disease for some patients. By making smart, evidence-based decisions in developing next generation immunotherapies, cancer should become an imminently treatable, curable and even preventable disease.

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1. Introduction

Cancer immunotherapy has become the fourth pillar of cancer care, complementing surgery, cytotoxic therapy, and radiotherapy [1]. The field has a long history that started with Coley's toxins [2] and Erlich's hypothesis that the immune system suppresses cancer development [3]. Thomas and Burnet expanded Erlich's idea by proposing the immune surveillance hypothesis [4], and Prehn and Main [5] subsequently demonstrated that carcinogen-induced tumours elicit tumour-specific immune responses. Intra-vesicular Bacillus Calmette Guerin was approved for superficial bladder cancer in 1990 [6], and the cytokines interferon- α and interleukin-2 were approved for melanoma and renal cell carcinoma in 1986 and 1992, respectively [7]. Schreiber more recently described immune-editing as a process that enables escape from immune surveillance to establish overt malignancy [8]. More specific cancer immunotherapies were approved in recent years, including preventive and therapeutic cancer vaccines [9,10], the first

immune checkpoint inhibitors [11–13], a bi-specific T-cell engager [14], and an oncolytic virus [15] (summarized in Fig. 1). Of these, immune checkpoint antagonists that target the PD-1 pathway have generated the most interest, with response rates across tumour types that average 20–30% [1]. With many more immunotherapy drugs under development, a major challenge for the field is how to prioritise the most promising of these many immuno-oncology agents alone or in combination immunotherapies designed to achieve therapeutic synergy.

A pressing challenge is transforming the majority of patients from immunotherapy non-responders to responders. This will likely require potent combination immunotherapies that effectively harness the cancer-immunity cycle described by Chen and Mellman [16]. Cancer therapies result in tumour cell death and release of tumour antigens, which are presented by dendritic cells in the tumour-draining lymph nodes to prime and activate tumour immunity. Tumour-specific T cells then gain access to the circulation and traffic to tumours,

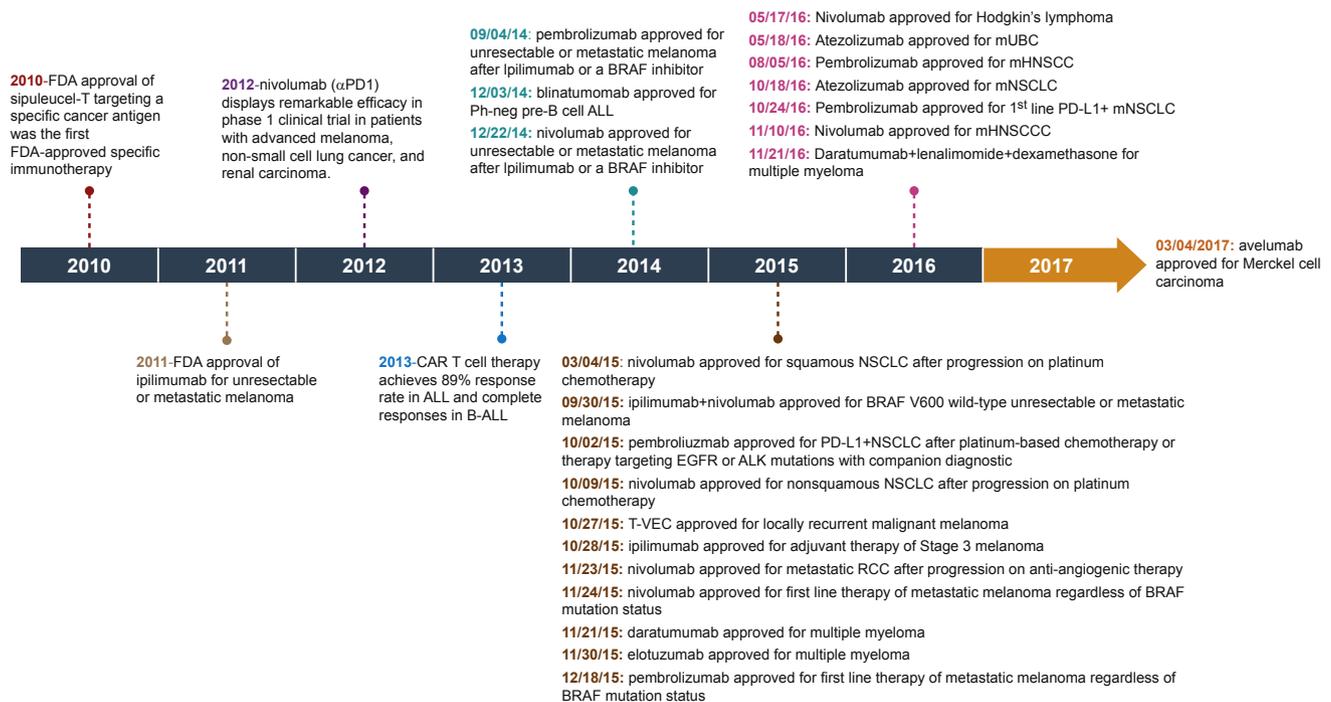


Fig. 1. The recent acceleration and expansion of cancer immunotherapy approvals. A timeline illustrates regulatory approvals in the United States since 2010.

where they infiltrate the tumour mass. T cell-mediated lysis of cancer cells releases more tumour antigens, thus perpetuating the cycle. Multiple opportunities for therapeutic intervention that enhance tumour immunity are possible at each step of this cycle (summarized in Table 1). Developing novel immunotherapeutic agents, defining synergistic drug combinations, and understanding the tumour microenvironment—defects in antigen processing and presentation, and the number, type, quality and distribution of immune cells in a tumour, and the pathways that regulate them—are critical for ongoing clinical success.

2. What have we learnt from immunotherapy in recent years?

Experience with ipilimumab (a CTLA-4 antagonist), nivolumab and pembrolizumab (PD-1 antagonists) and atezolizumab (a PD-L1 antagonist) in treating cancer has defined several key principles of cancer immunotherapy. First, checkpoint inhibitors engage T cells with inherent capacity for adaptability and memory. This mechanism of action underlies the durable responses

and long-term survival observed with these agents. Second, immunotherapy treats the immune system. It can work regardless of either tumour histology or the presence of driver mutations. Third, the side effects of checkpoint inhibitors are distinct from those of chemotherapy and targeted agents. Finally, the efficacy of immunotherapy can be improved by combining it with other treatment strategies [17].

2.1. Cancer immunotherapy has unique patterns of clinical benefit

2.1.1. The impact on overall survival is paramount

Ipilimumab prolongs overall survival (OS) with no impact on overall response rate (10%) or progression-free survival (PFS) in melanoma [11,18]. A recent phase III study comparing nivolumab to everolimus in metastatic kidney cancer showed no difference in median PFS (about 4.5 months), but the median OS for patients treated with nivolumab was longer (25 versus 19.6 months, HR 0.73, $P \leq 0.0148$) [19]. This clinical benefit pattern may be related to the immune response evolution over time, or to pseudo-progression, defined as an

Table 1
Summary of dynamic interactions between cancer and the immune system.

Process	Immune components involved	Opportunities for combination immunotherapy
Cancer cell death	Release of tumour antigens	Chemotherapies Radiation therapy Targeted therapies
Tumour antigen presentation	Dendritic cells, APCs	Cancer vaccines Interferon- α GM-CSF Anti-CD40 agonist TLR agonist STING agonist
Priming and activation	APCs and T lymphocytes	Anti-CTLA-4 Anti-CD137 agonist Anti-OX40 agonist Anti-CD27 agonist Interleukin-2 Interleukin-12
Trafficking of T cells to tumours	CTLs	Chemokine modulators
Infiltration of T cells into tumours	CTLs, endothelial cells	Bevacizumab Ramucirumab Endothelin B receptor Antagonist
Recognition of cancer cells by T cells	CTLs, tumour cells	CAR T cells TCR-modified T cells TIL therapy
Recognition of cancer cells by NK cells	NK cells, tumour cells	Anti-PD-1 Anti-PD-L1
Tumour cell lysis resulting in antigen release	T cells, NK cells, tumour cells	Anti-PD-1 Anti-PD-L1 IDO inhibitor A2AR antagonists

Abbreviations: APCs, antigen-presenting cells; GM-CSF, granulocyte-macrophage colony stimulating factor; TLR, toll-like receptor; STING, stimulator of interferon genes; CTLs, cytotoxic T lymphocytes; CTLA-4, cytotoxic T lymphocytes antigen-4; NK, natural killer; CAR, chimeric antigen receptor; TCR, T cell receptor; TIL, tumour-infiltrating lymphocyte; NK, natural killer; PD-1, programmed death-1; PD-L1, programmed death ligand-1; IDO, indoleamine dioxygenase; A2AR, adenosine A2A receptor.

increase in size of the tumour mass on imaging from immune infiltration rather than tumour growth. Atypical/non-conventional response patterns led to the development of immune-related response criteria (irRC), which preserve the potential of benefiting from immunotherapy despite apparent disease progression on imaging [20]. Thus, immunotherapy should be continued in the face of apparent disease progression by imaging if the patient is doing well, and until progressive disease is confirmed by a second scan 4 weeks later.

A meta-analysis of 5000 patients with advanced melanoma showed that ipilimumab rendered melanoma, a chronic disease in 20% of patients [21]. Early data with single-agent nivolumab [13,22,23], pembrolizumab [24], and combination ipilimumab/nivolumab [25] suggest that PD-1 antagonists could result in even more long-term responders. Relative to CTLA-4 blockade, PD-1/PD-L1 blockade appears to double the number of long-term responders. PD-1 blockade also appears to give higher ORRs and longer PFS than CTLA-4 blockade. First-line nivolumab has an ORR of about 43% and a median PFS of 5.4–6.9 months [26], whereas first- or second-line pembrolizumab has an ORR of about 33% and a median PFS of 4.1–5.5 months [27]. Combining CTLA-4 and PD-1/PD-L1 blockade is even more effective, and it is likely that overall survival will continue to improve with effective combination immunotherapies (Fig. 2).

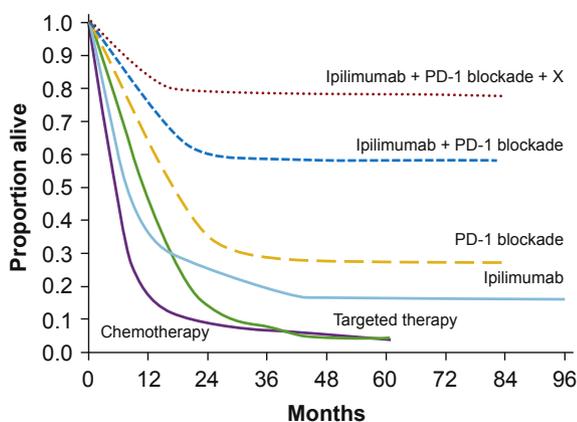


Fig. 2. A model illustrating the potential impact of single agent and combination cancer immunotherapies on survival. Cancer treatment regimens that include immunotherapy can raise the tail of the curve, increasing overall survival relative to standard cancer therapies. Targeted therapies enhance response rates relative to standard chemotherapies, but overall survival is minimally impacted. Single-agent immunotherapy is associated with long-term survival rates ranging from 10% (ipilimumab) to 20–30% (PD-1 blockade). Combination immunotherapy targeting the CTLA-4 (ipilimumab) and PD-1 pathways (nivolumab/pembrolizumab) is associated with long-term survival rates of 50–60%. Third-generation combination immunotherapy regimens (illustrated as ipilimumab + nivolumab + X) have the potential to further maximise overall survival, moving closer to cure.

2.1.2. Persistent responses after cessation of therapy

Patients who stop immune checkpoint blockade for reasons other than progression (primarily toxicity) may continue to benefit. Eighty-five percent of the patients who discontinued single-agent nivolumab due to toxicity had a complete or partial response, and 70% continued to respond despite stopping treatment [26]. Similarly, although grade 3–4 toxicity occurred in 55% of the patients treated with ipilimumab/nivolumab and led to treatment discontinuation in 30%, the median OS was not reached at ≥ 18 months follow-up for the population as a whole, or patients who discontinued treatment due to toxicity [28]. The optimal duration of immune checkpoint blockade thus remains unknown, and future studies should investigate this question.

2.1.3. Atypical patterns of response

Similar to anti-CTLA-4, unconventional responses also occur with anti-PD-1/PD-L1 therapy. About 8% of the patients treated with nivolumab [29,30] and 15% of the patients treated with pembrolizumab [31] developed new lesions but responded to treatment beyond progression (atypical response or pseudo-progression). Both irRC and classical RECIST criteria should be considered in assessing responses to prevent premature cessation of CTLA-4 and PD-1/PD-L1 blockade.

2.2. Immunotherapy targets a broad range of tumour types

Because cancer immunotherapy treats the immune system, it can be effective independent of tumour histology or driver mutations. In melanoma, ipilimumab has long-term benefit for cutaneous, ocular and mucosal melanomas, which have distinct biology [32–37]. There was no difference between BRAF- and NRAS-mutated melanoma in DoR or OS with ipilimumab [38]. Moreover, the efficacy of PD-1 blockade extends to multiple tumour types, including non-small-cell lung cancer (NSCLC), small-cell lung cancer (SCLC), kidney cancer, Hodgkin's lymphoma, head and neck cancer, oesophageal and gastric cancers, hepatocellular cancer, bladder cancer, breast cancer and others [1,39]. Thus, it is possible to achieve long-term survival in some patients across a range of distinct tumour types, where the different response rates across tumours reflect different immunogenicity.

2.3. The safety profile of immunotherapy is distinct

The safety profile of checkpoint inhibition differs from chemotherapy or targeted therapy. Immune-related adverse events (irAEs) result from immune activation once inhibition by CTLA-4 and/or PD-1 is released. The most frequent side effects of ipilimumab involve the skin (pruritus and cutaneous rash), gastrointestinal tract (colitis and diarrhoea), liver (autoimmune hepatitis) and

endocrine system (thyroid dysfunction and hypophysitis). Immune-related neuropathy, myositis, arthritis and uveitis also occur uncommonly. The safety profile of PD-1 blockade is similar and also includes pneumonitis. Pneumonitis is typically grade 1–2 and does not result in cessation of treatment. The incidence of grade 3–4 (severe) irAEs is 10–15% with ipilimumab, 5% with nivolumab or pembrolizumab, and 55% with ipilimumab/nivolumab (Fig. 3). Combination therapy did not cause new toxicities or deaths [26]. Early diagnosis and prompt treatment with steroids are critical for the effective management of irAEs. Algorithms have been developed to guide treating physicians [40].

2.4. Combination immunotherapy is more powerful

Different checkpoint inhibitors are combined to harness their distinct mechanisms of action. Anti-CTLA-4 acts during the priming phase, and anti-PD-1/PD-L1 is

thought to act primarily during the effector phase in the tumour microenvironment. Combining ipilimumab with nivolumab or pembrolizumab is more effective, but more toxic than either single agent in both melanoma and NSCLC, with responses related to PD-L1 expression in NSCLC but not melanoma [26,41–43]. Thus, a major priority for this combination is to maximise benefit and minimise toxicity. One promising strategy is to modify the dose and scheduling of ipilimumab. Using it at 1 mg/kg every 3 weeks for 4 cycles in combination with PD-1 blockade preserved the response (ORR 57%) with a 20% incidence of grade 3–4 irAEs [44]. Other immuno-oncology combinations with presumed complementary mechanisms of action are under rapid clinical development.

Immunotherapy can be combined effectively with chemotherapy, targeted therapy, and radiotherapy. The efficacy of chemotherapy combined with immunotherapy depends on the drug, and the relative timing of

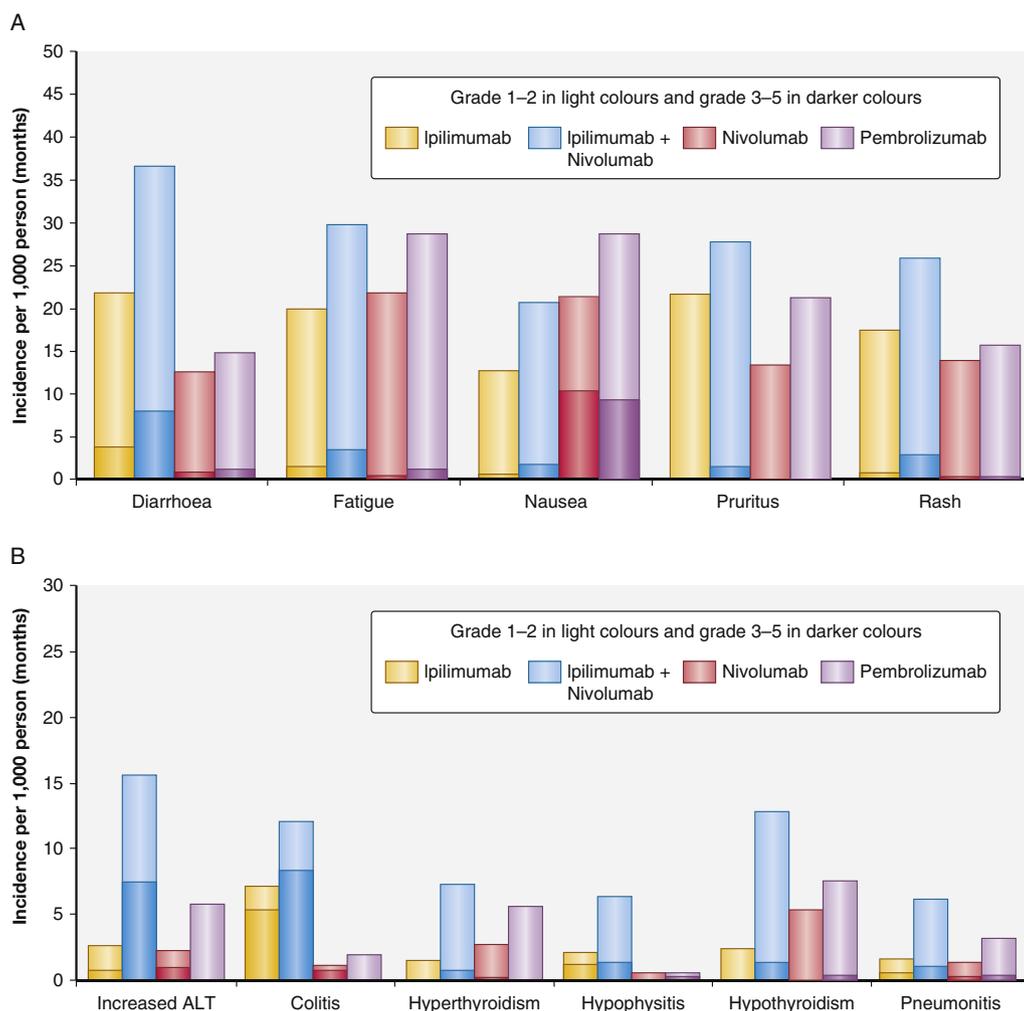


Fig. 3. Adverse events associated with ipilimumab, pembrolizumab, nivolumab or ipilimumab plus nivolumab. Events are illustrated as incidence per 1000 person-months. (A) The most common adverse events. (B) Immune-related adverse events of special interest.

Adapted by permission from Macmillan Publishers Ltd: Nat Rev Clin Oncol. 2016 Aug;13(8):473–86. Safety profiles of anti-CTLA-4 and anti-PD-1 antibodies alone and in combination. Boutros C, Tarhini A, Routier E, Lambotte O, Ladrerie FL, Carbonnel F, Izzeddine H, Marabelle A, Champiat S, Berdelou A, Lanoy E, Texier M, Libenciu C, Eggermont AM, Soria JC, Mateus C, Robert C.

immunotherapy and chemotherapy [45]. Dacarbazine combined with ipilimumab was more effective than chemotherapy alone in melanoma [18]. In NSCLC, the combination of ipilimumab with chemotherapy (paclitaxel and carboplatin) was evaluated on a concurrent schedule (four doses of ipilimumab plus chemotherapy followed by two doses of placebo plus chemotherapy) and a phased schedule (two doses of placebo plus chemotherapy followed by four doses of ipilimumab plus chemotherapy) [46]. Median PFS (5.1 and 4.1 months) and OS (12.2 and 9.7 months) were longer with the phased than with the concurrent regimen. There are similar data for SCLC [47]. More recently, a randomised phase 2 study evaluated the addition of pembrolizumab to carboplatin and pemetrexed for advanced non-squamous NSCLC, with a ORR of 55% for pembrolizumab with chemotherapy compared with 29% for chemotherapy alone [48]. Importantly, there was no apparent relationship between PD-L1 expression and response in this study. Combining targeted therapy with immunotherapy is another interesting approach. The addition of BRAF or BRAF and MEK inhibitors to ipilimumab is limited by a high incidence of side effects, and combinations involving anti-PD-1/PD-L1 agents might be more feasible. Increased understanding of the immunological effects of conventional and targeted cancer therapy is essential to guide the design of clinical studies testing them in combination with immunotherapy [49].

Radiotherapy is used for local tumour control and palliation of symptoms. The abscopal effect, where the regression of non-irradiated lesions occurs after the irradiation of an index lesion, is a long-standing observation in radiation oncology thought to reflect secondary activation of anti-tumour immunity [49–51]. Radiation can enhance tumour immunity at each step of the cancer immunity cycle. It releases tumour antigens and induces signals that promote antigen cross-presentation by dendritic cells [52–54], upregulates chemokines and vascular adhesion molecules that promote the recruitment of T cells into the tumour [55–57] and increases the expression of MHC molecules, stress-induced ligands, and death receptors on cancer cells [58–62]. In preclinical models, radiation doses between 2 and 60 Gy given as single or multiple fractions alone or given with immunotherapy agents promote the priming of tumour-specific T cells [60,63,64]. However, mechanisms underlying the impact of radiation dose and fractionation on immunogenic cell death and on the tumour microenvironment remain unclear, and the priming of anti-tumour T cells does not always result in an abscopal effect [65]. A phase 2 trial testing radiation with ipilimumab versus radiation alone in castrate-resistant prostate cancer patients used a single palliative dose of 8 Gy to a bone metastasis and showed no benefit of combination therapy [66]. In contrast, dramatic abscopal responses were reported in patients with lung cancer or melanoma treated with ipilimumab and

hypo-fractionated radiation given in doses of 9.5 Gy \times 3 or 6 Gy \times 5 [50,67]. The synergistic activity of hypo-fractionated radiation given with ipilimumab was recently confirmed in a phase 2 lung cancer trial [68]. Combinations of radiation with immunotherapy agents that target antigen-presenting cells have shown promising data in early trials with abscopal effects observed in close to a third of patients [69,70].

3. Current clinical challenges

The success of immunotherapy has generated new clinical questions, including the appropriate use of immunotherapy in earlier stage disease, optimising dose, schedule and duration of therapy, the best biomarkers for patient selection and the development of novel surrogate endpoints that reflect the impact of immunotherapy on OS early in treatment.

3.1. Adjuvant and neoadjuvant immunotherapies

Adjuvant immunotherapy is an attractive strategy for treating micrometastatic disease. IFN- α is approved for the adjuvant treatment of stage IIB–III melanoma (high-dose IFN- α [71] in USA and Europe, low-dose IFN- α for stage II melanoma [72] in Europe), and pegylated IFN- α for stage III melanoma in the USA [73]. The impact of adjuvant therapy with IFN- α has been modest, as meta-analyses of multiple randomised phase III trials revealed that improved relapse-free survival (RFS) has not led to a consistent impact on longer OS [74,75]. Tumour ulceration is a predictor of benefit from adjuvant IFN- α treatment [76–79]. Adverse outcomes may also occur, as several adjuvant vaccine trials were stopped early because of detrimental outcomes in the vaccine arm [80,81].

The Phase 3 EORTC 18071 trial randomised 951 patients with stage IIIa (>1 mm metastasis)/(stage IIB/IIC melanoma) to placebo or ipilimumab [82]. The trial demonstrated improved RFS with ipilimumab relative to placebo (HR 0.75, $p = 0.0013$), leading to approval by the FDA in 2015. Side effects were similar to those seen with the use of ipilimumab in metastatic melanoma. At median follow-up of 5.3 years, there was a 28% reduction in the relative risk of death (HR 0.72, $p = 0.001$), and the 5-year overall survival rate was 11% higher with adjuvant ipilimumab therapy than with placebo. A follow up trial comparing adjuvant therapy with ipilimumab (10 mg/kg) or nivolumab (3 mg/kg) for one year completed accrual in 2016 and will provide additional information about the dose and the duration of treatment with ipilimumab [83]. In addition, the EORTC 1325 trial randomised about 1000 patients with stage IIIa (>1 mm metastasis)/(stage IIB/IIC melanoma) to pembrolizumab versus placebo and has completed accrual. Patients who received placebo had access to pembrolizumab at relapse, so this trial could answer the crucial question of whether patients should

receive pembrolizumab up-front, or if it should be reserved as first-line therapy for disease relapse [84]. Clinical trials examining the use of immune checkpoint therapy in the neoadjuvant setting are also underway in multiple cancer types.

3.2. Dose and schedule considerations

Increasing evidence demonstrates that higher doses of checkpoint inhibitors result in higher response rates, but this does not translate into longer OS. Since higher doses also result in a higher incidence of irAEs, the use of lower doses is preferred. The use of different ipilimumab doses has been extensively studied in phase I–II trials [85]. A randomised phase 3 trial directly compared 3 and 10 mg/kg ipilimumab in advanced melanoma [86]. At 43-month follow-up, median OS was 15.7 months (95% CI 11.6–17.8) with 10 mg/kg versus 11.5 months (95% CI 9.9–13.3) with 3 mg/kg (HR 0.84; $p = 0.04$). Patients treated with the high dose experienced higher rates of drug-related toxicity. The phase I study of nivolumab showed no major impact of dose on ORR, and the 3 mg/kg dose was selected because it achieved the highest number of responses [87]. The phase I study of pembrolizumab explored doses of 2 mg/kg every 3 weeks, 10 mg/kg every 2 weeks and 10 mg/kg every 3 weeks [12]. The ORR was higher with pembrolizumab 10 mg/kg every 2 weeks than with 2 mg/kg every 3 weeks, but there was no difference in OS. Pembrolizumab 2 mg/kg every 3 weeks was chosen for further study given its more favourable safety profile and similar impact on OS.

3.3. Towards new surrogate endpoints

OS has been the gold-standard clinical trial endpoint. However, it can take many years before a phase III study provides OS data. A central question is whether we can design clinical trials using surrogate endpoints predictive of OS. Possible surrogate endpoints include ORR, PFS, duration of response (DoR) or a composite of these endpoints.

PFS was a strong surrogate for OS in randomised, dacarbazine-controlled trials for advanced melanoma [88]. The use of PFS as an endpoint should minimise the risk of discarding potentially effective new treatments when OS endpoints are contaminated by post-progression therapy with other effective drugs. However, an OS landmark analysis may be preferred. The potential value of this approach is illustrated by the 1-year and 2-year OS rate of 46% and 24% with ipilimumab relative to historic 1-year survival rates of 25.5% in advanced melanoma [89]. Similarly, the mPFS might not provide complete information about drug activity. With BRAF/MEK inhibitor combination therapy and anti-PD-1 agents (nivolumab and pembrolizumab), OS data are very similar. However, the tyrosine kinase

inhibitors have a longer PFS and a higher ORR, whereas the anti-PD-1 agents have a longer DoR (23 months with nivolumab [13], and a 2-year DoR rate of 70% with pembrolizumab [90]). The PFS landmark analysis, a composite endpoint that includes ORR and DoR, might best predict the effect of immunotherapy on long-term survival. Drugs with a high ORR and a long DoR reflected by 2- or 3-year PFS may be the most promising agents to develop [91].

4. Considerations for clinical translation of novel combination immunotherapies

4.1. Role of preclinical models

Preclinical models cannot recapitulate the inherent complexity and heterogeneity of human disease, but they provide important tools for defining the mechanism of action of immunotherapies, evaluating the potential synergy of immunotherapy combinations and optimising scheduling. Syngeneic murine models are well suited for evaluating the mechanism of action of immunotherapies because they yield rapid results and are relatively cost-effective. Murine models that employ human tumour xenografts lack a functional immune system to prevent the immune-mediated rejection of human tumours and should not be used to develop cancer immunotherapies. Mice that harbour a human immune system have been developed to evaluate cancer immunotherapies, but their ability to predict what will happen in the clinic remains unproven.

The majority of transplantable tumour models assess the effects of immunotherapy early after tumour implantation in mice with small tumours. This is unlikely to recapitulate the natural development of cancer in patients, where tumours develop over time and induce immune tolerance. Overcoming tumour-induced tolerance is a major hurdle that must be overcome to generate potent anti-tumour immunity in patients [92–95]. Genetically engineered mouse models (GEMMs) that develop tumours spontaneously more closely recapitulate both the natural tumour microenvironment and the tumour-specific immune tolerance that characterise human tumours. However, these models may be limited in that they typically do not have the high mutational burden that correlates with the response of some human tumours to immune checkpoint blockade.

An important objective of preclinical research is to identify biomarkers associated with improved clinical outcomes, particularly given the potential toxicity and high costs associated with immunotherapy in patients [41,96–99]. In preclinical models, changes in leucocyte phenotype and function can be serially evaluated in the peripheral blood, lymph nodes, spleen and tumour. Biomarker analyses may include profiling cell surface markers, cytokines and chemokines. Global gene

expression analyses (mRNA arrays, RNAseq, TCRseq) may aid in designing flow cytometry panels that can be validated in preclinical models before use in human studies. Conversely, insights from clinical trials, such as the compensatory induction of alternate checkpoint molecules following PD-1 blockade, may guide rational combinations for preclinical testing that can overcome therapeutic resistance.

4.2. The tumour immune microenvironment and immunotherapy

The striking activity of PD-1/PD-L1 antagonists reflects their ability to unleash T cells present in the tumour microenvironment. Developing strategies for profiling the tumour microenvironment to predict response and circumvent mechanisms of immune suppression and escape is the key to enhance the efficacy of next generation immunotherapies.

4.2.1. Reduced recognition of tumour cells by T cells

Defects in the antigen:MHC class I processing and presentation machinery hinder the ability of T cells to lyse tumours. This includes tumour antigen loss, altered expression of proteasome subunits, aminopeptidases, deficient expression of the peptide transporter associated with antigen processing (TAP) or tapasin, which facilitates peptide loading and downregulation or loss of MHC class I heavy chain and/or β_2 -microglobulin (β_2 -m). Alterations in these molecules are more pronounced in metastases compared with primary tumours and are associated with worse prognosis and shorter patient survival [100,101]. In breast cancers, a direct correlation between HER-2 overexpression and downregulation of HLA class I components was found [102]. Analyses of metastases from melanoma patients after immunotherapy showed that responding patients expressed high levels of HLA class I antigens and robust T cell responses, whereas progressing patients had lower HLA class I expression and reduced T cell responses [103]. Notably, HLA class I and/or antigen loss variants can develop during targeted immunotherapy, leading to immune escape [104]. There has recently been rekindled interest in the non-classical HLA class I antigen HLA-G, which inhibits both NK and CD8⁺ T cells. Overexpression of HLA-G in tumours is associated with poor prognosis and reduced T cell infiltrates [101,105,106]. Signalling networks also affect tumour cell immunogenicity. Structural alterations or downregulation of various type I and II IFN signalling components are frequently found in tumours [107–109]. Consistent with these data, acquired resistance to PD-1 blockade in melanoma patients was recently associated with defects in IFN receptor signalling (JAK1/2 mutations) and antigen presentation (β_2 -m mutations) [110]. Defects may also be related to epigenetic regulation, opening the door for restoring antigen presentation with

DNA methylation inhibitors and/or histone deacetylase inhibitors [111–113].

4.2.2. The tumour immune architecture

Increasing data suggest that the number, type, quality, and distribution of immune cells present in a tumour and the active pathways that regulate them are a critical determinant of patient outcome [114]. The immunoscore is a scoring system that quantifies the frequency of cytotoxic and memory T cells both within the tumour mass (CT) and in the tumour's invasive margin (IM) [115]. A higher immunoscore corresponds to better prognosis independent of other variables also known to affect overall survival. It is a stronger predictor of patient outcome than traditional staging by the TNM system for colorectal cancer (CRC) [116,117]. The immunoscore appears to also trump genomic predictors of outcome. CRCs with microsatellite instability (MSI) CRCs have a higher response to pembrolizumab than CRCs that are microsatellite stable (MSS), though some MSS tumours also respond [118]. Notably, a prominent immune gene expression signature is found in MSI tumours, and also in a subset of MSS tumours [119]. MSI tumours are characterised by increased frameshift mutations, genetic evidence of immune-editing, higher densities of both T helper type 1 and effector-memory T cells, more proliferating T cells, greater PD-1/PD-L1 expression and higher immunoscores. Selecting patients for PD-1/PD-L1 blockade based on PD-L1 expression by tumour cells and/or infiltrating immune cells can also enrich for responses to PD-1/PD-L1 antagonists. Pembrolizumab is approved for the second-line treatment of PD-L1-expressing NSCLC [24] and is superior to chemotherapy for the first-line treatment of NSCLCs that express high levels of PD-L1 [120]. In contrast, nivolumab was not superior to chemotherapy for the first-line therapy of NSCLC with lower levels of PD-L1 expression [121]. These data highlight the importance of developing informative predictive biomarkers of response to ensure that patients get the best therapy for their cancer.

4.2.3. Mechanisms of immune suppression in the tumour microenvironment

Regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs) and tumour-associated macrophages accumulate during tumour growth and progression and can inhibit tumour immunity. Developing therapies that abrogate the activity of these cells with concomitant immune checkpoint blockade is an active area of clinical research, with multiple combination trials underway. In preclinical models, inhibiting the adenosine pathway in combination with anti-PD-1 or anti-CTLA-4 can significantly increase the response to immunotherapy [122–124]. These strategies are currently in clinical trials. Modulating tryptophan metabolism by inhibiting the enzyme indoleamine

dioxygenase (IDO) in combination with immune checkpoint blockade has also shown anti-tumour activity in preclinical models and is being tested in clinical trials [125]. Early data evaluating the activity of pembrolizumab with the IDO inhibitor epacadostat revealed a response rate of 53% with only 5% of patients discontinuing therapy due to grade 3 AEs [126]. Modulation of both stimulatory immune checkpoints (CD40, OX40, CD137) and other inhibitory immune checkpoints (LAG-3, TIM-3, B7-H3, TIGIT) alone and in combination with PD-1 blockade is also under clinical investigation.

5. Capitalising on the opportunities and confronting the challenges before us

It is clear that, independent of cancer histology, the effectiveness of cancer immunotherapy is limited to a subset of patients with inflamed tumours. Understanding the intricacies of effective tumour immunity will depend on the extensive integration of complex biological and clinical information [127]. Immune-mediated tissue destruction underlies tumour immunity, allograft rejection and autoimmune disease, and these seemingly disparate fields may inform one another. Factors that influence cancer immunity include the host genetic background, the mutational evolution of individual cancers, and environmental variables that affect immune homeostasis. It is likely that tumour rejection will result only when favourable conditions in each category synchronize [128], but it is possible that enhancing the contribution of one variable may reduce the need for others. For example, individuals predisposed to autoimmunity may be more likely to both reject their cancers in response to immunotherapy, and to develop significant irAEs.

Extraordinary roadblocks currently hamper further progress in cancer immunotherapy. These include the cost and complexity of comprehensive tissue studies that integrate multiple aspects of tumour biology and the limited availability of human tumour, blood, and tissue samples collected prospectively during hypothesis-

driven clinical studies. Comprehensive tissue studies will allow us to integrate the genetic makeup of the tumour-bearing host, the molecular and immunological landscape of individual cancers, and environmental factors (such as the gut microbiota) clearly shown to affect the immune system. Several strategies should enable us to overcome these roadblocks (Table 2).

One major strategy is to capitalise on information that is already available. The exponential growth of biomedical information generated by high-throughput methods and/or accessible through open sources is a major opportunity. However, the availability of massive amounts of data does not automatically translate into the solution to biomedical problems. Strategically mining the wealth of open-access data may prevent wasteful preclinical and clinical research, transforming currently available data into high-quality knowledge that supports hypothesis-driven clinical trial design. Jonas Salk's quote has never been more appropriate and relevant than today: "The answer to biological problems preexists, it is the question that needs to be discovered".

Another major strategy is defining the specific factors that lead to the immune detection of cancer cells and the key biomarkers associated with them. The biomarkers most relevant to immune checkpoint inhibitor therapy still need to be more clearly defined. Profiling classical and non-classical MHC molecule expression is also essential given the fundamental role that these molecules play in antigen presentation and in the modulation of immune cell interactions [129,130]. The role of shared tumour antigens, which has been over-shadowed recently by the role of tumour neo-antigens, must also be reassessed [131]. Increasing data suggest that mutational load may predict for response to immune checkpoint blockade [132,133]. However, this is not conclusively proven in humans. A significant percentage of tumours with a high mutational burden are immunologically silent. These tumours may have a low antigen presentation potential that short-circuits the immunogenic effects of tumour neo-antigens [110]. This hypothesis can only be tested

Table 2
Clinical development priorities for cancer immunotherapy.

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1. Innovative strategies for evaluating response to therapy early in treatment.
 2. Develop informative surrogate response endpoints that predict long-term survival
 3. Define informative predictive biomarkers of therapeutic response, resistance, and toxicity
 4. Refine strategies for the early recognition and management of expected and unexpected adverse events in combination trials
 5. Develop innovative strategies for evaluating the best combinations to test in the clinic, including the drugs, dose, and schedule
 6. Exploit big-data mining of available open-source data to improve the design of correlative studies
 7. Harness the power of archival samples derived from completed trials for retrospective hypothesis-driven or hypothesis-generating studies
 8. Create highly relevant preclinical models that reflect both tumour and immune biology observed in cancer patients
 9. Develop and improve tools for trial prioritisation
 10. Improve tools for patient selection and exclusion
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by profiling the somatic makeup of each tumour and simultaneously documenting the antigen-presenting machinery of that tumour. Furthermore, some tumours with few mutations may also respond to immunotherapy, perhaps via shared tumour antigens. Finally, accumulating data suggest a role for B cells in the tumour microenvironment that extends beyond producing antibodies to modulating immune cell activation and differentiation [134].

Another major strategy is to use highly informative preclinical models to effectively drive clinical translation. Better models are sorely needed, and more relevant “humanised” preclinical models should be developed and validated. Humanised models may best be used in conjunction with GEMMs to identify the most promising immunotherapies to test in cancer patients. Informative preclinical models should prioritise the best immunotherapy agents and combination regimens for clinical testing, accurately predict toxicities (particularly the additive or synergistic toxicities of combination immunotherapies) and define the best sequencing of the agents in combination immunotherapy regimens.

A final strategy is to apply the novel technologies of nanomedicine, new functional imaging techniques, and three-dimensional histopathological characterisation (as a few examples) to cancer immunotherapy. Some graphene-based nanoparticles have intrinsic immune stimulatory effects through direct interaction with the cellular pathogen sensor in immune cells, thus presenting an opportunity for immunologic synergy in the drug delivery method [135]. Functional imaging may differentiate tumour size changes related to immune infiltration versus cancer cell proliferation, thus identifying early responses and ameliorating the uncertainty of pseudo-progression. Technology enabling immunologic portraits of tumours in multiple dimensions should ultimately enable personalised immunotherapy for all the patients. Effectively meeting these challenges should enable us to transform cancer from a major burden on patients and society to a curable and even preventable disease.

Conflict of interest statement

LAE has/had consultant and advisory roles for Roche-Genentech, Celgene, Vaccinex, Astrazeneca, Amgen, Syndax, Molecuvax, and Peregrine and has received research funds from Roche-Genentech, EMD Serono, Maxcyte, Astrazeneca, Aduro, Corvus, and the Breast Cancer Research Foundation. PAA has/had consultant and advisory role for Bristol Myers Squibb, Merck Sharp and Dohme, Roche-Genentech, Novartis, Inc, Amgen, Array, Merck Serono, Pierre Fabre and received research funds from Bristol Myers Squibb, Roche-Genentech, and Array. SD has/had consultant and/or advisory roles for Eisai, Inc, Lytix Biopharma, Nanobiotix, and EMD Serono. AME has/had consultant and/or advisory roles

for Actelion, Bristol Myers Squibb, Merck, HalioDx, Novartis, Agenus, Sanofi, GlaxoSmith Kline, and Pfizer. WLR has received commercial research grants, consulting fees, and/or royalties from Bristol-Myers Squibb, Merck, Galectin Therapeutics, IRX Therapeutics, Tesaro, and Nektar Therapeutics. The remaining authors declare no conflict of interest.

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