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REVIEW-THEMED SECTION

Immune checkpoint blockade in solid organ tumours: Choice, dose and predictors of response

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Immune checkpoint blockade has transformed outcomes across solid organ tumours. Monoclonal antibodies targeting the negative inhibitory cytotoxic T lymphocyte-associated protein 4 and programmed-death 1/programmed death-ligand 1 axis can lead to deep and durable responses across several tumour streams in the advanced setting. This immunotherapy approach is increasingly used earlier in the treatment paradigm. A rapidly evolving regulatory, reimbursement and drug development landscape has accompanied this novel class of immunotherapy. Unfortunately, only a small proportion of patients respond meaningfully to these agents. Here we review how the underlying tumoural genomic, histological and immunological characteristics interact within various patient phenotypes, leading to variations in response to checkpoint blockade. Concurrently, we outline the clinical trial and real-world evidence that allows for appropriate selection of agent, dose and schedule in solid organ malignancies. An exploration of current trends in basic and translational research in immune checkpoint blockade accompanies a commentary on future clinical directions for checkpoint blockade in oncology.

KEYWORDS

checkpoint blockade, cytotoxic T lymphocyte-associated protein 4, genotype, immunotherapy, programmed death 1, programmed death-ligand 1, pharmacology, phenotype

1 | INTRODUCTION

The advent of immune checkpoint blockers (ICBs) has signalled a paradigm shift in the treatment of many solid organ tumours. Monoclonal antibodies targeting T-cell immune checkpoints can break cancer induced immune tolerance and transform outcomes for patients. T-cell inhibitory checkpoints, cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and programmed-death 1 (PD-1), together with tumour/stromal expressed programmed death-ligand 1 (PD-L1) have all been successfully targeted within the last decade. CTLA-4, an inducible negative coregulator of T-cell activation, competitively binds to B7 ligand on antigen presenting cells, leading to an inhibitory signal that dampens early T-cell activation[1] and the removal of this brake[2] facilitates T-cell priming and subsequent tumour response.[3] PD-1, a broadly dispersed immune inhibitory receptor, interacts with its ligands PD-L1 or PD-L2 to inhibit the effector phase of T cells[4] and is commonly upregulated in T-cell exhaustion.[5]

This review will explore the underlying how the underlying pharmacology of ICBs interacts with tumoural histological, genomic and immunological characteristics within variable patient phenotypes in order to better understand choice, dose and schedule of agents. Despite the widespread use of these agents across solid malignancies, there remains an unmet medical need for the 70% of patients who do not respond or relapse post-initial response.[6] There is a lack of suitable predictive biomarkers to help guide therapy at present and we will explore the current biomarker landscape.

2 | A SHIFTING LANDSCAPE

The rapidity of clinical progress since the first Food and Drug Administration (FDA) approval of an ICB, **ipilimumab** (YERVOY Bristol-Myers-Squibb) for treatment refractory, unresectable/metastatic melanoma[7] has resulted in a shifting drug development and regulatory landscape. Subsequent to ipilimumab, 3 anti PD-1—**pembrolizumab** (KEYTRUDA, MSD), **nivolumab** (OPDIVO, Bristol-Myers-Squibb) and **cemiplimab-rwlc** (LIBTAYO, Regeneron)—and 3 anti-PD-L1—**atezolizumab** (TECQNTRIQ, Roche), **durvalumab** (IMFINZI, Astra Zeneca) and **avelumab** (BAVENCIO, Merck KGaA)—ICBs have been approved by the FDA. Total development time from investigational new drug application to new drug approval has shortened for ICBs compared to other recent anticancer therapies: 60.77 *vs* 81.4 months.[8] Given the similar approval phase lengths, the shortened time to the clinic has been due to the ability of regulatory authorities to grant ICBs "breakthrough therapy designation", permitting consideration of data from early phase trials as the basis for approval.[8,9] This designation facilitates expedited approval, if preliminary clinical evidence suggests substantial improvement over available therapies, in an area of unmet medical need.

Multiple ICBs have utilised the accelerated approval pathway, based on surrogate end points likely to predict clinical benefit,[10] including progression-free survival (PFS).[11] Overall survival (OS) data are immature for many of these studies, and most FDA approvals are contingent on the provision of postmarketing commitments, e.g. final study reports for these pivotal trials. Criticism has been levelled at this process given a number of meta-analyses finding a low level of correlation between surrogate end points and OS,[12,13] although the validation studies used in these analyses were performed before the development of ICBs.

The mechanism of action of ICBs leads to unique patterns of response such as pseudoprogression, first seen with ipilumumab.[14] Here, response occurred after initial conventional response evaluation criteria in solid tumours (RECIST) imaging-defined progression. This was due to anti-CTLA-4 induced trafficking of effector T cells to tumour sites prior to their clinical activity, leading to a temporary lymphocytic infiltration.[14,15] Other nonclassical tumour kinetics seen include delayed response and a mixed response prior to a sustained durable response.[16] It has been postulated that the modified World Health Organisation criteria used in the pivotal initial ipilimuamb trial[7] and RECIST v1.1, the imaging basis for all other pivotal ICB trials, may underestimate the benefit of these agents in approximately 15% of patients.[17] Subsequently, 4 separate immune-related response criteria have been developed beyond RECIST.[18–21] As a consensus guideline, immune-related RECIST has the most significant traction. This introduced unconfirmed progressive disease, which permits treatment beyond progression, requiring diagnosis of confirmed progressive disease by subsequent imaging assessment 4–8 weeks later. Patients can also be assigned introduced unconfirmed progressive disease multiple times providing there is no confirmed progressive disease.[15]

3 | EVIDENCE OF EFFICACY

3.1 | Melanoma

Melanoma has long been sensitive, in part, to utilisation of the immune system as a treatment modality.[22] High-dose recombinant interleukin-2, a cytokine mediating T-cell growth, was previously the only approved immunotherapy for metastatic disease, based on an objective response rate (ORR) of 15.9% (43/270),[23] with duration of response (DOR) that plateaued at 36 months.[24]

In 2011, ipilimumab an IgG1 anti-CTLA-4 mAb was the first ICB to show a median OS (mOS) benefit in patients with refractory metastatic melanoma.[7] This trial randomised previously treated patients to 4 cycles (induction) of ipilimumab plus glycoprotein 100 (gp100, a peptide vaccine), ipilimumab alone or gp100 alone. This saw a 3.6-month ipilimumab mOS benefit over gp100 (10 *vs* 6.4 months, hazard ratio [HR] 0.68, $P < .001$). Ipilimumab was moved into the first-line setting with a higher dose (10 mg kg^{-1} cf 3 mg kg^{-1} in later trials) plus dacarbazine compared to dacarbazine alone.[25] An increased response at a higher dose was seen at the expense of a higher rate of immune-related adverse events (irAEs). The trial showed durable responses, with 20% 3-year survival,[26] despite marginal improvements in mOS over dacarbazine (11.2 *vs* 9.1 months, HR: 0.72, $P < .001$). The clinical benefit for a subset of patients, despite a lack of change in traditional measures of response via RECIST, further increased the importance of a novel immunotherapy focused imaging criteria.[5]

2014 saw the first FDA approval for an anti-PD-1 mAb, pembrolizumab, based on the phase Ib KEYNOTE-001 study. This IgG4 humanised biologic was trialled via an adaptive study design with multiple expansion allowing simultaneous evaluation across tumour types,[10] leading to approvals in metastatic melanoma and metastatic nonsmall cell lung cancer (NSCLC) alongside a companion PD-L1 diagnostic assay. Unresectable or metastatic melanoma patients refractory to ipilimumab and, if $BRAF^{v600}$ mutation positive, a BRAF inhibitor, were randomised to 2 dose levels with both schedules providing an ORR of 26%.[27] Again, these responses proved to be durable, with mOS in all KEYNOTE-001 melanoma patients (naïve and pretreated) recently reported at 23.8 months with 34% 5-year survival.[28] 15 months post first approval, the indication for pembrolizumab was expanded to the first-line after KEYNOTE-006. This took patients who had received ≤1 line of therapy and randomised them to pembrolizumab or ipilimumab induction. One-year survival was prolonged, 10 mg kg^{-1} every 2 weeks (Q2W; 74.1 *vs* 58.2%, HR 0.63, $P < .0005$ *vs* ipilimumab) 10 mg kg^{-1} Q3W (68.4 *vs* 58.2%, HR 0.69, $P = .0036$ *vs* ipilimumab).[29] This led to the early termination of the trial to allow ipilimumab treated patients to cross over. Recent 5 year *post-hoc* exploratory outcomes showed that mOS was significantly improved in the combined pembrolizumab group (32.7 *vs* 15.9 months with ipilimumab, HR 0.73, $P = .00049$).[6]

Nivolumab, another IgG4 subclass anti-PD1 mAb was also first approved in melanoma, based on CHECKMATE-037. This compared nivolumab with investigator's choice of chemotherapy agents (ICC).

Inclusion criteria mirrored KEYNOTE-001 cohorts. Initial FDA approval was based on a significant improvement in response rate over ICC (31.7%, 95% confidence interval [CI] 23.5–40.8 *vs* 10.6%, CI 3.5–23.1).[30] Median OS results were similar between the groups (15.7 *vs* 14.4 months in ICC, HR 0.95), probably due to the permission of crossover post progression (41% of patients in ICC group *vs* 11% in nivolumab received subsequent anti-PD-1/PD-L1) and high numbers of patients who dropped out once assigned to ICC (23 *vs* 1% when randomised to nivolumab) who subsequently received pembrolizumab.[31] Nivolumab was brought to the first-line after CHECKMATE-066, which took treatment-naïve patients and randomised them nivolumab against dacarbazine. Similarly to KEYNOTE-006, this study was stopped early after a median follow up of 16.2 months once mOS was noted[32] with follow-up showing a persistent mOS benefit in the nivolumab arm (37.5 *vs* 11.2 months HR 0.46 $P < .01$).[33]

The trend to combine up-front CTLA-4 (ipilimumab) and PD-1 (nivolumab) blockade was heralded by CHECKMATE-067. Preclinical work suggested synergistic benefit with combined blockade of these receptors leading to enhanced antitumour responses.[34] As expected, from an understanding of T-cell trafficking, ipilimumab increased intratumoural CD8+ T cells, whilst nivolumab downregulated activation of PD-1 in these effector T cells.[35] Patients were randomised to nivolumab *vs* nivolumab + ipilimumab induction followed by nivolumab maintenance *vs* ipilimumab induction alone, across BRAF expression. Recent data have shown the established a 5-year survival of 52% with the doublet, the only treatment for metastatic melanoma where the mOS is >5 years (mOS not reached [NR], 95% CI 38.2–NR with the doublet *vs* 36.9 months, 95% CI 28.2–58.7; with nivolumab *vs* 19.9 months, 95% CI 16.8–24.6 with ipilimumab; HR doublet *vs* ipilimumab 0.52).

3.2 | NSCLC

Nivolumab was approved by the FDA in 2015 for the second-line treatment of advanced or metastatic NSCLC, based on phase III trials that showed its efficacy across all nonsmall cell histologies. CHECKMATE-017 took refractory patients with advanced or metastatic NSCLC, with a squamous histology and randomised them to nivolumab *vs* docetaxel. ORR was improved with nivolumab (20 *vs* 9%, $P = .008$) with mOS also improved significantly (9.2 *vs* 6 months, HR 0.59, $P < .001$).[36] CHECKMATE-057 was a similar second-line study of nivolumab in patients with refractory advanced or metastatic NSCLC, with nonsquamous histologies comparing the same dosing regimen of nivolumab *vs* docetaxel. Similar improved response rates (19 *vs* 12%, $P = .02$) and mOS benefits were seen (12.2 *vs* 9.4 months, HR 0.73, $P = .002$) with nivolumab over docetaxel.[37] Interestingly, again no PFS difference was noted between the 2 groups (2.3 *vs* 4.2 months, HR 0.92, $P = .39$), potentially driven by improved outcomes in patients who had an epidermal growth factor receptor (EGFR) driver mutation with chemotherapy, or a delay in ICB induced response.

KEYNOTE-010 generated the first randomised data for pembrolizumab at 2 dose levels and schedules *vs* docetaxel, across advanced or metastatic NSCLC (mNSCLC) histologies. All participants required tumours harbouring PD-L1 expression ≥1% (Dako 22C3 Agilent Technologies USA) for inclusion, a first use of a biomarker as a prerequisite inclusion criterion. In a prespecified stratified subgroup of PD-L1 tumour proportion score (TPS) ≥50%, clinically meaningful mOS improvements were seen (2 mg kg^{-1} pembrolizumab 14.9 *vs* 8.5 months docetaxel, HR 0.54, $P = .0002$; 10 mg kg^{-1} pembrolizumab 17.3 *vs* 8.5 months docetaxel, HR 0.61, $P < .0001$). Again, there was a lack of a PFS benefit in the overall population, which may have been driven by a group of patients who rapidly progressed on ICBs, termed hyperprogressive disease (HPD) skewing results and seen in up to 9% of patients.[38]

The first anti-PD-L1 ICB, atezolizumab, an IgG1 humanised mAb, entered this second-line treatment landscape, across all mNSCLC histologies, without a specific PD-L1 expression requirement. Patients were randomised to a flat-dose atezolizumab schedule, a first for an ICB, *vs* docetaxel. Survival was improved (mOS 13.8 *vs* 9.6 months, HR 0.73, $P = .0003$) and this benefit was maintained regardless of PD-L1 expression or tumour histology (HR 0.73 for squamous and non-squamous subgroups). Indeed, the high rate of crossover (17%) may have underestimated the eventual mOS benefit.[39]

4 | EFFECT ON CLINICAL PRACTICE

The explosion of ICB clinical trial data has led treating oncologists to have choice between agents across tumour streams at various lines of therapy. A careful analysis of trial design, patient selection and subsequent reported efficacy is required to correctly individualise ICB selection based on unique patient and tumoural characteristics.

4.1 | Melanoma

The genomic revolution in oncology has afforded clinicians the luxury of choice between ICB and BRAF and MEK targeted therapy (TT) in the BRAF mutant metastatic melanoma setting. Presence of a BRAF mutation within metastatic melanoma has been shown to be associated with a higher 5–year survival when treated with first-line doublet ICB when compared to BRAF wild type disease, (60 *vs* 48%).[40] Increasing evidence has shown poorer subsequent outcomes with ICB in patients who received first-line BRAF/MEK TT[41] with the ABC trial showing dramatically reduced efficacy for doublet ICB in patients with brain metastases progressing on BRAF/MEK agents.[42] A number of mechanisms have been proposed for cross-resistance to ICB post-TT.[43] Clinicians often have to balance the propensity for a rapid but brief metabolic shutdown with TT[44,45] compared to a slower acting but potentially more durable benefit with ICB when selecting therapy for melanoma. It is important to note that there is currently a lack of randomised data comparing the sequencing of ICB *vs* TT approaches.[46]

Considering the increased toxicity profile and higher treatment discontinuation rate of doublet ICB over single agent anti-PD-1,[47] it is imperative for clinicians to expose patients to doublet over single-agent ICB judiciously, especially given the only 8% absolute improvement in 5-year survival (52 *vs* 44%).[40] Variables such as a normal lactate dehydrogenase, lung only metastases[48] and a baseline sum of tumour dimensions <102 mm[49] were found to be prognostic markers for a durable OS with single agent pembrolizumab and may guide clinicians to safely chose single agent ICB in this context. Contrastingly, in high-risk disease involving metastases to sanctuary sites such as the brain, doublet ICB has proved to be superior to single agent in terms of ORR and 24-month OS (63 *vs* 51%),[42] suggesting a need for escalation of therapy if performance status and comorbidities permit.

4.2 | NSCLC

A landmark event in the treatment paradigm was the ability to use ICBs in the first-line mNSCLC setting, obviating the need for up-front chemotherapy. The validation of the companion diagnostic IHC assay as a predictive biomarker in KEYNOTE-001 and 010, allowed the design of KEYNOTE-024,[50] with the first fixed-dose combination of pembrolizumab used *vs* ICC of a platinum doublet. All patients were PD-L1 high, defined as ≥50% PD-L1 TPS.[50] Improvements in ORR and PFS have translated into significant improvements in mOS in recently published longer follow up (30 *vs* 14.2 months with ICC, HR 0.63, *P* = .002).[51] A similar design used nivolumab in the first-line setting in CHECKMATE-026 also against ICC of a platinum doublet. This trial showed no difference in mOS (13.7 *vs* 13.8 months with chemotherapy, HR 1.07, 95% CI 0.86–1.33) in the intention-to-treat cohort, with no differences in PFS, ORR or OS seen in the PD-L1 >50% subgroup either,[52] suggesting higher efficacy of pembrolizumab in this PD-L1 high cohort. Subtle differences in the assay cut-offs defining PD-L1 positivity existed (22C3 antibody and ≥ 50% PD-L1 TPS with KEYNOTE-024 *vs* 28–8 clone and ≥5% with CHECKMATE-026). The heterogeneity of PD-L1 testing protocols should be noted, with KEYNOTE-024 mandating fresh tissue after diagnosis of metastatic disease, whereas CHECKMATE-026 permitted archival tissue. A lack of balance between baseline characteristics in the 2 studies may also have contributed to the negative result of CHECKMATE-026, with a higher percentage of never-smokers (11 *vs* 3%) in CHECKMATE-026, a population that have canonically lower tumour mutational burden (TMB) potentially more resistant to ICB.[53] The fact that 27.5% of patients treated with nivolumab had progressive disease as their best overall response (BOR; cf 9.9% with ICC), suggests that there is a group of patients with HPD that ideally need to be identified prior to treatment with ICBs.[38]

An approach combing traditional cytotoxic chemotherapy with ICB has emerged in the first-line locally advanced/metastatic NSCLC setting. This method is backed by a strong scientific rationale, with chemotherapy leading to the apoptosis of tumour cells, increasing tumour neoantigen load.[54] Some chemotherapeutic agents can increase human leucocyte antigen 1 expression on tumour cells,[55] with other agents shown to upregulate PD-L1 expression in a murine tumour model.[56] There is an increasing understanding that expression of PD-L1 is a dynamic phenomenon, susceptible to host and exogenous factors and sensitive to the effect of ICBs and other systemic therapies within the milieu of the tumour immune microenvironment (TIME).[57] KEYNOTE-189 randomised untreated patients with metastatic nonsquamous NSCLC to pemetrexed in combination with pembrolizumab and cisplatin/carboplatin *vs* pemetrexed in combination with placebo and cisplatin/carboplatin.[58] Recent survival data have shown a longer mOS in the ICB containing triplet (22 *vs* 10.7 months with double chemotherapy alone HR 0.56 *P* < .00001), maintained across all PD-L1 expression levels.[59] The study found that the greatest relative benefit of combination ICB and chemotherapy was seen in the subgroup with PD-L1 >50%, but the question remains for physicians and patients of whether this group of patients requires the addition of chemotherapy and the resultant higher toxicity, given the results of KEYNOTE-024 and in the absence of direct comparison. The use of bevacizumab, a mAb against vascular endothelial growth factor (VEGF), combined with atezolizumab and chemotherapy also found promising results in the first-line setting with mNSCLC in IMpower150. A 3-arm design tested the addition of atezolizumab and/or bevacizumab to a chemotherapy backbone of carboplatin and paclitaxel. An improvement in response rate translated to an improvement in mOS in driver mutation wild-type patients in the quadruplet *vs* bevacizumab + chemotherapy (19.2 *vs* 14.7 months, HR 0.78, *P* = .002). This was the first study that showed improved OS with an ICB for patients who had progressed following targeted therapy for EGFR-mutated tumours (mOS NR *vs* 17.5, HR 0.3,9 95% CI 0.14–1.07 for atezolizumab, bevacizumab, carboplatin and paclitaxel *vs* bevacizumab, carboplatin and paclitaxel, P-value not provided),[60] suggesting the quadruplet Impower 150 regimen preferential in this post-EGFR space. Please refer to Table 1 for descriptors of all pivotal trials leading to FDA registration for ICBs in melanoma and NSCLC.

4.3 | Other solid organ tumours

The TIME is a poorly characterised, complex determinant of the response to ICB. VEGF affects the immune cell infiltrate into tumours, directly inhibiting dendritic cell growth,[61] potentiating CD8+ T-cell exhaustion[62] and preventing T-cell infiltration through endothelial cells.[63] All these actions contribute to an immunologically *cold* (cytotoxic T lymphocyte infiltrated-excluded) tumour.[64] A selective tyrosine kinase inhibitor (TKI) axitinib, with potential to overcome this VEGF immunosupressive effect, was combined with pembrolizumab (KEYNOTE-426) in the first-line setting in patients with metastatic clear cell renal cell carcinoma (mccRCC), with sunitinib, a first-line TKI as the comparator. The pembrolizumab/axitinib combination has shown positive OS data at 12 months (89.9% *vs* 78.3% with sunitinib, HR 0.53, *P* < .0001) with this OS benefit maintained only in the International Metastatic Renal Cell Cancer Database Consortium intermediate- and poor-risk groups.[65] A doublet ICB approach has also been utilised in the first-line setting in mccRCC, with a combination of

TABLE 1 Pivotal registrational trials of ICB in the metastatic setting: melanoma & NSCLC.

		Regimen update doses and schedules	Number patients	Baseline characteristics	Objective response rate	Overall survival
Ipilimumab	1st line	(Ipilimumab 10 mg/kg + dacarbazine) *vs* dacarbazine	502	Unresectable stage III or stage IV treatment naïve BRAF not assessed	33.2 *vs* 30.2%, *P* = .41	11.2 *vs* 9.1 months, HR 0.72, *P* < .001
	2nd line	Ipilimumab 3 mg/kg *vs* glycoprotein 100	676	Unresectable stage III or stage IV, disease progressed post dacarbazine/temozolomide/foemustine/carboplatin/interleukin-2 BRAF not assessed	10.9% *vs* 1.5%, *P* = .001	10 *vs* 6.4 months, HR 0.68, *P* < .001
Nivolumab	1st line	Nivolumab 3 mg/kg Q2W *vs* dacarbazine CHECKMATE 066	418	Unresectable stage III or stage IV treatment naïve without a BRAF mutation	40 *vs* 13.9%, *P* < .001	37.5 *vs* 11.2 months, HR 0.46, *P* < .01
	2nd line	Nivolumab 3 mg/kg Q2W *vs* (dacarbazine or carboplatin + paclitaxel) CHECKMATE-037	370	Unresectable stage III or stage IV, disease progressed post ipilimumab and if BRAFv600 mutation positive a BRAF inhibitor	31.7 *vs* 10.6%, *P*-value not provided	15.7 *vs* 14.4 months, HR 0.95, *P* = 0.716[a]
Pembrolizumab	1st line	(Pembrolizumab 10 mg/kg Q2W or Q3W) *vs* ipilimumab 3 mg/kg Q3W (4 doses) KEYNOTE-006	834	Unresectable stage III or stage IV, ≤1 systemic therapy and if BRAFv600 mutation positive a BRAF inhibitor not necessary if normal LDH and no rapidly progressive disease	Q2W (33.7 *vs* 11.9% *P* < .001 *vs* ipilimumab) and Q3W (32.9 *vs* 11.9% *P* < .001 *vs* ipilimumab	32.7 *vs* 15.9 months, HR 0.73, *P* = .00049
	2nd line	Pembrolizumab 2 mg/kg Q3W *vs* 10 mg/kg Q3W KEYNOTE-001	173	Unresectable stage III or stage IV, disease progressed post ipilimumab and if BRAFv600 mutation positive a BRAF inhibitor	26% at 2 mg/kg and 10 mg/kg dose	23.8 months (naïve and pretreated)
Ipilimumab & Nivolumab	1st line	Nivolumab 3 mg/kg Q2W *vs* Nivolumab 1 mg/kg Q3W + Ipilimumab 3 mg/kg Q3w (4 doses) followed by Nivolumab 3 mg/kg Q2W *vs* Ipilimumab 3 mg/kg Q3W (4 doses)	945	Unresectable stage III or stage IV, treatment naïve across BRAF mutant and wild-type	57.6% doublet *vs* 43.7% with single agent nivolumab *vs* 19% with single agent ipilimumab, *P* < .001 for both nivolumab containing arms over ipilimumab	Doublet NR *vs* 19.9 months HR 0.54, *P* < .0001 *vs* ipilimumab monotherapy). Doublet *vs* nivolumab monotherapy (NR *vs* 36.9 months HR descriptive 0.84 [0.67–1.05])

TABLE 1 (Continued)

		Regimen update doses and schedules	Number patients	Baseline characteristics	Objective response rate	Overall survival
Nivolumab	2nd line	Nivolumab 3 mg/kg Q2W *vs* doctaxel CHECKMATE-017	272	Stage IV refractory squamous NSCLC	20 *vs* 9%, *P* = .008	9.2 *vs* 6 months, HR:0.59, *P* < .001
Nivolumab	2nd line	Nivolumab 3 mg/kg Q2W *vs* doctaxel CHECKMATE-057	582	Stage IV refractory nonsquamous NSCLC	19 *vs* 12%, *P* = .02	12.2 *vs* 9.4 months HR 0.73, *P* = .002
Pembrolizumab	1st line	Pembrolizumab 200 mg Q3W *vs* ICC of platinum doublet KEYNOTE-024	305	Stage IV refractory nonsquamous NSCLC without driver mutation ≥50% PD-L1TPS	44.8 *vs* 27.8%, *P* value not provided	30 *vs* 14.2 months with chemotherapy HR 0.63, *P* = .002
	2nd line	Pembrolizumab 2 mg/kg Q3W *vs* Pembrolizumab 10 mg/kg Q3W *vs* docetaxel KEYNOTE-010	1034	Stage IV refractory NSCLC PD-L1 expression ≥1%	18% at 2 mg/kg *vs* 18% at 10 mg/kg *vs* 9% docetaxel, *P* = .005 for 2 mg/kg and *P* = .0002 for 10 mg/kg	10.4 *vs* 8.5 months for pembrolizumab 2 mg/kg HR 0.71, *P* < .0008), 12.7 *vs* 8.5 months for pembrolizumab 10 mg/kg, HR 0.61, *P* < .0001
Atezolizumab	2nd line	Atezolizumab 1200 mg Q3W *vs* docetaxel OAK trial	1225	Stage IV refractory NSCLC	14 *vs* 13%, *P* value not provided	Median overall survival 13.8 *vs* 9.6 months, HR 0.73, *P* = .0003
Pembrolizumab & Pemetrexed & carboplatin/ cisplatin	1st line	[Pembrolizumab 200 mg Q3W + pemetrexed + cisplatin/carboplatin] *vs* [placebo + pemetrexed + cisplatin/carboplatin] KEYNOTE-189	616	Stage IV refractory nonsquamous NSCLC without driver mutation	47.6 *vs* 18.9%, *P* < .001	22 *vs* 10.7 months, HR .56, *P* < .00001
Atezolizumab & Bevacizumab & carboplatin & paclitaxel	1st line	[Atezolizumab 1200 mg Q3W + bevacizumab + paclitaxel + carboplatin *vs* [bevacizumab + carboplatin] *vs* [atezolizumab 1200 mg Q3W + paclitaxel + carboplatin] IMpower150	1202	Stage IV refractory nonsquamous NSCLC or post-EGFR/ALK progression with targeted therapy	63.5 *vs* 48%, *P* value not provided (ABCP *vs* BCP)	19.2 *vs* 14.7 months, HR 0.78, *P* = .002 (ABCP *vs* BCP)

[a]Median overall survival similar due to dropout, crossover and imbalance in baseline characteristics.

nivolumab and ipilimumab showing a 30 month OS rate of 60% with doublet ICB *vs* 47% with sunitinib (HR 0.66, 95% CI 0.54–0.80, $P < .0001$),[66] once again with the benefit seemingly restricted to the intermediate- and poor-risk groups. One of the challenges for physicians moving forwards will be the sequencing of these combination approaches, with real-world data such as increased second-line response rates post-doublet ICB *vs* ICB + TKI (45 *vs* 15%, $P = .04$, $n = 40$)[67] helping to guide treatment choice. Even though cross trial comparisons should be approached with caution, the significantly higher ORR seen in KEYNOTE-426 (59.3%) *vs* CHECKMATE-214 (42%) can help guide clinicians requiring an emergent response with their first-line of therapy towards an ICB and TKI combination over doublet ICB. Contrastingly, a preference for a deep durable response, as seen with a 9% complete response (CR) rate with doublet ICB[68] could lead a physician to choose this over a 5.8% CR rate with ICB + TKI.[65]

5 | TIMING OF IMMUNE CHECKPOINT BLOCKADE

5.1 | Consolidative setting

The encouraging results of ICB in advanced or metastatic NSCLC have led to their use earlier in the disease course, with durvalumab being used as a consolidation therapy post-definitive chemoradiotherapy, for patients with stage III NSCLC. An abscopal effect on tumour cells outside an irradiated field when combined with CTLA-4 inhibition[69] and the ability of combined ionising radiation and PD-L1 inhibition to prevent inhibitory myeloid derived suppressor cells from entering the TIME[70] provided the scientific basis for the PACIFIC trial. These patients had locally advanced or unresectable NSCLC that did not progress after at least 2 cycles of platinum based concurrent chemoradiotherapy. They were randomised to consolidative durvalumab *vs* placebo. OS data showed a benefit in the ITT population (HR 0.68, $P = .0025$)[71] with subsequent 3 year follow-up data showing a persistent significant mOS improvement in the durvalumab arm (NR 95% CI 38.4–NR *vs* 29.1, 95% CI 22.1–35.1 months with placebo, HR 0.69, 95% CI 0.55–0.86, *P* value not provided).[72] Subgroup analysis from this trial has suggested an improved PFS (HR 0.39, 95% CI 0.26–0.58) in patients started on durvalumab <2 weeks from completion of radiotherapy compared to those that had their last RT dose >2 weeks post (HR 0.63, 95% CI 0.49–0.80) suggesting early initiation is a critical variable influencing early ICB response in this setting.[71]

5.2 | The adjuvant/neoadjuvant setting

Meaningful attempts at cure for solid malignancies can often only be achieved via surgical and radiotherapeutic approaches combined with neoadjuvant or adjuvant systemic therapy. This paradigm has been utilised to good effect with ICBs, which now have evidence of OS data in the adjuvant setting in resected melanoma, EORTC 18071. This trial compared ipilimumab (at a higher dose of 10 mg kg^{-1} compared to 1–3 mg kg^{-1} used in the metastatic setting) to placebo and found in high-risk resected stage III patients an absolute OS benefit of 8.7% at 7 years (HR 0.73, 95% CI 0.60–0.89, $P = .002$).[73] Improved relapsed-free survival seen in other anti-PD-1 agents such as nivolumab (HR 0.65, 95% CI 0.51–0.83, $P < .001$ over ipilimumab)[74] and pembrolizumab (HR 0.67, 95% CI 0.42–0.69, $P < .001$ over placebo)[75] augur well for an eventual OS benefit upon maturity of the data.

A neoadjuvant approach to treatment has been shown to reduce baseline tumour burden, facilitate easier surgical resection, provide a translational window into disease biology and improve survival across tumour streams.[76] Three trials have shown that ICB in the neoadjuvant setting in resectable stage III melanoma are effective[77–79]; however, the regimens remain used in the research setting alone at present. Duration of neoadjuvant ICB has ranged from 3–12 weeks, with the likelihood of pathological CR increasing with longer duration of treatment, but having to be balanced against a higher chance of toxicity, delay to surgery and potential progression to unresectable disease. Current consensus suggests a 6–8-week duration of neoadjuvant systemic ICB in melanoma, to maintain equipoise between these factors.[76]

Only a low proportion of breast cancers,[76] often primarily the triple negative subtype, are classified as *hot* immunologically (cytotoxic T lymphocyte infiltrated-inflamed).[80] Recent data have shown that pembrolizumab in combination with carboplatin containing neoadjuvant chemotherapy led to an improvement in pathological CR, a validated surrogate endpoint (64.8%, 95% CI 59.9–69.5 *vs* 51.2%, 95% CI 44.1–58.3) compared with chemotherapy alone.[81]

6 | THE PHARMACOLOGY OF IMMUNE CHECKPOINT BLOCKADE

6.1 | Pharmacokinetics and pharmacodynamics

The pharmacokinetics (PK) of ICBs bear much similarity.[82] Linear characteristics were seen across all dose ranges although at lower doses nonlinear PK was noted (<0.1 mg kg^{-1} with pembrolizumab, <3 mg kg^{-1} with durvalumab and <10 mg kg^{-1} with avelumab).[83] All have similar: low volumes of distribution (4.72–6.9 L), confinement to the vascular compartment,[82] long half-lives and receptor-mediated nonspecific degradation and clearance mechanisms, ubiquitous throughout plasma and tissues.[84] The traditional approach of obtaining a maximum tolerated dose (MTD) from early phase trials to guide subsequent pivotal studies was not achieved. This was due to the lack of dose-limiting toxicities and the rapid seamless trial design of multiple expansion cohorts when promising efficacy was first noted.[10] Thus, PK/pharmacodynamic (PD) modelling and simulation studies were critical in the identification of appropriate regimens and dosing schedules for ICBs.[85]

Clinical trials have highly selective patient populations, with a recent study showing that 55% ($n = 256$) of patients eligible for real-

world clinical use of ICBs in melanoma would be ineligible for the corresponding pivotal trial.[86] Unsurprisingly the PK/PD studies used to guide pivotal trials may lead to an underestimation of the range of plasma exposures seen in a real-world context, with estimates of residual error ranging from 16 to 27%.[82] Serum pembrolizumab levels from 3 KEYNOTE studies (001, 002, 006) utilised a model-based approach to justify the absence of dose adjustments in sub-populations. In creating the pembrolizumab dataset, those that had *nonreportable serum concentrations*[87] were excluded, which may have provided valuable insight into interpatient variability.

6.2 | Dose and schedule selection

The most efficacious doses and schedules of ICBs may change as our appreciation of the underlying pharmacology grows, with dose individualisation coming to the fore, as it has for oral TKIs.[88,89] The traditional body weight-guided dosing, based on past experiences with other mABs and cytotoxic chemotherapy, was used across the initial phase trials of the first PD-1 inhibitors.[82] It is notable that the pivotal trials of pembrolizumab in melanoma used a variety of dosing regimens. The aforementioned limited data on linearity of PK and PD and reliance on in silico results to guide doses for future trials[90] have led to a number of adjustments to schedules and dosing of pembrolizumab, with a 200-mg flat Q3W dosing now FDA approved for melanoma.[91] Later pivotal trials of pembrolizumab used doses ranging from 50 to 400% of the phase I dose[8] contributing to multiple subsequent changes in dose and schedule. Flat exposure response and exposure–safety curves (up to a 10 mg kg^{-1} dose level) together with a lack of contribution of body weight to PK alterations have been used by manufacturers of pembrolizumab[92] and nivolumab[93] as rationale behind regulatory submissions for fixed dose regimens. Population based PK/PD modelling showed similarities of pembrolizumab exposure of 2 mg kg^{-1} Q3W and 200-mg Q3W, with clinical data utilising a fixed-dose regimen (KEYNOTE-024) confirming these results.[85] Similar in silico modelling has been used to gained EMA approval for 400-mg Q6W pembrolizumab.[94] A similar approach with nivolumab, initially based entirely in-silico modelling[85,93] led to FDA approval of a 240-mg Q2W and a 480-mg Q4W[95] regimen across tumours. The 480-mg Q4W dosing regimen was later supported by an open label extension of a phase III trial. Indeed payors across the world have noted limited clinical data to support the noninferiority claims of flat-dosing regimens compared to the pivotal weight-based dosing regimens.[96] A flat-dosing schedule has been suggested by sponsors as a method to ease logistics, minimise cost of vial wastage and prevent drug dispensing error.[83] Real-world data have suggested equivalent efficacy and toxicity between flat and weight based dosing across ICBs and tumour streams.[97] Health economic data have suggested increased cost to payors from flat-based dosing, given an average increased total dose of ICB delivered to each patient.[98] Often, the choice between a shorter interval weight-based dose *vs* a longer interval flat dose is made from a physician's assessment of need for frequent of review in elderly patients to identify toxicity quickly against the convenience to patient and impact on resource allocation from a longer treatment interval.

Receptor occupancy of PD-1 by nivolumab in circulating T cells was found to saturate at 0.3 mg kg^{-1} with flat exposure–response curves above 1 mg kg^{-1} Q2W,[99] far above the current recommended schedules. Indeed, only 2 neoadjuvant doses of nivolumab have led to a major pathological response in 45% (n = 20) of patients in a NSCLC trial.[100] It has been suggested that nivolumab could be a candidate for dose reduction.[82] No randomised trial data or regulatory authority supports ICB dose modification, but retrospective real-world evidence in resource poor settings has found that low-dose nivolumab (20–100 mg Q3W) had similar efficacy in a metastatic NSCLC cohort.[101]

The drug development evolution of ipilimumab is a lesson in appropriate dose selection, with a previous phase III trial in metastatic melanoma showing an improvement in mOS with a 10- *vs* 3-mg kg^{-1} Q3W dosing (15.7 *vs* 11.5 months, HR 0.84, P = .04), albeit at a cost of higher toxicity with ≥ grade 3 adverse events in the higher dose group (34% *vs* 18% n = 362).[102] This suggests a relationship between dose, survival benefit and toxicity with ipilimumab, not seen in the approved dosing ranges of anti-PD-1/PD-L1 ICBs.[103] The current approved doses in the melanoma context for metastatic disease remains 3 mg kg^{-1}, but the 10 mg kg^{-1} regimen is approved in the adjuvant setting.

7.3 | Therapeutic drug monitoring

Even though no schedule or dose modification can at present be formally recommended for ICB, an emerging body of evidence suggests that therapeutic drug monitoring (TDM) may be an approach to identify patients less likely to respond to ICB. Any valid TDM approach for monoclonal antibody requires a large interpatient variability, low intra-individual variability, a valid assay and an exposure–response relationship.[104] Work on PK modelling, prompted by regulatory authorities,[83] has found clearance of ICBs varies over time.[105,106] Changes in BOR were associated with decreased maximal clearance change,[107] despite a lack of trial data showing any between plasma exposure (AUC steady state at 6 weeks) to pembrolizumab and OS, over a 5-fold dose range (2 mg $kg^{-1}$and 10 mg kg^{-1} Q3W).[108]

Despite pivotal trial data suggesting no significant exposure response/survival relationship at clinically used doses, increasing prospectively collected data from real-world practice has found a statistically and clinically significant negative clearance response relationship in patients with mNSCLC treated with nivolumab.[109] A cohort of mNSCLC patients treated with an identical weight based dosing of Q2W nivolumab found, within 10 weeks of ICB initiation, that responders (n = 15) had 73% higher (p = 0.002) trough concentrations than nonresponders, and those with the highest trough concentrations had significantly longer mOS (NR *vs* 306 days p = 0.001). This echoes our own work finding that patients who had rapid disease progression whilst being treated with pembrolizumab for metastatic melanoma had consistently low (<10 μg/mL) trough levels of drug,

compared to levels persistently >20 μg/mL in those who responded, with a significant difference between the mean trough of responders and nonresponders (29.11 *vs* 15.7 μg/mL, $P < .001$).[110]

Previous trial data suggesting an association between rapid baseline plasma clearance and poor OS[111,112] have been argued to merely represent rapidly progressing patients, with a higher baseline clearance associated with disease markers for cancer cachexia syndrome,[111] suggesting that sicker patients have higher malignancy-induced catabolic clearance and a lower chance of response. Given the number of confounders at play, both patient (altered catabolic state) and malignancy related (histopathology, tumour burden and receptor expression), it is difficult to tease out whether higher drug exposure is the cause or effect of tumour response, especially given the aforementioned flat exposure–response curves.[112,113] However, given the consistent data emerging out of practice, further study is warranted to identify whether a TDM for ICB may identify patients more likely to need dose or therapeutic escalation early in their treatment course.

7 | PHENOTYPIC PREDICTORS OF RESPONSE

7.1 | Age

Ineffective cytotoxic T cells linked to an age associated low grade chronic inflammatory state, immunosenescence,[114] together with an increased proliferation of exhausted T cells[115] have been suggested as mechanisms for age associated immune dysregulation. As with other oncological therapies, the pivotal ICB trials had low numbers of older adults included, with only 9% ($n = 272$) of patients treated with nivolumab in second-line NSCLC older than 75 years.[116] This subgroup was found not to benefit from ICB (unstratified HR 1.85 95% CI 0.65–3.32) with the small sample size, imbalance in performance status (PS) and lack of statistical adjustment for multiplicity all potential confounders.[36] Patients experiencing HPD, an accelerated tumour growth rate soon after anti PD-1/PD-L1 initiation have been found to be older (median age 65 *vs* 55 y in those not experiencing HPD $P = .007$) with correlation between age as a continuous variable and response (Spearman $\rho = 0.18$, $P = .036$).[38] A subsequent meta-analysis of 9 ICB trials, across melanoma, NSCLC and mccRCC ($n = 5265$) found consistent improvements in HR for OS between subgroups of younger (<65) and older patients (>65–70) over control arms. The individual randomised controlled trials that specifically included the subgroup effect of age in melanoma have found no variation in efficacy due to age across anti-CTLA-4 or anti-PD-1 agents.[116] No ICB has an FDA-mandated labelling restriction due to variations in geriatric efficacy.

The multimorbid, frail nature of many elderly patients has been noted to be associated with an increased likelihood of developing irAEs. The cumulative incidence of grade I–II irAEs are higher in patients >70 than their younger case-controls (72 *vs* 48%, $n = 220$, $P < .05$), although with similar rates of grade III/IV irAEs.[117] Real-world experience across 106 elderly (average age 74.4 range 65–90) metastatic patients has shown frailty being the most predictive risk factor for development of an irAE (odds ratio 3.03, $P = .006$).[118]

7.2 | Sex

Sex has long been characterised as a variable influencing the immune response, to self and nonself antigens.[119] A meta-analysis of 20 randomised ICB trials in metastatic patients across tumour streams ($n = 11\,000$) found a significantly improved HR for death compared to controls in men (HR 0.72, 95% CI 0.65–0.79 *vs* 0.86, 95% CI 0.79–0.93 with women, $P = .0019$).[120] A number of limitations exist to the extrapolation of this data to real-world practice; the significantly fewer women in these trials, together with unaccounted confounders such as variations in tumour genomic profiles and lifestyle factors that could lead to differences in tumour PD-L1 and TMB accounting for such variation in efficacy. Indeed real-world studies have shown no difference in outcomes in mccRCC.[121] A more recent meta-analysis, including trial data from the newer anti-PD-L1 ICBs, together with an exclusion of 3 trials that contrasted ICBs with other ICB controls (included in the Conforti meta-analysis[120]) found in 13 721 patients (67.9% men) no statistical difference between the sexes ($I^2 = 38\%$, $P = .60$).[122]

7.3 | Performance Status

Most pivotal trials of ICB excluded patients with an Eastern Cooperative Group PS of ≥2. This PS ≥ 2 cohort is a heterogenous group of patients who can make up to 40% of the incident population of mNSCLC.[123] The limited trial data to guide treatment choice include CHECKMATE-171, a single-arm open-label phase II study of second-line nivolumab in patients with metastatic squamous cell lung cancer, which found a shortened mOS of 5.2 months in this group.[124] Real-world registry data have supported this conclusion in the same agent and a broader indication (second-line mNSCLC), with subgroup analysis showing significantly reduced mOS based on PS (PS 0 NR *vs* PS 1 11.7 months *vs* PS 2 3.4 months, 95% CI 2.3–4.4).[125] The lack of trial data supporting ICB in a PS ≥ 2 cohort needs to be interpreted with caution as it is challenging to elucidate whether PS alone is predictive of poor response to ICB, or merely a prognostic reflection of the advanced burden of disease. Data from a pembrolizumab monotherapy study of PS 2 mNSCLC patients, found a group of treatment naïve patients with a PD-L1 expression >50% gained a mOS of 16.6 months with a similar toxicity level to pivotal trials,[126] suggesting that select subpopulations within the varied PS 2 cohort may indeed benefit from ICB. In metastatic melanoma, a retrospective review looking primarily at PS alone found a significantly reduced mOS (19.5 *vs* 1.8 m, HR 5.45, $P = <.0001$) in patients treated with ICB with a PS of 0–2 *vs* 2–3.[127] This suggests that caution is required when treating patients with a PS ≥ 2 in an unselected fashion.

7.4 | Autoimmune disease

Patients with pre-existing autoimmune disease are commonly excluded from clinical trials due to fears of potentiating irAEs with ICBs,[128] despite retrospective evidence across melanoma[129] and NSCLC[130] suggesting the development of irAEs or discontinuation due to them may lead to improved outcomes. Real world practice does not allow such selective patient exclusion, given that the incidence of autoimmune disease can be as high as 25% in renal and lung cancer.[131] Prospective registry data suggests that patients with pre-existing autoimmune disease at the time of ICB initiation have a higher chance of developing irAEs than their counterparts who lack these comorbidities, but with similar OS.[132] Incidence of new irAEs or flares of autoimmune disease is common in the limited observational real-world data available, in patients treated with ICBs who have autoimmune disease (75% *n* = 123). However, the majority of these patients were managed with first-line corticosteroids with only 16% requiring other immunomodulatory agents.[133]

7.5 | PD-L1 expression

7.5.1 | NSCLC

Although not a biomarker driven trial, in a substudy of KEYNOTE-001, pretreatment tumour biopsies were stained with an IHC PD-L1 stain (22C3 Dako) quantifying membranous staining for PD-L1 on tumour cells together with infiltrating mononuclear inflammatory cells, with results reported as a percentage of tumour cells exhibiting membranous staining, giving a TPS. Tumours with the highest levels of staining exhibited deeper and more durable responses. Patients with PD-L1 staining ≥1% had a 29.9 *vs* 12.6 month mOS (HR 0.76, $P < .001$).[134] NSCLC remains the only tumour where PD-L1 is used to confidently guide treatment eligibility, with benefits seen in ≥50% PD-L1 TPS patients as per KEYNOTE-024.[50] PD-L1 expression analysis (Dako 28–8) for nivolumab found a near doubled mOS in PD-L1-positive patients *vs* docetaxel at all prespecified expression levels, although this was a retrospective analysis.

7.5.2 | Melanoma

Data from a doublet ICB approach in melanoma, CHECKMATE-067 explored PD-L1 expression as a predictive biomarker. Both doublet ICBs and nivolumab monotherapy improved ORR, PFS and OS over ipilimumab regardless of PD-L1 cut-off used.[40] Indeed 5-year OS based PD-L1 expression using time-dependent receiver operated characteristic curves from CHECKMATE-067 showed an AUC of 0.53 (95% CI 0.46–0.6) suggesting PD-L1 has a poor discriminatory ability to predict survival.[40] The separation of survival curves between doublet and nivolumab monotherapy at a PD-L1 expression of <1% suggests this as a discrete cut-off where the risk–benefit of a doublet ICB approach should be weighed against nivolumab monotherapy, with monotherapy potentially preferred with PD-L1 >1%.[135] Given that response rates in PD-L1 negative tumours can be as high as 41% (CHECKMATE-067),[47] this renders PD-L1 expression alone an inefficient biomarker in melanoma, potentially missing those that may respond meaningfully to ICBs. Intratumoural heterogeneity in PD-L1 expression, variations in assays and antibodies, issues regarding fresh *vs* archival tissue and a variety of cut-offs to predict response are just some of the multiple remaining questions that remain with the use of PD-L1 as a predictive biomarker.[136]

8 | GENOTYPIC PREDICTORS OF RESPONSE

8.1 | Hyperprogressive Disease

HPD was first characterised as RECIST-defined progression at the first evaluation, a >2-fold increase in the tumour growth rate between commencement and first evaluation[38] and a time to treatment failure of <2 months.[137] HPD was not associated with a higher baseline tumour burden or any specific histopathology. HPD was associated with high metastatic burden and a poorer prognosis in patients treated with ICBs compared to those who did not experience HPD (mOS 3.4 *vs* 6.2 months, HR 2.18, 95% CI 1.29–3.69, $P = .003$).[138] Next-generation sequencing on a cohort of 155 patients with advanced solid malignancies before commencement of ICBs, found the most commonly altered genes to be *TP53* (41.9%]), followed by *CDKN2A/B* and *TERT* (23.9%). Of these 155 patients, 49 had a time-to-treatment failure of <2 months; mostly patients with melanoma (32.9%) or NSCLC (24.5%). Six patients had *MDMT2/4* amplifications and were all HPD patients.[137] At present, there is no confirmatory test to identify patients with HPD.

8.2 | PD-L1 copy number alterations

Proliferating tumour cells acquire amplifications or deletions across large breadths of DNA, termed copy number alterations (CNAs). These CNAs lead to a greater proportion of genetic alteration than any other type of somatic mutation.[139] Genomic datasets across tumour types have found tumours with PD-L1 copy number gains exhibit high PD-L1 expression levels and have a higher mutational load than their nonamplified counterparts.[140] PD-L1 gene amplification (a form of CNA) is independently associated with tumour PD-L1 expression and highly consistent between primary and metastatic site[141] in contrast to the spatial and temporal heterogeneity[142] seen with PD-L1 expression alone. When treated with standard induction chemotherapy, patients with classic Hodgkin's lymphoma (cHL) who exhibited higher levels of PD-L1/2 CNA had shortened PFS.[143] A prospective phase II trial utilising fluorescence in situ hybridisation (FISH) at 9p24 locus for PD-L1 and PD-L2 in patients with cHL receiving nivolumab found significant relationships between the level of 9p24 CNA and BOR, improved PFS,[144] and those with PD being more likely

to have lower 9p42.1 CNA.[145] This suggests that in cHL PD-L1/2 CNAs represent a poor prognostic variable, susceptible to influence by ICB. Examination of PD-L1/2 CNA as a tool in solid organ tumours has shown low prevalence of CNAs (0.7% in a large cohort of n = 118 187 samples[146]). In this small subset ORR of those treated with ICBs (6/13) was 66.7% with a median PFS of 15.2 months (range 1.6–>24.1 months) with a mOS NR (range 1.6–24.1 months). This compares favourably to the matched cohort response rate of 29.8% (45/151, P = .03) and, despite the small subsets, this urges further investigation into PD-L1 CNA amplification prospectively.[146] Retrospective analysis of tumour samples in patients with metastatic melanoma using IHC and PD-L1/9p24 FISH to detect PD-L1 (note not assessing PD-L2) CNA showed no predictive potential of IHC or FISH for response to ICBs,[147] urging caution at current use of PD-L1/2 CNA to guide clinical decision-making at present.

8.3 | Mismatch repair deficiency

Pembrolizumab was the first tissue agnostic drug approved in oncology, indicated for patients with unresectable or metastatic, microsatellite instability-high (MSI-HI) or mismatch repair-deficient tumours. This was based on durable responses across 5 single-arm multicohort trials, across tumour types including colorectal, endometrial and biliary, with response rates of 39.6% and a median DOR NR (range 1.6 + to 22.7+).[91] However, ORR was reduced in brain (0%) and pancreatic (19%) tumours, proving there are a few exceptions to this genotypic pan-tumour predictive biomarker.[148] MSI-HI has also been shown to be predictive with nivolumab treatment combined with ipilimumab for MSI-HI/mismatch repair deficient metastatic colorectal tumours that progressed post chemotherapy, with ORR of 49.2% and durable DOR (median NR range 1.9–23.2+).[149] These MSI-HI tumours have a dense CD8+ cytotoxic T-cell infiltration, with higher numbers of somatic mutations and neoantigens compared to their MSI-stable counterparts, potentially explaining these findings.[150]

8.4 | Tumour mutational burden

The total number of somatic mutations in a specific area of tumour exome defines the TMB.[151] Some of these mutations can occasionally translate and subsequently transcribe into the expression of neoantigens on the surface of tumour cells.[152] Rarely, these unique tumour-specific neoantigens are sufficiently immunogenic to stimulate an adaptive T-cell mediated immune response.[153] Removal of regulatory immune checkpoints via ICB could subsequently make these tumour cells vulnerable to an immune response. High TMB can be caused exogenously, e.g. by ultraviolet light or smoking, or endogenously due to specific driver mutations in mismatch repair genes, which lead to impaired production of DNA repair enzymes, with resultant genomic instability (MSI-HI tumours). Other well characterised contributory driver mutations are seen in DNA polymerase epsilon, where a loss of proofreading activity can lead to rapid accumulation of somatic alterations and subsequent increase in TMB.[151]

Correlation between TMB and subsequent neoantigen load has been established,[152] but this has not always translated into susceptibility to ICBs. There is a lack of standardisation in the approach to defining TMB, with variations in: breadth of genes sampled (whole exome sequencing *vs* specific cancer-related gene panel via next-generation sequencing), type of mutation noted (inclusion/exclusion of indels), use of concurrent patient testing to ensure exclusion of individual germline variations and total depth (average number of reads that align to a reference base[152]) of DNA sequenced. Previously, the only prespecified, validated TMB threshold that enriched for an enhanced ICB response was ≥10 mutations per megabase (mut/MB; FoundationOne CDx, equivalent to 200 mutations by whole exome sequencing).[154] However updated results from this CHECKMATE-227 trial in first-line NSCLC has shown no difference in HR for OS in the ≥10 mut/MB *vs* <10 mut/MB cohorts (HR 0.77, 95% CI 0.56–1.06 *vs* HR 0.78, 95% CI 0.61–1.00),[155] leading to the sponsor withdrawal of application to the FDA for this biomarker driven indication. Other studies evaluating TMB in the first-line NSCLC setting, have also failed to show any predictive ability.[156] An exploratory analysis of TMB from liquid biopsies, assessing for circulating tumour DNA from a phase III doublet ICB study in first-line NSCLC showed both a correlation with tissue TMB (Spearman ρ = 0.6) and a threshold of ≥20 mut/MB predicting for an OS benefit (21.9 months durvalumab + tremilimumab *vs* 10 months ICC, HR 0.49, 95% CI 0.32–0.74]).[157] However significant doubts remain about the validity circulating tumour DNA for assessment of TMB in terms of concordance and sensitivity.[151]

TMB does not take into account specific mutations known to affect ICB response (e.g. JAK2 and STK11[158]), allocates equivalent importance to each mutation despite significant heterogeneity in the quality of immunogenic neoepitopes potentially produced and is likely to have a variety of thresholds for different underlying histologies. It is the quality of the genetic changes and subsequent immunogenicity of the neoantigens created that is more important than a solely quantitative value such as TMB. Response to ICB depends broadly on both the tumour neoepitope burden and the surrounding TIME.[159] Transcriptomic approaches, such as specific gene expression profiles (GEP) create a molecular outline (gene signature) assessing the mRNA expression across inflammation associated genes.[160] Specific T-cell inflamed GEP are an emerging potential biomarker, alongside PD-L1 expression on tumour/tumour infiltrating immune cells to reflect a T-cell inflamed TIME. TMB and MSI reflect the underlying immunogenic potential of the tumour. The low correlation between TMB and GEP from the Cancer Genome Atlas (a molecular database)[159,161] allow potential stratification of the likelihood of ICB response across tumour types. Tumours that exhibit TMB^{hi}- GEP^{hi}- and PD-L1-positive expression have been shown to be the most immune sensitive with the highest likelihood of meaningful response to ICB.[159] Other components of the adaptive immune milieu, such as a higher incidence of distinct natural killer subsets[162] and intratumoural dendritic cells[163]

have also been predictive of response to ICB. Moving forward, it is likely that a composite of immune and intrinsic tumoural biomarkers will be required to accurately define predictors of response to ICBs.

9 | SUMMARY

The tremendous advances of ICBs have heralded an unprecedented explosion in immuno-oncology research, with an estimated 940 agents in clinical development, used in over 500 000 trial patients.[164] There is significant redundancy in the uncoordinated approach of industry, academic institutions, governments and supranational organisations, with 164 investigational agents directed at the PD-1/L-1 axis alone despite the approval of 7 agents already. The majority of active clinical trial programmes are examining combination approaches to antagonising the PD-1/L-1 axis with a range of other conventional therapies, e.g. TT, chemotherapy and radiotherapy.

Current data do not recommend alteration in dose or schedule of ICBs from their registrational approvals. However, as outlined previously, there are multiple clinical nuances to ICB use in terms of timing of administration, choice of drug/combination therapy and sequencing in the complex therapeutic landscape of solid organ malignancies. Any future changes in dose or schedule in clinical practice are likely to be driven by TDM studies to fully understand the PK/PD in a real-world setting whilst simultaneously identifying reliable predictive biomarkers for response. The extent of stromal involvement and inflammatory cytokine milieu are just some of a multitude of other host and tumoural factors necessitating full determination to help guide the next generation of biomarker driven clinical trials. The landscape is rapidly shifting and there remains much to achieve to translate promise from bench to bedside.

9.1 | Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY.

CONFLICT OF INTEREST

V.N.: travel (domestic): MSD, Bristol Myers Squibb. N.B.: research funding (investigator initiated trial) – Merck KGaA. M.G.: none declared. A.V.W.: travel (international): Bristol Myers Squibb, Amgen, Novartis. Research funding (investigator initiated trial) – Merck KGaA

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REFERENCES

1. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science*. 2018;359(6382):1350-1355.
2. Chambers CA, Kuhns MS, Egen JG, Allison JP. CTLA-4-mediated inhibition in regulation of T cell responses: mechanisms and manipulation in tumor immunotherapy. *Annu Rev Immunol*. 2001;19(1):565-594.
3. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. *Science*. 1996;271(5256):1734-1736.
4. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol*. 2008;26(1):677-704.
5. Carlino MS, Long GV. Ipilimumab combined with Nivolumab: a standard of Care for the Treatment of advanced melanoma? *Clin Cancer Res*. 2016;22(16):3992-3998.
6. Robert C, 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 Oncol*. 2019;20(9):1239-1251.
7. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010;363(8):711-723.
8. Jardim DL, de Melo Gagliato D, Giles FJ, Kurzrock R. Analysis of drug development paradigms for immune checkpoint inhibitors. *Clin Cancer Res*. 2018;24(8):1785-1794.
9. CDER Breakthrough Therapy Designation Approvals. 2018 [cited 2019 31st August]. Available from: https://www.fda.gov/media/95302/download
10. Kang SP, Gergich K, Lubiniecki GM, et al. Pembrolizumab KEYNOTE-001: an adaptive study leading to accelerated approval for two indications and a companion diagnostic. *Ann Oncol*. 2017;28(6):1388-1398.
11. Powles T, O'Donnell PH, Massard C, et al. Efficacy and safety of Durvalumab in locally advanced or metastatic urothelial carcinoma: updated results from a phase 1/2 open-label study. *JAMA Oncol*. 2017;3(9):e172411.
12. Haslam A, Hey SP, Gill J, Prasad V. A systematic review of trial-level meta-analyses measuring the strength of association between surrogate end-points and overall survival in oncology. *Eur J Cancer*. 2019;106:196-211.
13. Prasad V, Kim C, Burotto M, Vandross A. The strength of association between surrogate end points and survival in oncology: a systematic review of trial-level meta-analyses. *JAMA Intern Med*. 2015;175(8):1389-1398.
14. Di Giacomo AM, Danielli R, Guidoboni M, et al. Therapeutic efficacy of ipilimumab, an anti-CTLA-4 monoclonal antibody, in patients with metastatic melanoma unresponsive to prior systemic treatments: clinical and immunological evidence from three patient cases. *Cancer Immunol Immunother*. 2009;58(8):1297-1306.
15. Borcoman E, Nandikolla A, Long G, Goel S, Le Tourneau C. Patterns of response and progression to immunotherapy. *Am Soc Clin Oncol Educ Book*. 2018;38:169-178.
16. Hoos A, Eggermont AM, Janetzki S, et al. Improved endpoints for cancer immunotherapy trials. *J Natl Cancer Inst*. 2010;102(18):1388-1397.
17. Hodi FS, Hwu WJ, Kefford R, et al. Evaluation of immune-related response criteria and RECIST v1.1 in patients with advanced melanoma treated with Pembrolizumab. *J Clin Oncol*. 2016;34(13):1510-1517.
18. Wolchok JD, Hoos A, O'Day S, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. *Clin Cancer Res*. 2009;15(23):7412-7420.
19. Nishino M, Giobbie-Hurder A, Gargano M, Suda M, Ramaiya NH, Hodi FS. Developing a common language for tumor response to immunotherapy: immune-related response criteria using unidimensional measurements. *Clin Cancer Res*. 2013;19(14):3936-3943.

20. Seymour L, Bogaerts J, Perrone A, et al. iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. *Lancet Oncol*. 2017;18(3):e143-e152.
21. Hodi FS, Ballinger M, Lyons B, et al. Immune-modified response evaluation criteria in solid tumors (imRECIST): refining guidelines to assess the clinical benefit of cancer immunotherapy. *J Clin Oncol*. 2018;36(9):850-858.
22. Nixon NA, Blais N, Ernst S, et al. Current landscape of immunotherapy in the treatment of solid tumours, with future opportunities and challenges. *Curr Oncol*. 2018;25(5):e373-e384.
23. Atkins MB, Lotze MT, Dutcher JP, et al. High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. *J Clin Oncol*. 1999;17(7):2105-2116.
24. Rosenberg SA, Yang JC, Topalian SL, et al. Treatment of 283 consecutive patients with metastatic melanoma or renal cell cancer using high-dose bolus interleukin 2. *JAMA*. 1994;271(12):907-913.
25. Robert C, Thomas L, Bondarenko I, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med*. 2011;364(26):2517-2526.
26. Schadendorf D, Hodi FS, Robert C, et al. Pooled analysis of Long-term survival data from phase II and phase III trials of Ipilimumab in Unresectable or metastatic melanoma. *J Clin Oncol*. 2015;33(17):1889-1894.
27. Robert C, Ribas A, Wolchok JD, et al. Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial. *Lancet*. 2014;384(9948):1109-1117.
28. Hamid O, Robert C, Daud A, et al. Five-year survival outcomes for patients with advanced melanoma treated with pembrolizumab in KEYNOTE-001. *Ann Oncol*. 2019;30(4):582-588.
29. Robert C, Schachter J, Long GV, et al. Pembrolizumab versus Ipilimumab in advanced melanoma. *N Engl J Med*. 2015;372(26):2521-2532.
30. Weber JS, D'Angelo SP, Minor D, et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. *Lancet Oncol*. 2015;16(4):375-384.
31. Larkin J, Minor D, D'Angelo S, et al. Overall survival in patients with advanced melanoma who received Nivolumab versus Investigator's choice chemotherapy in CheckMate 037: a randomized, controlled, open-label phase III trial. *J Clin Oncol*. 2018;36(4):383-390.
32. 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.
33. Ascierto PA, Long GV, Robert C, et al. Survival outcomes in patients with previously untreated BRAF wild-type advanced melanoma treated with Nivolumab therapy: three-year follow-up of a randomized phase 3 trial. *JAMA Oncol*. 2019;5(2):187-194.
34. Okazaki T, Chikuma S, Iwai Y, Fagarasan S, Honjo T. A rheostat for immune responses: the unique properties of PD-1 and their advantages for clinical application. *Nat Immunol*. 2013;14(12):1212-1218.
35. Selby M, Engelhardt J, Lu LS, et al. Antitumor activity of concurrent blockade of immune checkpoint molecules CTLA-4 and PD-1 in preclinical models. *J Clin Oncol*. 2013;31(suppl 15):3061–3061.
36. Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. *N Engl J Med*. 2015;373(2):123-135.
37. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. *N Engl J Med*. 2015;373(17):1627-1639.
38. Champiat S, Dercle L, Ammari S, et al. Hyperprogressive disease is a new pattern of progression in cancer patients treated by anti-PD-1/PD-L1. *Clin Cancer Res*. 2017;23(8):1920-1928.
39. Rittmeyer A, Barlesi F, Waterkamp D, et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. *Lancet*. 2017;389(10066):255-265.
40. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Five-year survival with combined Nivolumab and Ipilimumab in advanced melanoma. *N Engl J Med*. 2019;381(16):1535-1546.
41. Kreft S, Gesierich A, Eigentler T, et al. Efficacy of PD-1-based immunotherapy after radiologic progression on targeted therapy in stage IV melanoma. *Eur J Cancer*. 2019;116:207-215.
42. Long GV, Atkinson V, Lo S, et al. Combination nivolumab and ipilimumab or nivolumab alone in melanoma brain metastases: a multicentre randomised phase 2 study. *Lancet Oncol*. 2018;19(5):672-681.
43. Hugo W, Shi H, Sun L, et al. Non-genomic and immune evolution of melanoma acquiring MAPKi resistance. *Cell*. 2015;162(6):1271-1285.
44. Ascierto PA, Agarwala SS, Botti G, et al. Perspectives in melanoma: meeting report from the melanoma bridge (November 29th-1 December 1st, 2018, Naples, Italy). *J Transl Med*. 2019;17(1):234–234.
45. Ascierto PA, Bastholt L, Ferrucci PF, et al. The impact of patient characteristics and disease-specific factors on first-line treatment decisions for BRAF-mutated melanoma: results from a European expert panel study. *Melanoma Res*. 2018;28(4):333-340.
46. Friedlander P. The use of baseline tumor size to prognosticate overall survival in stage IV melanoma patients treated with the PD-1 inhibitor pembrolizumab. *Ann Transl Med*. 2019;7(Suppl 1):S24–S24.
47. 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.
48. Weide B, Martens A, Hassel JC, et al. Baseline biomarkers for outcome of melanoma patients treated with Pembrolizumab. *Clin Cancer Res*. 2016;22(22):5487-5496.
49. Joseph RW, Elassaiss-Schaap J, Kefford R, et al. Baseline tumor size is an independent prognostic factor for overall survival in patients with melanoma treated with Pembrolizumab. *Clin Cancer Res*. 2018;24(20):4960-4967.
50. Reck M, Rodriguez-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.
51. Reck M, Rodriguez-Abreu D, Robinson AG, et al. Updated analysis of KEYNOTE-024: Pembrolizumab versus platinum-based chemotherapy for advanced non-small-cell lung cancer with PD-L1 tumor proportion score of 50% or greater. *J Clin Oncol*. 2019;37(7):537-546.
52. Carbone DP, Reck M, Paz-Ares L, et al. First-line Nivolumab in stage IV or recurrent non-small-cell lung cancer. *N Engl J Med*. 2017;376(25):2415-2426.
53. Champiat S, Ferte C, Lebel-Binay S, Eggermont A, Soria JC. Exomics and immunogenics: bridging mutational load and immune checkpoints efficacy. *Oncoimmunology*. 2014;3(1):e27817.
54. Bezu L, Gomes-de-Silva LC, Dewitte H, et al. Combinatorial strategies for the induction of immunogenic cell death. *Front Immunol*. 2015;6:187–198.
55. Liu WM, Fowler DW, Smith P, Dalgleish AG. Pre-treatment with chemotherapy can enhance the antigenicity and immunogenicity of tumours by promoting adaptive immune responses. *Br J Cancer*. 2010;102(1):115-123.
56. Peng J, Hamanishi J, Matsumura N, et al. Chemotherapy induces programmed cell death-ligand 1 overexpression via the nuclear factor-kappaB to Foster an immunosuppressive tumor microenvironment in ovarian cancer. *Cancer Res*. 2015;75(23):5034-5045.

57. Vilain RE, Menzies AM, Wilmott JS, et al. Dynamic changes in PD-L1 expression and immune infiltrates early during treatment predict response to PD-1 blockade in melanoma. *Clin Cancer Res*. 2017;23 (17):5024-5033.
58. Gandhi L, Rodriguez-Abreu D, Gadgeel S, et al. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. *N Engl J Med*. 2018;378(22):2078-2092.
59. Gadgeel S, Rodriguez-Abreu D, Speranza G, et al. Updated analysis from KEYNOTE-189: Pembrolizumab or placebo plus Pemetrexed and platinum for previously untreated metastatic nonsquamous non-small-cell lung cancer. *J Clin Oncol*. 2020;JCO1903136. 1505–1517.
60. Reck M, Jotte R, Mok TSK, et al. 104OIMpower150: an exploratory analysis of efficacy outcomes in patients with EGFR mutations. *Ann Oncol*. 2019;30(Supplement_2):mdz063-002.
61. Gabrilovich DI, Chen HL, Girgis KR, et al. Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. *Nat Med*. 1996;2(10):1096-1103.
62. Voron T, Colussi O, Marcheteau E, et al. VEGF-A modulates expression of inhibitory checkpoints on CD8+ T cells in tumors. *J Exp Med*. 2015;212(2):139-148.
63. Dirkx AE, Oude Egbrink MG, Castermans K, et al. Anti-angiogenesis therapy can overcome endothelial cell anergy and promote leukocyte-endothelium interactions and infiltration in tumors. *FASEB J*. 2006;20(6):621-630.
64. Binnewies M, Roberts EW, Kersten K, et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nat Med*. 2018;24(5):541-550.
65. Rini BI, Plimack ER, Stus V, et al. Pembrolizumab plus Axitinib versus Sunitinib for advanced renal-cell carcinoma. *N Engl J Med*. 2019;380 (12):1116-1127.
66. Motzer RJ, Rini BI, McDermott DF, et al. Nivolumab plus ipilimumab versus sunitinib in first-line treatment for advanced renal cell carcinoma: extended follow-up of efficacy and safety results from a randomised, controlled, phase 3 trial. *Lancet Oncol*. 2019;20(10): 1370-1385.
67. Dudani S, Graham J, Wells JC, et al. First-line Immuno-oncology combination therapies in metastatic renal-cell carcinoma: results from the international metastatic renal-cell carcinoma database consortium. *Eur Urol*. 2019;76(6):861-867.
68. Motzer RJ, Tannir NM, McDermott DF, et al. Nivolumab plus Ipilimumab versus Sunitinib in advanced renal-cell carcinoma. *N Engl J Med*. 2018;378(14):1277-1290.
69. Dewan MZ, Galloway AE, Kawashima N, et al. Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody. *Clin Cancer Res*. 2009;15(17):5379-5388.
70. Deng L, Liang H, Burnette B, et al. Irradiation and anti-PD-L1 treatment synergistically promote antitumor immunity in mice. *J Clin Invest*. 2014;124(2):687-695.
71. 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.
72. Gray JE, Villegas A, Daniel D, et al. Three-year overall survival with Durvalumab after Chemoradiotherapy in stage III NSCLC-update from PACIFIC. *J Thorac Oncol*. 2020;15(2):288-293.
73. Eggermont AMM, Chiarion-Sileni V, Grob JJ, et al. Adjuvant ipilimumab versus placebo after complete resection of stage III melanoma: long-term follow-up results of the European Organisation for Research and Treatment of Cancer 18071 double-blind phase 3 randomised trial. *Eur J Cancer*. 2019;119:1-10.
74. Weber J, Mandala M, Del Vecchio M, et al. Adjuvant Nivolumab versus Ipilimumab in resected stage III or IV melanoma. *N Engl J Med*. 2017;377(19):1824-1835.
75. Eggermont AMM, Blank CU, Mandala M, et al. Adjuvant Pembrolizumab versus placebo in resected stage III melanoma. *N Engl J Med*. 2018;378(19):1789-1801.
76. Amaria RN, Menzies AM, Burton EM, et al. Neoadjuvant systemic therapy in melanoma: recommendations of the international Neoadjuvant melanoma consortium. *Lancet Oncol*. 2019;20(7):e378-e389.
77. Blank CU, Rozeman EA, Fanchi LF, et al. Neoadjuvant versus adjuvant ipilimumab plus nivolumab in macroscopic stage III melanoma. *Nat Med*. 2018;24(11):1655-1661.
78. Rozeman EA, Menzies AM, van Akkooi ACJ, et al. Identification of the optimal combination dosing schedule of neoadjuvant ipilimumab plus nivolumab in macroscopic stage III melanoma (OpACIN-neo): a multicentre, phase 2, randomised, controlled trial. *Lancet Oncol*. 2019;20(7):948-960.
79. Amaria RN, Reddy SM, Tawbi HA, et al. Neoadjuvant immune checkpoint blockade in high-risk resectable melanoma. *Nat Med*. 2018;24 (11):1649-1654.
80. Nathan MR, Schmid P. The emerging world of breast cancer immunotherapy. *Breast*. 2018;37:200-206.
81. Schmid P., Cortés J., Dent R., Pusztai L., McArthur H.L., Kuemmel S., Bergh J., Denkert C., Park Y.H., Hui R., Harbeck N., Takahashi M., Foukakis T., Fasching P.A., Cardoso F., Jia L., Karantza V., Zhao J., Aktan G., O'Shaughnessy J.. KEYNOTE-522: Phase III study of pembrolizumab (pembro) + chemotherapy (chemo) vs placebo (pbo) + chemo as neoadjuvant treatment, followed by pembro vs pbo as adjuvant treatment for early triple-negative breast cancer (TNBC). *Annals of Oncology*. 2019;30 v853–v854. https://doi.org/10.1093/annonc/mdz394.003
82. Centanni M, Moes D, Troconiz IF, Ciccolini J, van Hasselt JGC. Clinical pharmacokinetics and pharmacodynamics of immune checkpoint inhibitors. *Clin Pharmacokinet*. 2019;58(7):835-857.
83. Sheng J, Srivastava S, Sanghavi K, et al. Clinical pharmacology considerations for the development of immune checkpoint inhibitors. *J Clin Pharmacol*. 2017;57(Suppl 10):S26-S42.
84. Keizer RJ, Huitema AD, Schellens JH, Beijnen JH. Clinical pharmacokinetics of therapeutic monoclonal antibodies. *Clin Pharmacokinet*. 2010;49(8):493-507.
85. Leven C, Padelli M, Carre JL, Bellissant E, Misery L. Immune checkpoint inhibitors in melanoma: a review of pharmacokinetics and exposure-response relationships. *Clin Pharmacokinet*. 2019;58(11): 1393-1405.
86. Donia M, Kimper-Karl ML, Hoyer KL, Bastholt L, Schmidt H, Svane IM. The majority of patients with metastatic melanoma are not represented in pivotal phase III immunotherapy trials. *Eur J Cancer*. 2017;74:89-95.
87. Ahamadi M, Freshwater T, Prohn M, et al. Model-based characterization of the pharmacokinetics of Pembrolizumab: a humanized anti-PD-1 monoclonal antibody in advanced solid tumors. *CPT Pharmacometrics Syst Pharmacol*. 2017;6(1):49-57.
88. Lucas CJ, Martin JH. Pharmacokinetic-guided dosing of new Oral cancer agents. *J Clin Pharmacol*. 2017;57(Suppl 10):S78-S98.
89. Bjarnason GA, Knox JJ, Kollmannsberger CK, et al. The efficacy and safety of sunitinib given on an individualised schedule as first-line therapy for metastatic renal cell carcinoma: a phase 2 clinical trial. *Eur J Cancer*. 2019;108:69-77.
90. >Elassaiss-Schaap J, Rossenu S, Lindauer A, et al. Using model-based "learn and confirm" to reveal the pharmacokinetics-pharmacodynamics relationship of Pembrolizumab in the KEYNOTE-001 trial. *CPT Pharmacometrics Syst Pharmacol*. 2017;6 (1):21-28.
91. Merck. Keytruda (Pembrolizumab) [package insert]. US Food and Drug Administration Website.

92. Freshwater T, Kondic A, Ahamadi M, et al. Evaluation of dosing strategy for pembrolizumab for oncology indications. *J Immunother Cancer*. 2017;5(1):43–52.
93. Zhao X, Suryawanshi S, Hruska M, et al. Assessment of nivolumab benefit-risk profile of a 240-mg flat dose relative to a 3-mg/kg dosing regimen in patients with advanced tumors. *Ann Oncol*. 2017;28 (8):2002-2008.
94. Lala M, Li M, Sinha V, de Alwis D, Chartash E, Jain L. A six-weekly (Q6W) dosing schedule for pembrolizumab based on an exposure-response (E-R) evaluation using modeling and simulation. *J Clin Oncol*. 2018;36(suppl 15):3062-3062.
95. Long GV, Tykodi SS, Schneider JG, et al. Assessment of nivolumab exposure and clinical safety of 480 mg every 4 weeks flat-dosing schedule in patients with cancer. *Ann Oncol*. 2018;29(11):2208-2213.
96. PBAC. Public Summary Document. Nivolumab: Injection concentrate for I.V. infusion 40 mg in 4 mL, Injection concentrate for I.V. infusion 100 mg in 10 mL; Opdivo®. In: PBAC, editor. 2019.
97. Mukherjee S, Ibrahimi S, Machiorlatti M, et al. Personalized dosing versus fixed dosing of immune checkpoint inhibitors: a cost analysis study. *Am J Ther*. 2018;25(6):e767-e768.
98. Meurant A, Michel G, Morin-Légier V, Delplanque R. 1ISG-008 flat doses of anti-PD1: what is the economic impact? *Eur J Hosp Pharm*. 2019;26(Suppl 1):A4-A.
99. Agrawal S, Feng Y, Roy A, Kollia G, Lestini B. Nivolumab dose selection: challenges, opportunities, and lessons learned for cancer immunotherapy. *J Immunother Cancer*. 2016;4(1):72–83.
100. Forde PM, Chaft JE, Smith KN, et al. Neoadjuvant PD-1 blockade in Resectable lung cancer. *N Engl J Med*. 2018;378(21): 1976-1986.
101. Yoo SH, Keam B, Kim M, et al. Low-dose nivolumab can be effective in non-small cell lung cancer: alternative option for financial toxicity. *ESMO Open*. 2018;3(5):e000332.
102. Ascierto PA, Del Vecchio M, Robert C, et al. Ipilimumab 10 mg/kg versus ipilimumab 3 mg/kg in patients with unresectable or metastatic melanoma: a randomised, double-blind, multicentre, phase 3 trial. *Lancet Oncol*. 2017;18(5):611-622.
103. Ascierto PA, Marabelle A. How do immune checkpoint-targeted antibodies work? The need for improved pharmacokinetic evaluation in early phase studies. *Ann Oncol*. 2018;29(11):2157-2160.
104. Oude Munnink TH, Henstra MJ, Segerink LI, Movig KL, Brummelhuis-Visser P. Therapeutic drug monitoring of monoclonal antibodies in inflammatory and malignant disease: translating TNF-alpha experience to oncology. *Clin Pharmacol Ther*. 2016;99(4): 419-431.
105. Baverel PG, Dubois VFS, Jin CY, et al. Population pharmacokinetics of Durvalumab in cancer patients and association with longitudinal biomarkers of disease status. *Clin Pharmacol Ther*. 2018;103(4): 631-642.
106. Liu C, Yu J, Li H, et al. Association of time-varying clearance of nivolumab with disease dynamics and its implications on exposure response analysis. *Clin Pharmacol Ther*. 2017;101(5):657-666.
107. Li H, Sun Y, Yu J, Liu C, Liu J, Wang Y. Semimechanistically based modeling of Pembrolizumab time-varying clearance using 4 longitudinal covariates in patients with non-small cell lung cancer. *J Pharm Sci*. 2019;108(1):692-700.
108. Coss CC, Clinton SK, Phelps MA. Cachectic cancer patients: immune to checkpoint inhibitor therapy? *Clin Cancer Res*. 2018;24(23):5787-5789.
109. Hurkmans DP, Basak EA, van Dijk T, et al. A prospective cohort study on the pharmacokinetics of nivolumab in metastatic non-small cell lung cancer, melanoma, and renal cell cancer patients. *J Immunother Cancer*. 2019;7(1):192–203.
110. Graves M, Navani V, Galettis P, Van Der Westhuizen A, Bowden N. Detecting plasma pembrolizumab concentrations in patients with melanoma using Liquid Chromatography/Mass Spectrometry. Society of Melanoma Research; Utah, USA 2019. p. 33. https://registration.sitesolutionsworldwide.com/synergy/v_1_/event_files/2019_SMR_Congress_Abstracts_with_Index.pdf
111. Turner DC, Kondic AG, Anderson KM, et al. Pembrolizumab exposure-response assessments challenged by association of cancer cachexia and catabolic clearance. *Clin Cancer Res*. 2018;24(23): 5841-5849.
112. Wang X, Feng Y, Bajaj G, et al. Quantitative characterization of the exposure-response relationship for cancer immunotherapy: a case study of Nivolumab in patients with advanced melanoma. *CPT Pharmacometrics Syst Pharmacol*. 2017;6(1):40-48.
113. Bajaj G, Gupta M, Feng Y, Statkevich P, Roy A. Exposure-response analysis of Nivolumab in patients with previously treated or untreated advanced melanoma. *J Clin Pharmacol*. 2017;57(12):1527-1533.
114. Fulop T, Larbi A, Witkowski JM, Kotb R, Hirokawa K, Pawelec G. Immunosenescence and cancer. *Crit Rev Oncog*. 2013;18(6): 489-513.
115. Lee KA, Shin KS, Kim GY, et al. Characterization of age-associated exhausted CD8(+) T cells defined by increased expression of Tim-3 and PD-1. *Aging Cell*. 2016;15(2):291-300.
116. Friedman CF, Wolchok JD. Checkpoint inhibition and melanoma: considerations in treating the older adult. *J Geriatr Oncol*. 2017;8(4): 237-241.
117. Herin H, Aspeslagh S, Castanon E, et al. Immunotherapy phase I trials in patients older than 70 years with advanced solid tumours. *Eur J Cancer*. 2018;95:68-74.
118. Silva CCHD. Treating the elderly with immune checkpoint inhibitors: real life experience from a large Brazilian center. *J Clin Oncol*. 2018; 36(suppl 15):e15077-e.
119. Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol*. 2016;16(10):626-638.
120. Conforti F, Pala L, Bagnardi V, et al. Cancer immunotherapy efficacy and patients' sex: a systematic review and meta-analysis. *Lancet Oncol*. 2018;19(6):737-746.
121. Graham J, Abdel-Rahman O, Choueiri TK, Heng DYC. International m RCCDC. Re: Fabio Conforti, Laura Pala, Vincenzo Bagnardi, et al. cancer immunotherapy efficacy and Patients' sex: a systematic review and meta-analysis. Lancet Oncol 2018;19:737-46: outcomes of metastatic renal cell carcinoma by gender: contrasting results from the international mRCC database consortium. *Eur Urol*. 2018; 74(6):e139-e140.
122. Wallis CJD, Butaney M, Satkunasivam R, et al. Association of Patient sex with Efficacy of immune checkpoint inhibitors and overall survival in advanced cancers: a systematic review and meta-analysis. *JAMA Oncol*. 2019;5(4):529-536.
123. Passaro A, Spitaleri G, Gyawali B, de Marinis F. Immunotherapy in non-small-cell lung cancer patients with performance status 2: clinical decision making with scant evidence. *J Clin Oncol*. 2019;37(22): 1863-1867.
124. Felip E, Ardizzoni A, Ciuleanu T, et al. CheckMate 171: a phase 2 trial of nivolumab in patients with previously treated advanced squamous non-small cell lung cancer, including ECOG PS 2 and elderly populations. *Eur J Cancer*. 2020;127:160-172.
125. Areses Manrique MC, Mosquera Martinez J, Garcia Gonzalez J, et al. Real world data of nivolumab for previously treated non-small cell lung cancer patients: a Galician lung cancer group clinical experience. *Transl Lung Cancer Res*. 2018;7(3):404-415.
126. Middleton G, Brock K, Savage J, et al. Pembrolizumab in patients with non-small-cell lung cancer of performance status 2 (PePS2): a single arm, phase 2 trial. *The Lancet Respiratory Medicine*. 2020. https://doi.org/10.1016/s2213-2600(20)30033-3

127. Wong A, Williams M, Milne D, et al. Clinical and palliative care outcomes for patients of poor performance status treated with antiprogrammed death-1 monoclonal antibodies for advanced melanoma. *Asia Pac J Clin Oncol*. 2017;13(6):385-390.
128. Carrera C, Potrony M, Puig S. Sex as a predictor of response to cancer immunotherapy. *Lancet Oncol*. 2018;19(8):e375.
129. Teulings HE, Limpens J, Jansen SN, et al. Vitiligo-like depigmentation in patients with stage III-IV melanoma receiving immunotherapy and its association with survival: a systematic review and meta-analysis. *J Clin Oncol*. 2015;33(7):773-781.
130. Komiya K, Nakamura T, Abe T, et al. Discontinuation due to immune-related adverse events is a possible predictive factor for immune checkpoint inhibitors in patients with non-small cell lung cancer. *Thorac Cancer*. 2019;10(9):1798-1804.
131. El-Refai SM, Brown JD, Black EP, Talbert JC. Immune checkpoint inhibition and the prevalence of autoimmune disorders among patients with lung and renal cancer. *Cancer Inform*. 2017;16:1176935117712520.
132. Danlos FX, Voisin AL, Dyevre V, et al. Safety and efficacy of anti-programmed death 1 antibodies in patients with cancer and pre-existing autoimmune or inflammatory disease. *Eur J Cancer*. 2018;91:21-29.
133. Abdel-Wahab N, Shah M, Lopez-Olivo MA, Suarez-Almazor ME. Use of immune checkpoint inhibitors in the treatment of patients with cancer and preexisting autoimmune disease: a systematic review. *Ann Intern Med*. 2018;168(2):121-130.
134. Daud AI, Wolchok JD, Robert C, et al. Programmed death-ligand 1 expression and response to the anti-programmed death 1 antibody Pembrolizumab in melanoma. *J Clin Oncol*. 2016;34(34):4102-4109.
135. Wolchok JD, Chiarion-Sileni V, Gonzalez R, et al. Overall survival with combined Nivolumab and Ipilimumab in advanced melanoma. *N Engl J Med*. 2017;377(14):1345-1356.
136. Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature*. 2014;515(7528):563-567.
137. Kato S, Goodman A, Walavalkar V, Barkauskas DA, Sharabi A, Kurzrock R. Hyperprogressors after immunotherapy: analysis of genomic alterations associated with accelerated growth rate. *Clin Cancer Res*. 2017;23(15):4242-4250.
138. Ferrara R, Mezquita L, Texier M, et al. Hyperprogressive disease in patients with advanced non-small cell lung cancer treated with PD-1/PD-L1 inhibitors or with single-agent chemotherapy. *JAMA Oncol*. 2018;4(11):1543-1552.
139. Heitzer E, Ulz P, Geigl JB, Speicher MR. Non-invasive detection of genome-wide somatic copy number alterations by liquid biopsies. *Mol Oncol*. 2016;10(3):494-502.
140. Budczies J, Bockmayr M, Denkert C, et al. Pan-cancer analysis of copy number changes in programmed death-ligand 1 (PD-L1, CD274) - associations with gene expression, mutational load, and survival. *Genes Chromosomes Cancer*. 2016;55(8):626-639.
141. Inoue Y, Yoshimura K, Mori K, et al. Clinical significance of PD-L1 and PD-L2 copy number gains in non-small-cell lung cancer. *Oncotarget*. 2016;7(22):32113-32128.
142. 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.
143. Roemer MG, Advani RH, Ligon AH, et al. PD-L1 and PD-L2 genetic alterations define classical Hodgkin lymphoma and predict outcome. *J Clin Oncol*. 2016;34(23):2690-2697.
144. Roemer MG, Ligon AH, Engert A, Younes A, Santoro A. Chromosome 9p24.1/PD-L1/PD-L2Alterations and PD-L1 Expression and Treatment Outcomes in Patients with Classical Hodgkin Lymphoma Treated with Nivolumab (PD-1 Blockade). *Blood*. 2016;128:2923–2923.
145. Younes A, Santoro A, Shipp M, et al. Nivolumab for classical Hodgkin's lymphoma after failure of both autologous stem-cell transplantation and brentuximab vedotin: a multicentre, multicohort, single-arm phase 2 trial. *Lancet Oncol*. 2016;17(9):1283-1294.
146. Goodman AM, Piccioni D, Kato S, et al. Prevalence of PDL1 amplification and preliminary response to immune checkpoint blockade in solid tumors. *JAMA Oncol*. 2018;4(9):1237-1244.
147. Perottet J, Le Goff E, Legoupil D, et al. PD-L1 copy number variation does not correlate with PD-L1 expression or response to anti-PD-1 immunotherapy in patients with advanced melanomas. *Appl Immunohistochem Mol Morphol*. 2020;28(2):161-165.
148. Diaz LA, Le D, Maio M, et al. 1174OPembrolizumab in microsatellite instability high cancers: Updated analysis of the phase II KEYNOTE-164 and KEYNOTE-158 studies. *Ann Oncol*. 2019;30(Supplement_5);475–532.
149. Overman MJ, Lonardi S, Wong KYM, et al. Durable clinical benefit with Nivolumab plus Ipilimumab in DNA mismatch repair-deficient/-microsatellite instability-high metastatic colorectal cancer. *J Clin Oncol*. 2018;36(8):773-779.
150. Zhao P, Li L, Jiang X, Li Q. Mismatch repair deficiency/microsatellite instability-high as a predictor for anti-PD-1/PD-L1 immunotherapy efficacy. *J Hematol Oncol*. 2019;12(1):54–54.
151. Buttner R, Longshore JW, Lopez-Rios F, et al. Implementing TMB measurement in clinical practice: considerations on assay requirements. *ESMO Open*. 2019;4(1):e000442.
152. Chabanon RM, Pedrero M, Lefebvre C, Marabelle A, Soria JC, Postel-Vinay S. Mutational landscape and sensitivity to immune checkpoint blockers. *Clin Cancer Res*. 2016;22(17):4309-4321.
153. Coulie PG, Van den Eynde BJ, van der Bruggen P, Boon T. Tumour antigens recognized by T lymphocytes: at the core of cancer immunotherapy. *Nat Rev Cancer*. 2014;14(2):135-146.
154. Hellmann MD, Ciuleanu TE, Pluzanski A, et al. Nivolumab plus Ipilimumab in lung cancer with a high tumor mutational burden. *N Engl J Med*. 2018;378(22):2093-2104.
155. Bristol-Myers Squibb Provides Update on the Ongoing Regulatory Review of Opdivo Plus Low-Dose Yervoy in First-Line Lung Cancer Patients with Tumor Mutational Burden ≥10 mut/Mb [press release]. 2019.
156. Update on the Phase III NEPTUNE trial of Imfinzi plus tremelimumab in Stage IV non-small cell lung cancer [press release]. Astra Zeneca 2019.
157. Peters S, Cho BC, Reinmuth N, et al. Abstract CT074: Tumor mutational burden (TMB) as a biomarker of survival in metastatic non-small cell lung cancer (mNSCLC): Blood and tissue TMB analysis from MYSTIC, a Phase III study of first-line durvalumab ± tremelimumab vs chemotherapy. *Cancer Research*. 2019;79(Suppl 13):CT074-CT.
158. Chan TA, Yarchoan M, Jaffee E, et al. Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic. *Ann Oncol*. 2019;30(1):44-56.
159. Cristescu R, Mogg R, Ayers M, et al. Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. *Science*. 2018;362(6411):eaar3593.
160. Walker MS, Hughes TA. Messenger RNA expression profiling using DNA microarray technology: diagnostic tool, scientific analysis or un-interpretable data? *Int J Mol Med*. 2008;21(1):13-17.
161. Portal TD. The Cancer Genmore Atlas [12th October 2019]. Available from: https://tcga-data.nci.nih.gov/docs/publications/tcga/
162. 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):18–18.

163. Barry KC, Hsu J, Broz ML, et al. A natural killer-dendritic cell axis defines checkpoint therapy-responsive tumor microenvironments. *Nat Med*. 2018;24(8):1178-1191.
164. Tang J, Shalabi A, Hubbard-Lucey VM. Comprehensive analysis of the clinical immuno-oncology landscape. *Ann Oncol*. 2018;29(1):84-91.

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