Ther Adv Med Oncol
2018, Vol. 10: 1–16
DOI: 10.1177/1758835918777427

Immune-checkpoint inhibitors in melanoma and kidney cancer: from sequencing to rational selection

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Abstract: Immune-checkpoint inhibitors (ICPIs), including antibodies against cytotoxic T-lymphocyte associated antigen 4 and programmed cell death protein 1, have been shown to induce durable complete responses in a proportion of patients in the first-line and refractory setting in advanced melanoma and renal cell carcinoma. In fact, there are several lines of both targeted agents and ICPI that are now feasible treatment options. However, survival in the metastatic setting continues to be poor and there remains a need for improved therapeutic approaches. In order to enhance patient selection for the most appropriate next line of therapy, better predictive biomarkers of responsiveness will need to be developed in tandem with technologies to identify mechanisms of ICPI resistance. Adaptive, biomarker-driven trials will drive this evolution. The combination of ICPI with specific chemotherapies, targeted therapies and other immuno-oncology (IO) drugs in order to circumvent ICPI resistance and enhance efficacy is discussed. Recent data support the role for both targeted therapies and ICPI in the adjuvant setting of melanoma and targeted therapies in the adjuvant setting for renal cell carcinoma, which may influence the consideration of treatment on subsequent relapse. Approaches to select the optimal treatment sequences for these patients will need to be refined.

Keywords: biomarkers, immune-checkpoint inhibitors, melanoma, renal cell carcinoma, resistance, sequencing, targeted therapies

Received: 4 February 2018; accepted in revised form: 25 April 2018

Introduction

Background

Cutaneous melanoma accounts for a significant proportion (5%) of new cancer diagnoses and has an incidence that has rapidly increased over the past decade.[1] For patients with metastatic disease, the prognosis is poor with an estimated less than 20% 5-year survival.[2] Renal cell carcinoma (RCC) accounts for up to 4% of all adult malignancies, and its incidence is increasing.[3] While the majority of patients present with localised disease, 15–20% of patients present with metastatic disease and a similar percentage of patients will experience recurrence at some point following treatment with radical intent.[3] Metastatic RCC (mRCC) is rarely curable and major tumour shrinkage is very uncommon, even with modern treatments. Of historical relevance, the only demonstrated complete responses seen in the advanced setting in these diseases (5–10%) were in patients treated with high-dose interleukin 2 (IL-2).[4,5] The observed responses in what was assumed to be incurable disease provided the foundation for our understanding of the importance of the immunological milieu in which these tumours develop and accelerated the subsequent development of immune checkpoint inhibitors (ICPIs). As these drugs became licensed therapies in melanoma, an expanding interest in their potential role in other solid tumours including mRCC has developed. In the next section, we will outline the available evidence for ICPIs in both advanced melanoma and RCC.

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Current role of ICPIs in advanced melanoma and kidney cancer

Classes of ICPIs include monoclonal antibodies against cytotoxic T-lymphocyte associated antigen 4 (CTLA-4) (ipilimumab, tremelimumab), programmed cell death protein 1 (PD-1) (nivolumab, pembrolizumab), and programmed cell death protein 1 ligand 1 (PD-L1) (atezolizumab, avelumab, and durvalumab). Table 1 summarises the pivotal immunotherapy trials leading to the European Medicines Agency and US Food and Drug Administration (FDA) licensing of some of these agents in the first-line or refractory setting in advanced melanoma and RCC.

In metastatic melanoma, ipilimumab was the first ICPI to be licensed, first in the pretreated patient setting in comparison to gp100 peptide vaccination alone or in combination,[6] and then in the first-line setting in which superiority in combination with dacarbazine over dacarbazine alone was demonstrated.[7] Nivolumab's single agent efficacy in the second-line[8] and first-line settings,[9] respectively, was subsequently proven. Pembrolizumab showed better efficacy and tolerability compared with ipilimumab[10] and is currently licensed as a single agent for any line of treatment in the US and Europe. However, the first-line combination of ipilimumab and nivolumab is usually advocated for those patients deemed fit enough to derive the significant overall response rate (ORR) benefit compared with single agent ipilimumab or nivolumab.[11]

In advanced RCC, nivolumab was approved in both the US and Europe for the treatment of patients who have received at least one line of prior antiangiogenic therapy, based on improved overall survival (OS) compared with the mammalian target of rapamycin (mTOR) inhibitor everolimus.[12] Recently, the combination of nivolumab and ipilimumab has shown superior overall survival (OS) compared with sunitinib in the first-line setting.[13]

Approaches to extending survival in advanced melanoma and RCC

Despite these outstanding trial results which have shifted treatment goals for a significant proportion of patients, there is clearly scope for improvements in patient selection and a reduction in toxicities in order to enhance an individual's quality of life. The next sections will outline some of the methodologies being instigated within clinical trials to achieve these goals. These include enhanced biomarker selection which is aimed at permitting the intelligent combination of ICPI with chemotherapy, targeted therapies and other immunotherapies. Although selecting the most suitably matched treatment adjuvantly may reduce the need for or delay treatment in the advanced setting, smart scheduling trial designs may also be utilised to reduce the development of resistance and the duration of toxicities.[14]

Identifying predictive biomarkers of responsiveness to ICPIs

Identifying predictive biomarkers of ICPIs' responsiveness remains a particularly pressing concern for oncologists working with IO drugs. One of the factors confounding decision making with ICPIs is pseudoprogression, which makes it difficult to assess whether to continue treatment in the setting of apparent tumour growth. Indeed, even with iRECIST modified tumour measurement guidelines,[15] a proportion of patients are continued on treatment due to perceived clinical benefit. Equally, overall response rate (ORR) and progression-free survival (PFS) may be poor surrogate markers of OS within immunotherapy studies.[16] Treatment decisions then should ultimately be centred around which patients should start immunotherapy, but also for how long treatment should be continued despite evidence of progression, or conversely, apparent disease control. This uncertainty underpins the importance of identifying novel biomarkers which will permit tailored decision making for patients treated with IO drugs.

A meta-analysis of six studies in both early and advanced RCC supports the negative prognostic value of high PD-L1 expression in this disease.[17] In addition to this prognostic information, some IO studies currently use PD-L1 expression as a stratifying factor of response to ICPIs (Table 1). Many of these studies have, however, either not looked at or failed to show a consistent differential ORR or OS benefit in patients with higher PD-L1 expression. This may be due to the variable sensitivity of the companion diagnostic used to quantify PD-L1 expression. Many different cells express PD-L1 and the significance of expression on nontumour cells is not known. Most of these studies perform PD-L1 on archived tissue providing only a perspective of PD-L1 status in one part of the tumour, and may not be representative of the changing tumour microenvironment (TME) in other parts or in metastases. A retrospective

Table 1. Pivotal trials of immune-checkpoint inhibitors (ICPIs) in metastatic melanoma and advanced renal cell carcinoma (RCC).

	Trial	Outcome	Subpopulation deriving benefit (PD-L1 expression)	Reference
Metastatic melanoma				
Ipilimumab (ipi)	Pretreated; HLA-A*0201–positive patients ipi (3 mg/kg) *versus* ipi+gp100 *versus* gp100	2-year OS rate: 21.6% *versus* 23.5% *versus* 13.7%		Hodi *et al.*[6]
	First line; ipi (10 mg/kg) + DTIC *versus* placebo +DTIC	3-year OS rate: 20.8% *versus* 12.2%		Robert *et al.*[7]
Nivolumab (nivo)	CheckMate 037 After ipi-BRAF-inhibitor; nivo or ICC	ORR: 31.7% *versus* 10.6%	Nivo treated ORR 43.1% (>5%) *versus* 20.3% (<5%)	Weber *et al.*[8]
	CheckMate 066 First line; nivo plus placebo *versus* DTIC in BRAF WT melanoma	1-year OS rate: 72.9% *versus* 42.1%	Nivo treated ORR 52.7% (>5%) *versus* 33.1% (<5%)	Robert *et al.*[9]
Pembrolizumab (pembro)	KEYNOTE-006: ⩾1 previous line; pembro 10 mg/kg every 3 weeks *versus* every 3 weeks × 2 years *versus* ipi 3 mg/kg × 4	2-year OS rate: 55.5% *versus* 55.1% *versus* 43%		Schachter *et al.*[10]
ipi+nivo	CheckMate 067 First line; ipi 3 mg/kg × 4 followed by placebo *versus* nivo 1 mg/kg + ipi 3 mg/kg × 4 followed by nivo 3 mg/kg *versus* nivo 3 mg/kg with placebo × 4 followed by nivo 3 mg/kg	ORR: 19% *versus* 57.6% *versus* 43.7%	Highest differential in combination treated subgroup ORR 72.1% (>5%) *versus* 54.8% (<5%)	Larkin *et al.*[11]
Advanced RCC				
nivo	Pretreated; CheckMate 025 nivo 3 mg/kg every 2 weeks *versus* everolimus	mOS 25.0 *versus* 19.6 months		Motzer *et al.*[12]
ipi and nivo	First line; CheckMate 214 nivo (3 mg/kg)+ipi 1 mg/kg every 3 weeks × 4 followed by nivo 3 mg/kg *versus* sunitinib	Low risk ORR 29% *versus* 52% PFS 15.3 *versus* 25.1 months High and intermediate risk ORR 41.6% *versus* 26.5% PFS 11.6 *versus* 8.4 months mOS: not reached *versus* 26 months	Intermediate/poor risk metastatic RCC, in patients with PD-L1 expression ⩾1% ORR: 58% *versus* 22% PFS: 22.8 *versus* 5.9 months	Motzer *et al.*[13]

DTIC, dacarbazine; ICC, investigator's choice of chemotherapy; mOS, median overall survival; ORR, overall response rate = complete responses and partial responses; OS, overall survival; PD-L1, programmed cell death protein 1 ligand 1; PFS, progression-free survival.

analysis of PD-L1 expression of 55 patients' samples confirmed this disparity between primary RCC and mRCC.[18] Clearly, a more dynamic approach to biomarker identification is required.

Although retrospective analysis of tumour biopsies by immunohistochemistry (IHC) and whole exome sequencing (WES) for novel biomarkers of interest such as tumour mutational burden (TMB)

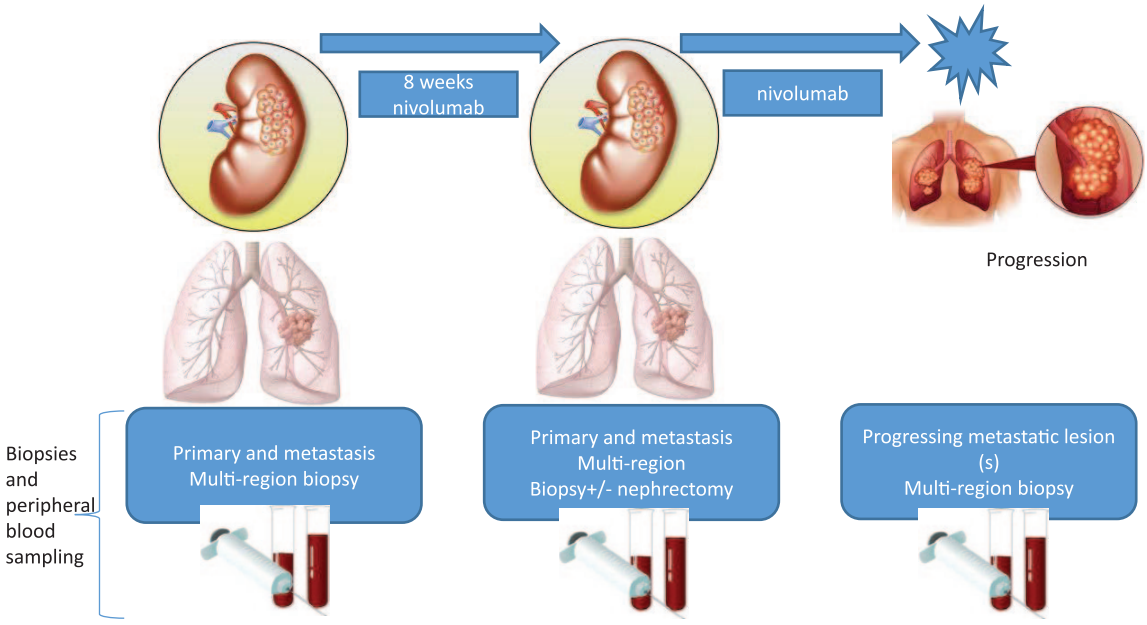

Figure 1. A schematic representation of ADAPTeR trial design. Nivolumab is initiated for 8 weeks prior to surgery for nephrectomy-suitable patients with oligometastatic renal cell carcinoma (RCC). Patients go on to have nephrectomy and are maintained on nivolumab therapy until progression. For biomarker identification, and validation, biopsies and peripheral blood samples for biomarker analysis are obtained pretreatment, at the time of surgery and at the time of progression.

and tumour infiltrating lymphocytes (TILs), respectively, is being carried out within many current trials, a more universal approach for data acquisition is needed. One example of a study incorporating prospective tissue collection with predefined endpoints is provided by the renal cancer TRACERX consortium's work,[19] which has published a provisional analysis of features identified in pretreatment tumour WES thought more likely to correlate with recurrence and survival.[20] Following on from this, more research should be conducted into enhancing our understanding of mechanisms of resistance to ICPIs. This should take a multipronged approach, including the prioritisation of comprehensive genomic profiling in order to identify novel genetic alterations which may drive this resistance. Equally important, given the increasingly significant role the TME is likely to play in the response obtained from ICPIs, are the novel laboratory methodologies to interrogate the immune milieu that will need to be developed. ADAPTeR is one such trial design which incorporates the acquisition of tumour and bloods samples at baseline and over the course of ICPI treatment [ClinicalTrials.gov identifier: NCT02446860] (Figure 1). The study is focused on the identification of novel neoantigens as well as immune-related signatures together with the evolution of T-cell subsets, immune checkpoint molecules and T-cell receptor repertoire within the TME, and the validation of cell-free DNA as both a predictive biomarker and in the identification of clonal expansions.[21]

In the next section an outline of some of the established and hypothesised innate and acquired mechanisms of resistance to ICPIs will be provided followed by an extrapolation of how each of these may be translated into the development of more reproducible biomarkers. Factors which are thought to predispose to ICPI resistance include the lack of sufficiently immunogenic mutation-associated neoantigens (MANAs), defective mechanisms for tumour antigen presentation, genetic or epigenetic alterations within immunogenic signalling pathways, and an immunosuppressed TME.[22] All these factors broadly converge to obstruct the intended unmasking of immunogenicity by ICPI. Figure 2 provides an overview of some of the well studied ICPI resistance mechanisms.

TMB and neoantigens. It has become established that those tumours with a high TMB including melanoma and non-small-cell lung cancer (NSCLC) tend to aggregate in the higher centiles of responsiveness to PD-1/PD-L1 blockade[23] and

in fact may be better biomarkers of response than PD-L1 expression across tumour types.[24] The search for new cancer genes has exploded through initiatives such as The Cancer Genome Atlas (TCGA) and International Cancer Genome Consortium (ICGC), but is now being dwarfed in magnitude of ambition by the AACR Project GENIE Consortium, which recently published some of the preliminary analysis of their phase I work.[25] The advancement provided by high-throughput technologies has permitted the substantial lowering of sequencing costs and the refinement of these genomic analyses. Indeed, recent data have shown TMB estimates from panel data closely correlated with true whole-exome mutation counts.[26] New and more rapid technological approaches have, in addition, permitted more specific subsets of mutations being identified. For example, in a recent large-scale analysis of TCGA, RNAseq and ICPI clinical response data across tumour types, RCC showed the highest number of insertion deletion (indel) mutations, which are less well characterised than nonsynonymous single nucleotide variants.[27] In a separate subanalysis of this study, a review of ICPI response data revealed the number of frameshift indels to be significantly associated with ICPI response across separate melanoma cohorts.[27] Allied to TMB is the association of altered neoepitope expression with acquired resistance to ICPIs. By comparing the neoantigen landscape of matched pretreatment and relapsed biopsies from patients with NSCLC after treatment with anti-PD-1 or an anti-PD-1 plus anti-CTLA-4 antibody combination, a number of lost neoantigens were identified.[28] The lost neoantigens had higher affinities for their major histocompatibility complex (MHC) variants and elicited stronger T-cell receptor responses in lymphocytes compared with neoantigens that were retained or gained in the relapsed tumour. Immune editing defines the dynamic balance between immune surveillance and tumour progression, leading to elimination, equilibrium or escape. The specific immune escape function that loss of neoantigens may provide is becoming evident.[22] Cancers evolve by a reiterative process of clonal expansion, diversification and selection within the adaptive landscape of the tumour microenvironment. Clearly, a separate consideration apart from the number of neoantigens is the clonality of these neoantigens within individual tumours and the prognostic information their degree of heterogeneity may provide. Large-scale analyses of neoantigen heterogeneity and its downstream effect on immune surveillance support the potential for its development into a predictive biomarker of ICPI responsiveness.[29]

Defective neoantigen presentation. The loss of β2 microglobulin (B2M) is one mechanism of acquired resistance to immunotherapy which results from defective antigen presentation.[30,31] B2M loss interferes with MHC class I heavy chain folding, leading to a loss of its receptor localization and interruption of downstream signalling, which would otherwise propagate T-cell activation and recruitment. Tumour downregulation of MHC class I molecules is an alternative mechanism of tumour immune escape which renders antitumour T-cell responses ineffective.[32] Further refinement of the influence of MHC class I alterations has been provided in a subanalysis of neoantigen expression in patients with NSCLC.[33] This suggests that up to 45% of patients had demonstrated loss of heterozygosity of the human leucocyte antigen (HLA) locus. Apart from variations in MHC class I haplotype, gene polymorphisms may also influence tumour immune responses. For example, a sequence polymorphism in the gene coding for toll-like receptor 4 (TLR4) prevents its binding to the DAMP (damage-associated molecular pattern), HMGB1.[34] A retrospective analysis of patients with breast cancer revealed that carriers of the defective protein relapsed more quickly after radiotherapy and chemotherapy than those carrying the normal TLR4 receptor,[35] which may be linked to the differential immunogenicity these therapies would provide in patients with wild-type *versus* mutant TLR4 receptors. These findings support the idea that a proficient tumour antigen presentation pathway is required for a successful response to ICPIs. Nevertheless, one could argue that antigen-loss variants could be bypassed when cytotoxic T lymphocytes (CTLs) destroy myeloid-derived suppressor cells (MDSCs), which cross present tumour neoantigens, or alternatively by MHC class II mediated CD4 T-cell immune recognition,[36] thus providing support for further research into antigen expression and recognition pathways. In addition, post-translational modifications of neopeptides, which may be identified by techniques including mass spectrophotometry of the immune peptidome, may be relevant as mechanisms of altered antigen presentation.

Altered oncogenic signalling. Another mechanism of resistance may be altered oncogenic signalling within tumour cells. Much research has been focused on defects in the interferon (IFN)

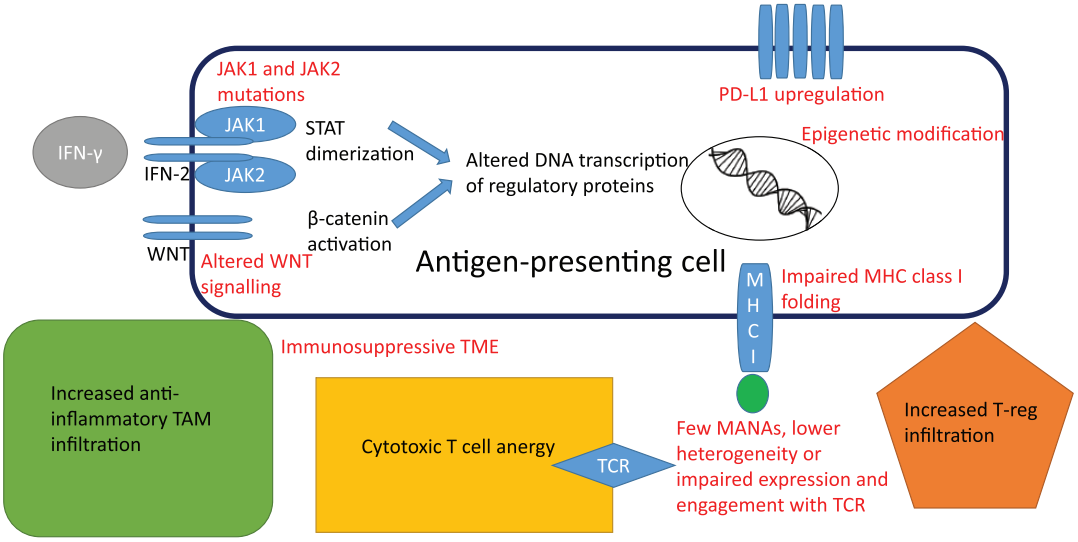

Figure 2. Schematic representation of mechanisms of innate and acquired resistance to immune-checkpoint inhibitors (ICPIs). Interferon (IFN)-γ signals *via* the type 2 IFN (IFN-2) receptor which relies on Janus kinase 1/2 (JAK1/2) phosphorylation to initiate signal transducer and activator of transcription (STAT) dimerization and transcriptional alterations on nuclear translocation. Similarly WNT phosphorylation cascades initiate alterations in β-catenin signalling. Alterations in this pathway or mutations in JAK1/2 lead to transcriptional modification of proteins which may result in, for example, impaired major histocompatibility complex (MHC) class I folding, or programmed cell death protein 1 ligand 1 (PD-L1) upregulation. Alternatively, epigenetic modification may alter regulatory protein function. Impaired MHC class I folding impairs T-cell receptor (TCR) recognition of mutation-associated neoantigens (MANAs) or fewer tumour mutations and their associated MANAs or less heterogeneity, a greater degree of which would normally induce cytotoxic T-cell binding, and the initiation of an effective antitumour response. In addition, an increased infiltration of regulatory T-cell (T-reg) and anti-inflammatory tumour-associated macrophages (TAMs) within the tumour microenvironment (TME) limit the induction of an adequate immune response.

signalling pathways.[37] In a small WES analysis case series of four matched melanoma tumour biopsies at baseline and on relapse after an initial response to PD-1 targeted therapy, the authors identified JAK1, JAK2 and B2M alterations as significant alterations which may have contributed to the development of ICPI resistance in three-quarters of matched tumour samples.[38] The role of JAK1, JAK2 and B2M alterations has also been validated in other studies.[39,40] In preclinical models, alterations in the WNTβ-catenin signalling pathway have been shown to result in a curtailment in the recruitment of dendritic cells (DCs) and CTLs.[41] An alternative mechanism may be phosphatase and tensin homolog (PTEN) loss, with PTEN loss in tumour cells associated with increased secretion of immunosuppressive cytokines, and autophagy inhibition with resulting loss of T-cell infiltration and cell-mediated cytotoxicity.[42] It is becoming evident that other receptor tyrosine kinases may also drive immune escape, for example, correlation between activation of the epidermal growth factor receptor (EGFR) pathway and upregulation of immune checkpoint proteins and anti-inflammatory cytokines has been demonstrated.[43] During ICPIs, multiple inhibitory checkpoints might also be upregulated because of IFN signalling,[44] ultimately leading to therapeutic failure. For instance, T-cell immunoglobulin and mucin domain 3 (TIM-3) upregulation has been detected in growing lesions from patients with NSCLC who initially had a partial response to anti-PD-1 therapy.[45] Many immunologists concede that IFN insensitivity induced through other means, including *via* epigenetic silencing of IFN-signalling components or *via* increased expression of negative immune regulators, might be as important as oncogenic signalling alterations. Indeed, loss-of-function mutations in the tumour suppressor *PBRM1* gene encodes a subunit of chromatin remodelling complex which is now thought to play a role in predicting response to ICPIs in RCC.[46] PBRM1 loss in this disease is thought to alter tumour cell expression profiles and may play an important role in influencing

ICPI responsiveness. An enhanced understanding of negative immune regulators within the TME has demonstrated its critical role in the development of ICPI resistance.

TME alterations. Growing evidence suggests that greater infiltration of MDSCs, including CD68+ or CD163+ specific tumour-associated macrophages (TAMs), correlates with ICPI resistance.[47] *In vivo* studies have also demonstrated that suppression of CD103+ DCs recruitment by β-catenin signalling results in primary resistance to ICPIs.[48] Chemokines such as type 1 IFN play an important role in optimal T-cell recruitment to tumours following activation of the stimulator of IFN genes (STING) pathway in the BATF3 lineage of DCs,[49,50] with curtailment in type 1 IFN activity resulting in reduced T-cell infiltration. Alternatively, metabolic processes within the TME have also been shown to reduce the immunogenicity intended to be unmasked by ICPIs. For example, a high concentration of serum lactate dehydrogenase is known to correlate with primary resistance to ICPIs.[51] These findings might be explained by the inability of CD8 T cells to export lactate in the presence of a high extracellular concentration of tumour-derived lactate, which blunts aerobic glycolysis.[52] Alterations in angiogenesis may also play a role in ICPI resistance. One effect of enhanced vascular endothelial growth factor (VEGF) signalling is the selective culling of CTLs.[53]

Gut microbiome. The intriguing associations between microbiome diversity and nonresponse to immune checkpoint inhibitors have been recently described.[54,55] It is becoming apparent that primary resistance to ICPIs can be due to specific gut microbiome profiles and that antibiotics which alter this profile may limit the clinical benefit of ICPIs. In keeping with this, faecal microbiota transplantation from ICPI-responding patients into germ-free or antibiotic-treated mice improved ICPI antitumour efficacy.[55] The mechanisms by which specific enteric bacteria including *Akkermansia muciniphila* modify the immune response during immunotherapy remain largely obscure, although the potential cross reactivity between microbial and tumour antigens is thought to enhance DC priming.[56] A lot more work is needed in this field in order to harmonize the profiling, analysis and interpretation of faecal microbiome diversity.

ICPI combination strategies

The success of ICPIs, particularly in melanoma but increasingly in other solid tumours including RCC, has initiated a flurry of preclinical research into and clinical trials of combination strategies. Some of the reasons for resistance against ICPI have been outlined and provide a justification for how combination therapies may be used to enhance the efficacy of these agents. The optimal combination approaches in the treatment of advanced melanoma and RCC has specific considerations which will be discussed in the next sections.

ICPIs and chemotherapy. The rationale for the enhancement of the antitumour efficacy of chemotherapeutics by their upregulation of immunogenicity has been known for some time. Anthracyclines are, for example, thought to be particularly immunogenic.[35] Many chemotherapeutics induce immunogenic cell death (ICD) by the combination of exposure of DAMPs, their recognition by TLRs and DC activation.[57] Paradoxically, the potential for chemotherapy to create an excess of subclonal neoantigens could potentially be detrimental to patient outcomes.[29] Thus, its role in cancers with a known higher TMB with a predicted sensitivity to ICPI alone needs to be measured against the potential of chemotherapy to be more toxic but ineffectual in curbing disease progression in combination with ICPIs.

Currently, the treatment for cutaneous melanoma rarely involves chemotherapeutics, but noncutaneous melanoma subgroups have historically shown mixed responses to chemotherapies. In a subset of mucosal melanoma, vulvar-vaginal melanoma with wild-type KIT, tumours were more likely to express markers suggestive of alkylating-agent and anthracycline sensitivity but also to express PD-L1.[58] Therefore, the combination of ICPIs with anthracyclines may, for example, be a rational approach for this subpopulation. In certain patients with targetable and accessible superficial cutaneous melanomas, the combination of ICPIs with locally applied electrochemotherapy may be efficacious.[59]

With specific reference to RCC, classical chemotherapeutics have historically demonstrated limited efficacy in this disease.[60] Some preclinical models support the 5-fluorouracil (5-FU)-induced increased exposure of the DAMP, HMGB1 from cell lines followed by the enhanced activity of 5-FU with anti-PD-L1 blockade in

xenograft models.[61] Although the effect of the combination of chemotherapeutics such as cyclophosphamide with peptide vaccination[62,63] has been investigated, study designs which combine ICPIs and chemotherapy are not well documented in the literature.

ICPIs and targeted therapies. Targeted therapeutic approaches which may be particularly amenable to combination with immunotherapies in melanoma (BRAF/MEK) and RCC (angiogenesis inhibitors) are discussed below.

Melanoma

BRAF V600E/K-mutant melanoma. The mitogen-activated protein kinase (MAPK) signalling axis is a crucial oncogenic driver and has been the subject of intense research resulting in the approval of the v-raf murine sarcoma viral oncogene homolog B1 (BRAF)/mitogen-activated protein kinase kinase (MEK) inhibitors for use in patients with unresectable or metastatic melanoma harbouring BRAF V600 mutations. Expression of immune signalling proteins, microphthalmia-associated transcription factor (MITF), and melanocyte lineage antigens including gp100 are upregulated following BRAF/MEK inhibition.[64–66] This combination also contributes to a disruption in signalling between tumour cells and the anti-inflammatory TAM population, which would otherwise function to impair effector T-cell tumour entry.[67] These findings support the idea that combined BRAF/MEK inhibition could enhance immune recognition of tumours and provides a rationale for their combination with ICPI. At present, a selection of ongoing clinical trials presented in Table 2 are exploring the safety and efficacy of continuous administration of BRAF, ME, and ICPI. Initial reports on these combinations have suggested that they are well tolerated, with response rates similar to those observed with BRAF/MEK inhibition alone.[68]

BRAF-non V600E/K and other MAPK/TK signalling/CDK disrupted pathways in melanoma. *U*nlike BRAF inhibitors, immunotherapies are also licensed in BRAF-non-V600E melanoma and have therefore become the first line of therapy for most patients in this subgroup. However, there may still be a role for signalling inhibitors in selected patients. MEK inhibitors including binimetinib have shown single-agent efficacy compared with dacarbazine in a phase III trial of NRAS mutant melanoma.[69] There is some evidence that even in rare BRAF non-V600E mutant/NRAS wild type melanoma, MEK inhibitors may demonstrate efficacy.[70,71] In addition, targeting alternative pathways has demonstrated preclinical activity, including with cyclin-dependent kinases 4 and 6 (CDK4/6) inhibitors[72] or PI3K/AKT pathway inhibitors.[73] Due to the stated efficacy of ICPIs in this disease, there is thus a rationale to combine these approaches.

RCC

Antiangiogenics. Emerging evidence suggests that antiangiogenic therapies may have immunomodulatory effects in addition to their known antiangiogenic effects. One recent preclinical study showed that during antiangiogenic therapy, PD-L1 is upregulated by IFN-γ-expressing T cells in refractory tumour mouse models,[74] suggesting a rationale for combining them with ICPIs. In addition, although there are data to suggest that angiogenesis inhibition may favour an immunosuppressive tumour microenvironment,[75] low doses of an anti-VEGFR-2 blocker may, for example, result in a trend towards normalized tumour microvasculature permitting greater infiltration of CTLs and anti-tumorigenic M1 macrophages.

Indeed, several clinical studies are ongoing in patients with advanced RCC with combinations of ICPIs and VEGF pathway inhibitors (Table 2). Preliminary results have shown encouraging clinical activity in terms of PFS and ORR.[76] IMmotion 150 which randomizes patients to receive either sunitinib or atezolizumab with or without bevacizumab [ClinicalTrials.gov identifier: NCT01984242], with crossover permitted to the combination on progression, has reported provisional phase II results. These showed no significant differences between ORR and PFS in the intention-to-treat population, but a trend towards a prolonged PFS in patients treated with first-line atezolizumab and bevacizumab (14.7 months) compared with sunitinib (7.8 months) in those patients in whose tumours PD-L1 expression was confirmed in over 1% of immune cells.[77] Interestingly, in those patients in whom an initial response to treatment with atezolizumab compared with sunitinib was demonstrated, there was an observed trend towards a prolonged PFS (12.6 *versus* 8.3 months) if subsequently treated with the combination of atezolizumab and bevacizumab on progression. An expanded confirmatory randomized phase III trial of IMmotion 150 has recently reported interim results. In the intention-to-treat analysis, a

Table 2. Combinations of immune-checkpoint inhibitor (ICPIs) with targeted therapy in melanoma and renal cell carcinoma (RCC).

Drug class MOA	Target	Drug	ICPI	Phase	Clinical Trials.gov identifier
Melanoma					
Transcriptional signalling	BRAF/MEK inhibition	Dabrafenib and trametenib	Ipilimumab (ipi) and nivolumab (nivo)	III	NCT02224781
		Dabrafenib and trametenib	Pembrolizumab (pembro)	I/II	NCT02130466
		Dabrafenib and trametenib or trametenib alone	Durvalumab	I/II	NCT02027961
		Vemurafenib and cobimetinib	Atezolizumab	III	NCT02908672
Epigenetic modulation		Azacytidine	pembro	II	NCT02816021
RCC					
Angiogenesis	VEGFA/ VEGFR/MET	Sunitinib/bevacizumab	Atezolizumab	III	NCT02420821 (ASCO 2017)
		Sunitinib/axitinib	Avelumab	III	NCT02684006
			pembro	III	NCT02853331
		Lenvatinib/ levantanib+everolimus/sunitinib	pembro	III	NCT02811861
		Cabozantanib	ipi+ nivo/nivo	III	NCT03141177

ICPI, immune checkpoint inhibitor; MOA, mechanism of action.

statistically significantly different median PFS was 11.2 months for atezolizumab + bevacizumab compared with 8.4 months for sunitinib. In the PD-L1+ group, confirmed ORR rates were 43% and 35% for atezolizumab plus bevacizumab *versus* sunitinib, respectively. Other trials comparing sunitinib *versus* avelumab with axitinib, pembrolizumab with axitinib, pembrolizumab with lenvatinib with or without everolimus, or nivolumab with cabozantinib with or without ipilimumab are ongoing in advanced RCC (Table 2).

IO/IO drug combinations. In the setting of metastatic melanoma, recurrence after prior anti-PD-1 therapy irrespective of prior response to PD-1 inhibition, CTLA-4 inhibition, demonstrated efficacy in some patients.[78] This study highlights the potential benefit of alternative approaches in the unmasking of the immune system's antitumour efficacy. In the next section, we discuss the rationale for and evolution of newer strategies of combining immune system targeted therapies in melanoma and RCC.

Targeting the TME. Tumour development is associated with the generation of an immunosuppressive tumour milieu consisting of multiple cell types, extracellular matrix and metabolic mediators.[57] Each of these components potentially represents a hurdle to CTLs and their antitumour immune responses. One example of a TME metabolic mediator showing promise is the potent indoleamine-2,3 dioxygenase (IDO) inhibitor, epacadostat. The IDO family of heme-dioxygenases catalyse the catabolism of tryptophan to kynurenine and other metabolites that drive maintenance of an immunosuppressive TME in many cancers.[79] IDO inhibition synergizes with ICPIs in preclinical models in their activation of intratumoural CD8+ T cells.[41] Based on the provisional positive results of trials combining epacadostat with pembrolizumab,[80] a phase III trial of

pembrolizumab in combination with epacadostat (ECHO-301) in metastatic melanoma has completed recruitment and the results are awaited [ClinicalTrials.gov identifier: NCT02752074]. As some efficacy of this approach was also demonstrated in RCC, the combination of another IDO mediator, vorinostat, with pembrolizumab is being trialled specifically in RCC and urothelial neoplasms [ClinicalTrials.gov identifier: NCT02619253]. Alternative TME approaches are expanding but remain in early phase trial development.

T-cell agonists and immune checkpoint inhibitors. The reversal of anergic or exhausted T cells by ICPIs may allow these cells to be more potently activated by targeting with immune-activating antibodies, potentiating their antitumour activity.[81] Alternatively, blockade of other identified immune checkpoint molecules (B7/H3, TIM-3, LAG-3) may be hypothesized to provide enhanced efficacy over PD-1, PD-L1 and CTLA-4 inhibitors alone. Most of these agents are in early phase trials across solid tumour indications, although the combination of pembrolizumab with an anti-LAG-3 antibody is an example of a trial recruiting patients with advanced melanoma [ClinicalTrials.gov identifier: NCT02676869]. A clinical trial currently recruiting patients with advanced RCC stratifies patients to receive an alternative checkpoint molecule inhibitor added to their anti-PD-1 inhibitor treatment if at least a partial response to treatment is not attained [ClinicalTrials.gov identifier: NCT02917772].

Other T-cell activation techniques: vaccine therapies and alterations in gut microbiota. Vaccine therapies seek to exploit cellular immune responses to cancer antigens.[82] Such antigens may be delivered to the host immune system as peptides or *via* DCs which when activated act as powerful immune activators, though this is rapidly ablated by CD8+ lymphocytes. The combination with pembrolizumab for unresectable melanoma showed objective responses in a phase I trial[83] and the combination is being further investigated in a phase III trial against the vaccine on its own [ClinicalTrials.gov identifier: NCT02965716]. Preliminary data from the phase II trial investigating ipilimumab *versus* ipilimumab and T-VEC showed superior ORR of the combination over ipilimumab alone.[84] Clinical data in advanced RCC are limited. After objective responses to the administration of the multipeptide vaccine, IMA901, were shown to be associated with improved OS in patients with advanced RCC, a phase III trial comparing the addition of IMA901 with sunitinib and sunitinib alone was set up but failed to demonstrate an improvement in OS.[85] Similarly, although the rocapuldencel-T (AGS-003)/sunitinib combination had demonstrated immunological responses associated with extended OS,[86] this combination was not associated with improved OS compared with sunitinib alone in the expanded phase III trial.[87] Other phase II combination trials with vaccines which induce a significant immunological response will likely continue to drive further research into this approach. For example, in one phase II trial of 28 patients, the combination of DC vaccination with injection of cytokine-induced CD8+ T cells was well tolerated with an ORR of 39% demonstrated.[88] The combination of multipeptide dendritic vaccination with anti-PD-1 therapy is being tested in a phase II trial in solid tumours including advanced RCC [ClinicalTrials.gov identifier: NCT02886897].

A better understanding of gut microbiota diversity will permit selection of approaches that will facilitate the development of adjunctive therapies, including appropriate antibiotic or probiotic formulations or commensal antigens/vaccines with molecular mimicry to tumour antigens.[22] The development of vaccines against specific tumour neoantigens is likely to refine their efficacy,[89] and together with anti-PD-1 antibodies and granulocyte–monocyte cell-stimulating factor (GM-CSF) may enhance their activity.[90] An example of a personalised vaccine strategy utilized in patients with advanced melanoma includes a trial of vaccination with up to 20 predicted personal tumour neoantigens with the aim to expand pre-existing neoantigen-specific T-cell populations and induce a broader repertoire of T-cell specificities. Within this trial, of six vaccinated patients, four had no recurrence at 25 months after vaccination, while two with recurrent disease were subsequently treated with anti-PD-1 therapy and experienced complete tumour regression.[91]

Adjuvant therapies to prevent metastatic disease

An alternative approach to improving outcomes in advanced melanoma and RCC is to introduce effective adjuvant therapies which may reduce the incidence of progression to advanced disease or extend the disease-free interval in subgroups of patients.

Melanoma. Adjuvant ipilimumab has been shown to improve relapse-free survival (RFS) and OS when initiated in resected stage 3 melanoma, but more than half of patients experience significant toxicity with treatment-related deaths reported.[92] Nivolumab may be a less toxic but effective alternative with treatment in resected, stage 3B/C melanoma resulting in significantly longer RFS with lower rates of severe toxicity compared with ipilimumab.[93] Although the single agent vemurafenib has shown an improvement in RFS in excised BRAF V600E-mutant melanoma,[94] the combination of dabrafenib plus trametinib significantly lowered the risk of recurrence in a similar group of resected patients with BRAF V600E/K mutations compared with placebo.[95]

RCC. To date, three adjuvant-targeted trials have reported in patients with RCC. ASSURE reported no improvement in RFS in patients treated with sorafenib or sunitinib *versus* placebo, even in high risk for recurrence groups.[96] S-TRAC showed a RFS benefit for adjuvant sunitinib *versus* placebo (6.8 *versus* 5.6 years).[97] Although a 31% decrease in the risk of recurrence in the PROTECT study was observed, the study did not meet the primary DFS endpoint.[98] Apart from possible differences in drug efficacy or variations in the standardizations of blinded review, one of the central differences between the positive trial S-TRAC, and ASSURE and PROTECT was that S-TRAC mainly enrolled patients at higher risk for RCC recurrence.[99] At least three trials of neoadjuvant or adjuvant ICPIs are open for recruitment and include IMmotion010 [Clinical Trials.gov identifier: NCT03024996], PROSPER [ClinicalTrials.gov identifier: NCT03055013] and KEYNOTE-564 [ClinicalTrials.gov identifier: NCT03142334].

The question raised by the successes of many of these adjuvant therapies is how this may affect the treatment selection for those patients who go on to develop metastatic disease. Better trial designs, for example, a multiarm, multistage, randomized control platform that foresees the possibility of the development of resistance and stratifies accordingly, will become more important. This will permit the assessment of several agents against a single control with the cessation of recruitment to research arms that do not show a predefined advantage and thereby accelerate treatment evaluation. An example of one such approach is the Renal Adjuvant MultiPle Arm Randomised Trial (RAMPART) trial which has not yet opened to recruitment.

Sequencing therapies

Scheduling and dosing regimens relating to optimal tolerability and the potential for treatment-free periods are important considerations in both melanoma and RCC, diseases in which there are now several lines of treatment available. The slow clearance of therapeutic antibodies might make it difficult to manipulate the administration schedule of ICPIs, although the durability of response after single doses of ICPI is well documented.[100] Pharmacokinetically, dabrafenib and trametinib could be intermittently dosed.[100] Although the influence of treatment breaks on the development of resistance needs to be considered, there is also a counterargument that intermittent therapy may give more durable disease control.[101] If utilised in an optimum sequence of ICP/BRAK/MEK-i, this strategy may permit the triple combination to be used as a well tolerated, intermittently administered regimen. Sequential combination immunotherapy and targeted therapy (SECOMBIT) is a trial which has been set up to address this specific question.[102] Additionally, the phase II trial combination of the BRAF/MEK-inhibitors, vemurafenib/cobimetinib, followed by immunotherapy with atezolizumab is recruiting [ClinicalTrials.gov identifier: NCT02902029].

In RCC, in order to improve treatment tolerability, modifications to schedules with the VEGF inhibitor sunitinib, for example with a 4-week on, 2-week off, or 2-week on, 1-week off schedule, has already been successfully trialled.[103] There are a few trial designs which attempt to answer the question of the most appropriate sequence of targeted therapy and ICPIs. For example, a trial sequencing ICPIs *versus* further antiangiogenic therapy after at least stabilization of disease on antiangiogenic therapy is recruiting patients [ClinicalTrials.gov identifier: NCT02959554]. AXINIVO is a phase II trial randomizing patients to receive first-line axitinib *versus* nivolumab with crossover permitted on progression [ClinicalTrials.gov identifier: NCT03172754]. TITAN is another phase II study which attempts to address the question of the potential benefit of adding in ipilimumab boosts in those patients who have demonstrated a measureable response to nivolumab [ClinicalTrials.gov identifier: NCT02917772].

Conclusions and perspective

Although there have been significant advances in the treatment of both advanced melanoma and RCC at least in part by the expansion in the use of

ICPIs, 5-year survival rates in these diseases remain poor. This review outlines approaches to increase the 'tail of the curve' of survival for our patients. One approach is the improvement in our identification of biomarkers of response so that we can select the most biologically suitable first line of treatment for each patient. Intelligent trial designs including ADAPTeR which incorporate biomarker validation are essential to identifying mechanisms of ICPI resistance. The rationale for specific ICPI combinations with chemotherapy, targeted agents and other IO drugs is provided. Although adjuvant therapies particularly in melanoma are showing significantly improving RFS, more widespread adoption of these therapies will impact on the sequencing of therapies for those who relapse with metastatic disease. Currently, in melanoma and kidney cancer we have few high-quality data to guide the sequencing of ICPIs with other therapies. However, because sequencing may be a more tolerable and cost-effective way to extend survival for selected patients, trial designs which intelligently test sequencing of immunotherapy with targeted agents should be prioritized.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of interest statement

James Larkin obtains Institutional research support from: BMS, MSD, Novartis, Pfizer, Consultancy fees from: Eisai, BMS, MSD, GSK, Kymab, Pfizer, Novartis, Roche/Genentech, Secarna, Pierre Fabre, EUSA Pharma, Funding support from: NIHR RM/ICR Biomedical Research Centre for Cancer. Other authors declare that there is no conflict of interest.

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