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LEADING ARTICLE

Clinical Impact of Checkpoint Inhibitors as Novel Cancer Therapies

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Abstract Immune responses are tightly regulated via signaling through numerous co-stimulatory and co-inhibitory molecules. Exploitation of these immune checkpoint pathways is one of the mechanisms by which tumors evade and/or escape the immune system. A growing understanding of the biology of immune checkpoints and tumor immunology has led to the development of monoclonal antibodies designed to target co-stimulatory and co-inhibitory molecules in order to re-engage the immune system and restore antitumor immune responses. Anti-cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) antibodies were among the first to be tested in the clinic, and ipilimumab was the first immune checkpoint inhibitor approved for an anticancer indication. Agents targeting the programmed death 1 (PD-1) pathway, either PD-1 or one of its ligands, programmed death ligand 1, are in active clinical development for numerous cancers, including advanced melanoma and lung cancer. Understanding the different mechanisms of action, safety profiles, and response patterns associated with inhibition of the CTLA-4 and PD-1 pathways may improve patient management as these therapies are moved in to the clinical practice setting and may also provide a rationale for combination therapy with different inhibitors. Additional immune checkpoint molecules with therapeutic potential, including lymphocyte activation gene-3 and glucocorticoid-induced tumor necrosis factor receptor-related gene, also have inhibitors in early stages of clinical development. Clinical responses and safety data reported to date on immune checkpoint inhibitors suggest these agents may have the potential to markedly improve outcomes for patients with cancer.

Key Points

Immune checkpoint inhibitors are designed to interrupt inhibitory immune signals and restore immune responses against tumors.

Numerous immune checkpoint inhibitors are in advanced stages of development and show activity across multiple tumor types, including advanced melanoma and advanced non-small-cell lung cancer.

Understanding the mechanism-associated adverse events and response patterns is important to the management of patients as these drugs are moved into the clinical practice setting.

1 Introduction

Rudolph Virchow may have been one of the first physicians in modern times to observe the link between the immune system and malignancy in what he termed "lymphoreticular infiltrates". These infiltrates were leukocytes surrounding malignant tumors, and he hypothesized that proinflammatory states might induce normal tissues to become malignant [1]. Since then, we have learned a great deal about how the immune system responds and reacts to tumors, which tumor-specific antigens are recognized as foreign, and how immune responses can be manipulated and harnessed to enhance tumor cell killing.

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Recently, it has been recognized that, on its own, tumor peptide presentation by major histocompatibility complex (MHC) to T-cell receptors is inadequate for successful T-cell activation and immune destruction of cancer cells. Co-regulatory signals, either inhibitory or stimulatory, are also required [2, 3]. T cells play a critical role in cell-mediated tumor immunity, and do so through an intricate counterbalance of co-stimulatory and co-inhibitory cell-to-cell signals between various components of the immune system. This system of checks and balances is necessary not only to allow a powerful destructive response against both pathogens and malignancies, but also to prevent immune responses from being generated against normal tissues. Critical 'checkpoints' control and fine-tune the immune system through regulation of this complex network of co-stimulatory and co-inhibitory signaling [3]. In this paper, we review some of the important immune checkpoint molecules elucidated to date, as well as efforts to block these molecules in order to shift the balance towards antitumor immunity. We also describe some of the complexities and challenges encountered using these checkpoint inhibitors in the clinic.

2 Cytotoxic T-Lymphocyte-Associated Antigen (CTLA)-4

2.1 Background

More than 40 years of research has led to the development of a two-signal theory of T-cell activation: antigenic stimulation of the T-cell receptor (TCR) (signal 1) together with co-stimulation by other molecules on the cell surface (signal 2) [2, 3]. One of the key co-stimulatory mechanisms involves the interaction of CD28 on the surface of the T cell with B7 molecules CD80 or CD86 on antigen-presenting cells. CTLA-4, a transmembrane glycoprotein with considerable homology to CD28, binds to the same B7 ligands, as such (Fig. 1). Upon TCR stimulation by antigens, T cells express CTLA-4, which can bind B7 molecules; however, unlike CD28, CTLA-4 inhibits T-cell responses and is important for maintenance of immune tolerance. Expression of CTLA-4 raises the activation threshold and attenuates clonal expansion; thus, a productive T-cell response ensues only upon a net co-stimulatory signal.

2.2 Efficacy of CTLA-4 Inhibitors

2.2.1 Ipilimumab

Ipilimumab, one of the best-studied monoclonal antibodies targeting CTLA-4 (Table 1 [4–16]), has been evaluated in a clinical trial program of more than 2,000 patients with a variety of solid tumors [4, 5, 17–19]. Ipilimumab (Yervoy®), administered every 3 weeks for four doses, gained US FDA approval in 2011 for the treatment of unresectable or metastatic melanoma, based on data from two phase III randomized trials showing improvement on median overall survival (OS) over control arms in patients with melanoma [4, 5, 20]. One of the pivotal phase III trials evaluated ipilimumab with or without gp100 vaccine in previously treated patients with advanced melanoma. Although the best overall response rates were modest, 10.9 % in the ipilimumab-alone group and 5.7 % in the ipilimumab plus gp100 vaccine group, some patients in both groups maintained an objective response for at least 2 years [4]. In this trial, the 3-year OS rate for ipilimumab monotherapy was 20 % [4], which compares favorably with the 3-year OS rate of 17 % for historical control patients receiving standard of care chemotherapy in a separate clinical trial [21] (Table 2 [4, 5, 7, 18, 19, 21–33]). The other pivotal phase III trial was conducted in treatment-naïve patients with metastatic melanoma and compared ipilimumab plus dacarbazine versus dacarbazine plus placebo [5]. Although the dose and schedule were slightly different, the rate of best overall response was 15 % in the ipilimumab plus dacarbazine group versus 10 % for the dacarbazine plus

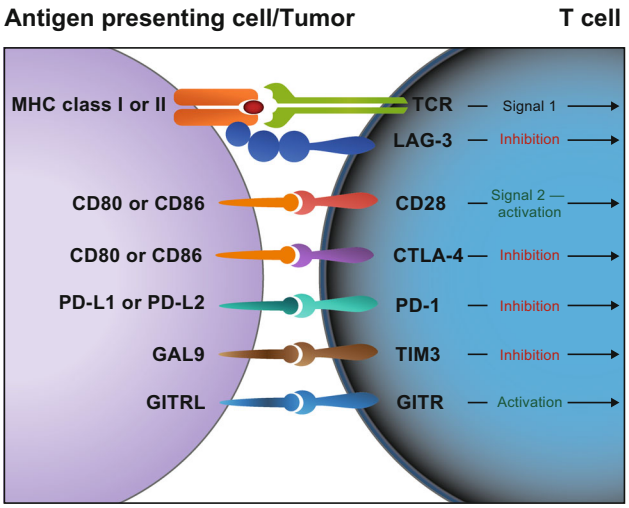

Fig. 1 T-cell activation and immune checkpoint pathways. T-cell activation requires two signals: (1) presentation of antigenic peptides by MHC to the TCR and (2) co-stimulation, typically via CD28:CD80 or CD28:CD86 ligation. Immune checkpoint pathways comprising receptors on T cells and ligands on antigen-presenting cells and/or tumors fine-tune immune responses via T-cell activation or inhibition. *CTLA-4* cytotoxic T-lymphocyte-associated antigen 4, *GAL9* galectin-9, *GITR* glucocorticoid-induced TNF receptor-related gene, *GITRL* glucocorticoid-induced TNF receptor-related gene ligand, *LAG-3* lymphocyte activation gene-3, *MHC* major histocompatibility complex, *PD-1* programmed death-1, *PD-L1* programmed death ligand 1, *PD-L2* programmed death ligand 2, *TCR* T-cell receptor, *TIM3* T-cell immunoglobulin and mucin domain 3, *TNF* tumor necrosis factor

Table 1 Immune checkpoint inhibitors in clinical development [4–16]

Name	Company	Description of agent
Ipilimumab [4, 5]	Bristol-Myers Squibb	Human IgG1 mAb against CTLA-4
Tremelimumab [6]	MedImmune/AstraZeneca	Human IgG2 mAb against CTLA-4
Pembrolizumab (MK-3475) [7]	Merck	Humanized IgG4 mAb against PD-1
Nivolumab (BMS-936558) [8]	Bristol-Myers Squibb	Human IgG4 mAb against PD-1
Pidilizumab (CT-011) [9]	CureTech	Humanized IgG1 mAb against PD-1
AMP-224 [10]	Amplimmune; GlaxoSmithKline	PD-L2-IgG recombinant fusion protein
MPDL3280A [11, 12]	Genentech/Roche	Human IgG mAb against PD-L1
BMS-936559 [13]	Bristol-Myers Squibb	Human IgG4 mAb against PD-L1
MEDI4736 [14]	MedImmune/AstraZeneca	Human mAb against PD-L1
IMP321 [15]	Immutep	Soluble LAG-3 Ig fusion protein and MHC class II agonist
TRX518 [16]	GITR, Inc	Humanized mAb against GITR

CTLA-4 cytotoxic T-lymphocyte-associated antigen 4, *GITR* glucocorticoid-induced tumor necrosis factor receptor-related gene, *IgG* immunoglobulin G, *LAG-3* lymphocyte activation gene-3, *mAb* monoclonal antibody, *MHC* major histocompatibility complex, *PD-1* programmed death 1, *PD-L1* programmed death ligand 1, *PD-L2* programmed death ligand 2

Table 2 Preliminary efficacy data with immune checkpoint inhibitors or controls from individual (not head-to-head) trials[a] [4, 5, 7, 17, 18, 21–33]

Advanced tumor setting	Agent or control	Median PFS	Median OS	Survival rate	Reference(s)
Melanoma	CTX (CTX-naïve pts)	ND	9.1–10.7 months	1-year: 36 % 3-year: 12–17 %	[5, 21]
	Ipilimumab	2.9 months	10.1 months	3-year: ≈20 %	[4]
	Tremelimumab	ND	12.6 months	3-year: 21 %	[21]
	Pembrolizumab	5.5 months	NR	1-year: 69 %	[7]
	Nivolumab	3.7 months	17.3 months	1-year: 63 % 3-year: 41 %	[22]
	Pidilizumab	1.9 months	ND	1-year: 65 %	[23]
	Nivolumab plus ipilimumab	27 weeks	40 months	1-year: 85 % 2-year: 79 %	[24]
NSCLC	CTX (CTX-naïve pts)	4.2 months	8.3 months	1-year: 39 % 2-year: 18 %	[18]
	Pembrolizumab	10–27 weeks[b]	51 weeks	ND	[25–27]
	Nivolumab (previously-treated pts)	2.3 months	9.9 months	1-year: 42 % 3-year: 24 %	[28]
	Nivolumab (CTX-naïve pts)	36.1 weeks	NR	1-year: 75 %	[29]
RCC	Sorafenib	3.6–5.7 months	11.0–19.2 months	3-year: ≈25 %	[30–32]
	Nivolumab	2.7–4.2[c] months	18.2–24.7[c] months	2.5-year: ≈35 %	[33]
CRPC	Placebo	3.1 months	10.0 months	1-year: 40 % 2-year: 15 %	[17]
	Ipilimumab	4.0 months	11.2 months	1-year: 47 % 2-year: 26 %	[17]

CRPC castration-resistant prostate cancer, *CTX* chemotherapy, *ND* no data, *NR* not reached, *NSCLC* non-small-cell lung cancer, *OS* overall survival, *PFS* progression-free survival, *pts* patients, *RCC* renal cell carcinoma

[a] Important: data are not from head-to-head trials, and the trials differ by patient characteristics, patient numbers, and length of follow-up, therefore direct comparisons across trials and agents have limited validity; trials in tumor types with PFS and OS data were included

[b] Based on differing studies and data-cuts

[c] Dose-dependent

placebo group, while the median duration of response was 19.3 versus 8.1 months for the dacarbazine plus placebo group. Responses lasting at least 2 years were observed in both treatment groups. The 3-year survival rate with ipilimumab plus dacarbazine was significantly higher than dacarbazine plus placebo: 20.8 versus 12.2 % ($P < 0.001$).

Ipilimumab was evaluated as adjuvant therapy following complete resection of stage III melanoma in a phase III trial in patients at high risk of recurrence [34]. Patients receiving ipilimumab had a significantly increased median OS as compared with patients receiving placebo: 26.1 versus 17.1 months ($P = 0.0013$). The 3-year rates of recurrence-free survival were 47 % for ipilimumab and 35 % for placebo.

Preclinical studies suggest that chemotherapy can induce the release of tumor-specific antigens, thereby initiating T-cell activation and sensitizing tumor cells to T-cell-mediated killing [35]. These observations provided the rationale for combining immunotherapy with cytotoxic agents to improve responses in patients with melanoma, and also led to the initiation of clinical trials evaluating ipilimumab with chemotherapy in lung cancer. A phase II, randomized study provided evidence that sequential ipilimumab is more effective than concurrent ipilimumab when administered with paclitaxel/carboplatin in chemotherapy-naïve stage IIIB/IV patients with non-small-cell lung cancer (NSCLC). The median OS with sequential ipilimumab, concurrent ipilimumab, and the control regimen was 12.2, 9.7, and 8.3 months, respectively. In this trial, patients with squamous histology exhibited better outcomes (median immune-related progression-free survival [irPFS] and OS) with sequential ipilimumab dosing than did patients with non-squamous histology [18]. Based on these findings, a phase III trial evaluating OS in patients with squamous NSCLC receiving sequential ipilimumab after chemotherapy was initiated (Table 3 [16]).

Ipilimumab is also being investigated in the setting of metastatic castration-resistant prostate cancer (mCRPC). In a phase III trial of ipilimumab versus placebo in post-docetaxel patients with mCRPC receiving a single dose of radiotherapy, the primary endpoint of OS was not reached; however, pre-specified subset analyses suggested that ipilimumab may be more active in patients with favorable prognostic factors, including no visceral disease, alkaline phosphatase <1.5 upper limit of normal, and hemoglobin ≥11 g/dL [17]. Results from this study support the investigation of ipilimumab in the ongoing phase III, CA184-095 study among chemotherapy-naïve patients with mCRPC (Table 3 [16]).

2.2.2 *Tremelimumab*

Tremelimumab is a fully human immunoglobulin G (IgG)-2 monoclonal antibody targeting CTLA-4 [6] (Table 1 [4–16]). Tremelimumab provided durable responses in 6.6 % of patients in a phase II trial of patients with advanced melanoma, as compared with the objective response rates (ORRs) of 5.7 and 10.9 % seen in the phase III trial of ipilimumab with or without vaccine [4, 6]. However, the phase III trial of tremelimumab monotherapy failed to demonstrate a statistically significant survival advantage over chemotherapy in first-line treatment of patients with metastatic melanoma [21]. Patient selection criteria, dosing regimen, and use of ipilimumab as salvage therapy for patients in the control arm were potential reasons for the lack of survival benefit.

Tremelimumab showed evidence of activity against previously treated malignant mesothelioma in a small ($N = 29$) phase II single-arm trial [36]. Four patients had partial responses, and 11 patients had stable disease of median duration 7.7 months (range 2.6–16.6+), with a median OS of 11.3 months. Based on these results, a phase II trial of tremelimumab in malignant mesothelioma has been initiated (Table 3 [16]).

2.3 Safety of CTLA-4 Inhibitors

The cumulative safety data across many trials show that agents that inhibit CTLA-4 are generally safe, with unique, but usually manageable, side effects that are linked to their mechanism of stimulating immune responses. Multiple phase II and III trials have characterized these immune-related adverse events (irAEs) of CTLA-4 inhibition. Overall, irAEs were observed in 58–63 % of patients treated with ipilimumab, with 5–26 % of patients experiencing grade 3/4 irAEs [4, 17, 37] (Table 4 [4, 7, 8, 12–14, 17, 21, 23, 26, 28, 36, 38–40]). In the phase III trial investigating ipilimumab treatment with or without vaccine, skin-related irAEs (including pruritus, rash, and erythema) and gastrointestinal irAEs (including diarrhea and colitis) were the most common, occurring in 29–44 % of patients; endocrine disorders were reported in 4–8 % of patients [4]. Some of the more rare adverse events (AEs) (≤1 % for each) reported during treatment with ipilimumab include uveitis, conjunctivitis, and neuropathy [37]. Interestingly, when ipilimumab was given with dacarbazine, immune-mediated grade 3/4 hepatitis occurred in 32 % of patients, while the rates of gastrointestinal events, such as colitis, were lower than expected based on previous trials [5]. As adjuvant therapy, ipilimumab had a safety profile generally consistent with that seen in patients with advanced melanoma, although the incidence of some irAEs (e.g. endocrinopathies) was higher. Also, five patients (1 %) in the ipilimumab arm died due to treatment-related AEs versus 0 patients in the placebo group [34]. Tremelimumab has a similar irAE profile to ipilimumab. The most common irAEs with tremelimumab were

Table 3 Ongoing phase II and III clinical trials of immune checkpoint inhibitors [16][a]

Target Treatment approach Tumor type	Trial phase	Treatment setting	Trial stage (no. patients)	Clinical endpoints	Estimated primary/final completion date	Trial identifier
CTLA-4						
Ipilimumab[b]						
Lung cancer						
Ipilimumab + etoposide/platinum vs. etoposide/platinum	III	Extensive-disease SCLC	Recruiting ($n = 1{,}100$)	Primary: OS Secondary: OS, irPFS, BORR, DOR	Nov 2015/Mar 2017	NCT01450761
Ipilimumab + paclitaxel/carboplatin vs. paclitaxel/carboplatin	III	Stage IV/recurrent squamous NSCLC	Recruiting ($n = 920$)	Primary: OS Secondary: OS, PFS, BORR	Apr 2015/Dec 2016	NCT01285609
Melanoma						
Ipilimumab	I/II	Uveal melanoma	Recruiting ($n = 141$)	Primary: MTD Secondary: OS	Nov 2017	NCT01585194
Ipilimumab + temozolomide	II	Metastatic melanoma	Ongoing ($n = 64$)	Primary: 6-month PFS	May 2015	NCT01119508
Ipilimumab vs. chemotherapy (retreatment)	II	Advanced melanoma with progression after initial disease control with ipilimumab	Ongoing ($n = 138$)	Primary: OS Secondary: DCR, BORR, QOL	Jul 2016	NCT01709162
Ipilimumab 3 mg/kg vs. 10 mg/kg	III	Unresectable or metastatic melanoma	Ongoing ($n = 700$)	Primary: OS Secondary: PFS, BORR, DCR, DOR, DSD	Sep 2015/Dec 2016	NCT01515189
Ipilimumab vs. placebo	III	High-risk stage III melanoma after surgical removal	Ongoing ($n = 950$)	Primary: RFS Secondary: OS, distant metastases-free survival, safety, QOL	July 2013/April 2015	NCT00636168
Ipilimumab vs. nivolumab vs. ipilimumab + nivolumab	III	Previously untreated advanced melanoma	Recruiting ($n = 915$)	Primary: OS Secondary: PFS, ORR, PD-L1 biomarker, QOL	Oct 2016/Oct 2017	NCT01844505
Ipilimumab vs. high-dose IFN-α-2b	III	High-risk stage III or IV melanoma after surgical removal	Recruiting ($n = 1{,}500$)	Primary: Recurrence-free survival, OS Secondary: Safety, QOL	May 2018	NCT01274338
Prostate cancer						
Ipilimumab + sipuleucel-T	II	Progressive metastatic prostate cancer	Ongoing ($n = 66$)	Primary: Safety, immune responses Secondary: PSA response, clinical response, immune markers	Aug 2014/Aug 2015	NCT01804465
Ipilimumab vs. placebo	III	Metastatic prostate cancer	Ongoing ($n = 600$)	Primary: OS Secondary: PFS, time to pain progression, time to subsequent non-hormonal systemic therapy, safety	Jan 2015/Nov 2015	NCT01057810
Gastric cancer						
Ipilimumab vs. standard of care	II	Unresectable or metastatic gastric and gastroesophageal cancer	Recruiting ($n = 114$)	Primary: irPFS Secondary: PFS, OS, irBORR	Jul 2014/Mar 2015	NCT01585987

Table 3 continued

Target Treatment approach Tumor type	Trial phase	Treatment setting	Trial stage (no. patients)	Clinical endpoints	Estimated primary/final completion date	Trial identifier
Tremelimumab						
Mesothelioma						
Tremelimumab vs. placebo	II	Unresectable malignant mesothelioma	Recruiting ($n = 180$)	Primary: OS Secondary: DCR, PFS, ORR, DOR, safety, QOL, PK	Jun 2015	NCT01843374
PD-1						
Pembrolizumab (*MK-3475*)						
Lung cancer						
Pembrolizumab vs. docetaxel	II/III	Previously treated PD-L1-positive NSCLC	Recruiting ($n = 920$)	Primary: OS, PFS, safety Secondary: ORR, DOR	Sep 2015/Jan 2020	NCT01905657
Melanoma						
Pembrolizumab vs. chemotherapy[c]	II	Advanced melanoma with progression after prior therapy	Ongoing ($n = 510$)	Primary: PFS, OS Secondary: ORR, DOR	Mar 2015/Jan 2016	NCT01704287
Pembrolizumab vs. ipilimumab	III	Unresectable or metastatic melanoma	Recruiting ($n = 645$)	Primary: PFS, OS Secondary: ORR	Jul 2014/Mar 2016	NCT01866319
Melanoma or NSCLC with untreated brain metastases						
Pembrolizumab	II	Melanoma or NSCLC with untreated brain metastases	Recruiting ($n = 64$)	Primary: Response Secondary: Brain metastases response	Dec 2018/Mar 2018	NCT02085070
Colon cancer						
Pembrolizumab	II	Colon cancer	Recruiting ($n = 71$)	Primary: irPFS, irOR Secondary: OS, irPFS, PFS, BORR, DCR, safety, biomarkers	Jun 2017	NCT01876511
Nivolumab						
Lung cancer						
Nivolumab	II	Advanced or metastatic squamous cell NSCLC with ≥ 2 prior systemic regimens	Ongoing ($n = 100$)	Primary: IRC-assessed ORR Secondary: Investigator-assessed ORR	Feb 2014/Feb 2015	NCT01721759
Nivolumab + epigenetic priming	II	Recurrent metastatic NSCLC	Recruiting ($n = 120$)	Primary: Tumor response Secondary: PFS, TTP, OS, safety	Jan 2015/Aug 2015	NCT01928576
Nivolumab vs. docetaxel	III	Previously treated advanced or metastatic squamous cell NSCLC	Ongoing ($n = 264$)	Primary: IRC-assessed ORR, OS Secondary: IRC-assessed PFS, PD-L1 biomarker, DOR, TTR, QOL	Aug 2014/Aug 2015	NCT01642004
Nivolumab vs. docetaxel	III	Previously treated advanced or metastatic non-squamous cell NSCLC	Ongoing ($n = 574$)	Primary: OS Secondary: ORR, PFS, PD-L1 biomarker, DRSPR	Nov 2014/Nov 2015	NCT01673867

Table 3 continued

Target Treatment approach Tumor type	Trial phase	Treatment setting	Trial stage (no. patients)	Clinical endpoints	Estimated primary/final completion date	Trial identifier
Melanoma						
Nivolumab + ipilimumab vs. ipilimumab	II	Previously untreated, unresectable or metastatic melanoma	Ongoing ($n = 150$)	Primary: ORR Secondary: PFS, ORR and PFS in BRAF mutant patients, QOL	Jul 2014/May 2015	NCT01927419
Nivolumab + ipilimumab	II	Advanced or metastatic melanoma	Recruiting ($n = 100$)	Primary: Safety Secondary: ORR, PR	Aug 2014/Jan 2019	NCT01783938
Nivolumab vs. dacarbazine or carboplatin/paclitaxel	III	Advanced melanoma with progression after ipilimumab	Ongoing ($n = 390$)	Primary: ORR, OS Secondary: PFS, PD-L1 biomarker, QOL	May 2015/Jan 2016	NCT01721746
Nivolumab vs. dacarbazine	III	Previously untreated unresectable or metastatic melanoma	Recruiting ($n = 410$)	Primary: OS Secondary: PFS, ORR, PD-L1 biomarker, QOL	Sep 2015/Nov 2015	NCT01721772
Nivolumab or nivolumab + ipilimumab vs. ipilimumab	III	Previously untreated unresectable or metastatic melanoma	Recruiting ($n = 915$)	Primary: OS Secondary: PFS, ORR, PD-L1 biomarker, QOL	Oct 2016/Oct 2017	NCT01844505
RCC						
Nivolumab	II	Advanced or metastatic clear-cell RCC	Ongoing ($n = 150$)	Primary: PFS Secondary: PFS, BORR, OS	May 2013/Jun 2014	NCT01354431
Nivolumab vs. everolimus	III	Pre-treated advanced or metastatic clear-cell RCC	Recruiting ($n = 822$)	Primary: OS Secondary: PFS, ORR, DOR, PD-L1 biomarker, safety, DRSPR	Feb 2016	NCT01668784
Other						
Nivolumab or nivolumab + ipilimumab vs. bevacizumab	II	Recurrent glioblastoma	Recruiting ($n = 260$)	Primary: Safety, OS Secondary: PFS, ORR, OS	Jan 2018	NCT02017717
Nivolumab	II	Relapsed or refractory diffuse large B-cell lymphoma	Recruiting ($n = 120$)	Primary: ORR Secondary: DOR, CRR, PFS, ORR	Feb 2016	NCT02038933
Nivolumab	III	Recurrent or metastatic head and neck carcinoma	Recruiting ($n = 180$)	Primary: PFS, OS Secondary: ORR	Jun 2016/Jun 2017	NCT02105636
Pidilizumab (*CT-011*)						
Prostate cancer						
Pidilizumab + sipuleucel-T + cyclophosphamide	II	Advanced prostate cancer	Recruiting ($n = 57$)	Primary: Feasibility, immune efficacy Secondary: PFS, OS	Dec 2014/Dec 2017	NCT01420965
Hematologic malignancies						
Pidilizumab + vaccine	II	AML	Recruiting ($n = 75$)	Primary: Toxicity Secondary: Immune response, tumor regression, TTP	Sep 2014	NCT01096602

Table 3 continued

Target Treatment approach Tumor type	Trial phase	Treatment setting	Trial stage (no. patients)	Clinical endpoints	Estimated primary/final completion date	Trial identifier
Pidilizumab + lenalidomide	II	Relapsed or refractory MM	Recruiting ($n = 53$)	Primary: MTD, OR, Secondary: TTP, OS, PK	Jun 2017	NCT02077959
PD-L1						
MPDL3280A						
Lung cancer						
MPDL3280A	II	PD-L1-positive locally advanced or metastatic NSCLC	Recruiting ($n = 130$)	Primary: ORR Secondary: ORR, DOR, PFS, safety, PK	May 2015	NCT01846416
MPDL3280A vs. docetaxel	II	Advanced or metastatic NSCLC after platinum failure	Recruiting ($n = 300$)	Primary: OS Secondary: OR, PFS, safety, QOL	Mar 2016/Mar 2017	NCT01903993
MPDL3280A	II	PD-L1-positive advanced or metastatic NSCLC	Recruiting ($n = 300$)	Primary: ORR Secondary: DOR, PFS, OS, safety, PK	Mar 2018	NCT02031458
MPDL3280A vs. docetaxel	III	Advanced or metastatic NSCLC after platinum failure	Recruiting ($n = 850$)	Primary: OS Secondary: Safety, OR, PFS, DOR	Jun 2018	NCT02008227
Other						
MPDL3280A or MPDL3280A + bevacizumab vs. sunitinib	II	Previously untreated locally advanced or metastatic RCC	Recruiting ($n = 150$)	Primary: PFS Secondary: irPFS, OR, DOR, OS, PK	Mar 2016	NCT01984242
MPDL3280A	II	Locally advanced or metastatic urothelial bladder cancer	Recruiting ($n = 330$)	Primary: ORR Secondary: DOR, PFS, OS, safety, PK	Nov 2015/Jul 2016	NCT02108652
MEDI4736						
Lung cancer						
MEDI4736	II	Locally advanced or metastatic NSCLC with ≥ 2 prior systemic regimens	Recruiting ($n = 210$)	Primary: ORR Secondary: DOR, PFS, DCR, OS, DSR, safety, PK	Apr 2015/Jan 2016	NCT02087423
MEDI4736 vs. docetaxel (sub-study)	II	Advanced squamous NSCLC	Recruiting ($n =$ undefined for substudy)	Primary: PFS, OS Secondary: ORR, safety, irPFS, irOR	Jun 2022	NCT02154490

Table 3 continued

Target	Treatment approach	Tumor type	Trial phase	Treatment setting	Trial stage (no. patients)	Clinical endpoints	Estimated primary/final completion date	Trial identifier
	MEDI4736 vs. placebo following concurrent chemoradiation	III	Stage III unresectable NSCLC	Recruiting ($n = 880$)	Primary: OS, PFS Secondary: OS, DOR, ORR, PFS	May 2017/Nov 2020	NCT02125461	

AML acute myelogenous leukemia, *BORR* best overall response rate, *CRR* complete remission rate, *CTLA-4* cytotoxic T-lymphocyte-associated antigen 4, *DCR* disease control rate, *DMFS* distant metastases-free survival, *DOR* duration of response, *DRSPR* disease-related symptom progression rate, *DSD* duration of stable disease, *DSR* deep sustained response, *IFN* interferon, *irBORR* immune-related best overall response rate, *IRC* independent review committee, *irOR* objective response using immune-related response criteria, *irPFS* immune-related progression free survival, *MM* multiple myeloma, *MTD* maximum tolerated dose, *NSCLC* non-small-cell lung cancer, *OR* overall response, *ORR* objective response rate, *OS* overall survival, *PD-1* programmed death-1, *PD-L1* programmed death ligand 1, *PFS* progression-free survival, *PK* pharmacokinetics, *PR* progression rate, *PSA* prostate specific antigen, *QOL* quality of life, *RCC* renal cell carcinoma, RFS recurrence-free survival, *SCLC* small cell lung cancer, *TTP* time to progression, *TTR* time to response

[a] Active (as of July 2014) phase II and III trials with planned enrolment of ≥50 patients are listed

[b] Due to the high volume of ipilimumab trials, only those with Bristol-Myers Squibb listed as the sponsor or collaborator are included

[c] Investigator-choice chemotherapy (carboplatin + paclitaxel, paclitaxel alone, dacarbazine, or temozolomide)

gastrointestinal (18 % grade 3–5), dermatologic (rash 33 % all grades, 2 % grade 3–5), and endocrine (thyroid/pan-hypopituitarism/adrenal insufficiency 7 %) in nature [21] (Table 4 [4, 7, 8, 12–14, 17, 21, 23, 26, 28, 36, 38–40]).

irAEs for CTLA-4 inhibitors tend to occur during the induction period or first 12 weeks of therapy, but, in rare cases, can occur in the weeks and even months following discontinuation of therapy [37]. Grade 2 irAEs are usually responsive to interruption of therapy and institution of low-dose steroids (0.5 mg/kg/day of prednisone), and grade 3 and higher irAEs are generally responsive to high-dose steroids (1–2 mg/kg/day of prednisone or equivalent), although the steroid-refractory patients (reported as 2–8 % of patients) [20, 41] may require the use of alternate immunosuppressive agents, such as infliximab (5 mg/kg) once every 2 weeks until symptom resolution, followed by a prolonged steroid taper [42]. Prompt recognition, management, and monitoring of grade 2 and higher irAEs are critical for the successful resolution of these toxicities, although such events can often take weeks to months to return to baseline. Algorithms for the management of irAEs with ipilimumab have been published by Fecher et al. [37]. Unfortunately, prophylactic steroids and other preventive strategies to avoid irAEs have not shown clinical benefit to date [37]. An interesting and consistent observation is that a higher incidence of irAEs, particularly grade 3/4 irAEs, has been associated with a higher proportion of patients eventually achieving an objective response [43]. However, as grade 3/4 irAEs can be life threatening, it is recommended that patients experiencing severe irAEs discontinue ipilimumab [20, 37].

3 Programmed Death-1 (PD-1) and Programmed Death Ligand 1 (PD-L1)

3.1 Background

PD-1 (CD279) is also a co-inhibitory molecule that plays an important role in the balance of tumor immunity and inflammatory reactions [3, 44]. However, in contrast to CTLA-4, PD-1 appears to play a greater role in limiting and modulating the activity of T cells in peripheral tissues and organs during inflammatory responses in an effort to prevent host tissue damage. PD-1 expression is induced on activated T cells, and the interaction between PD-1 and one of its ligands—typically PD-L1 (B7-H1, CD274) or PD-L2 (B7-DC, CD273)—on the surface of tumors leads to a diminished antitumor response and has been associated with a poorer patient outcome [44]. High levels of PD-1 expression on antigen-experienced $CD8^+$ T cells are associated with the loss of effector functions, including the ability of T cells to proliferate and express interleukin (IL)-

Table 4 Preliminary safety data of CTLA-4, PD-1, and PD-L1 targeting agents[a] [4, 7, 8, 12–14, 17, 21, 23, 26, 28, 36, 38–40]

Agent name	Setting	Phase	Dosing/description	Patients (*N*)	Treatment-related AEs	Grade 3/4 treatment-related AEs	Potential immune-related AEs[b]
Anti-CTLA-4 agents							
Ipilimumab [4]	Unresectable stage III or IV melanoma	III	3 mg/kg q3w, for up to 4 doses	131	80 % ($n = 105$), including fatigue 42 %, nausea 35 %, diarrhea 33 %, decreased appetite 27 %, vomiting 24 %, constipation 21 %; 4 TRD	Grade 3: 19 % ($n = 25$), including fatigue 7 %, diarrhea 5 %, dyspnea 3 %, anemia 3 % Grade 4: 4 % ($n = 5$), including dyspnea 1 %	Any grade: 61 % ($n = 80$), including diarrhea 28 %, pruritus 24 %, rash 19 %, colitis 8 %, endocrine disorders 8 % Grade 3: 12 % ($n = 16$), including diarrhea 5 %, colitis 5 %, endocrine disorders 2 % Grade 4: 2 % ($n = 3$)
Ipilimumab [17]	mCRPC with bone metastasis	III	10 mg/kg q3w, for up to 4 doses, after radiotherapy	393	75 % ($n = 295$); 4 TRD	ND	Any grade: 63 % ($n = 249$) Grade 3/4: 26 % ($n = 101$)
Tremelimumab [21]	Treatment-naïve, unresectable stage IIIc or IV melanoma	III	15 mg/kg once q90d	325	All cause[c]: 96 % ($n = 312$), including diarrhea 51 %, nausea 34 %, fatigue 33 %, rash 33 %, pruritus 31 %, vomiting 23 %, decreased appetite 21 %; 7 TRD	All cause[b]: 52 % ($n = 170$), including diarrhea 18 %, fatigue 6 %, nausea 4 %, vomiting 4 %, decreased appetite 4 %, abdominal pain 4 %	Any grade AEs included thyroid disorders 5 %, ocular disorders 4 %, hypothalamus and pituitary disorders 2 % Grade 3/4: 1 % each for thyroid disorders, hypothalamus and pituitary disorders, adrenal insufficiency, hepatitis, pancreatitis
Tremelimumab [36]	CTX-resistant advanced malignant mesothelioma	II	10 mg/kg once q90d	29	Grade 1/2: 90 % ($n = 26$), including GI AEs 66 %, dermatologic AEs 48 %, fever 34 %	3 % ($n = 1$)	None reported[d]
Anti-PD-1 agents							
Pembrolizumab [7]	Advanced melanoma	I	10 mg/kg q2/3w, or 2 mg/kg q3w	411	83 % ($n = 341$), including fatigue 36 %, pruritus 24 %, rash 20 %, diarrhea 16 %, arthralgia 16 %, nausea 12 %, vitiligo 11 %	12 % ($n = 51$), including fatigue 2 %; all others <1 %	Any grade hypothyroidism 8 %, pneumonitis 3 %, hyperthyroidism 1%, colitis <1 %, hepatitis <1 % Individual grade 3–4 events each occurred in <1 %
Pembrolizumab [26]	NSCLC previously treated with ≥1 systemic regimens	I	10 mg/kg q2/3w	217	64 %, including fatigue 20 %, arthralgia 9 %, decreased appetite 9 %, pruritus 8 %, diarrhea 7 %	10 %, including fatigue, arthralgia, nausea, each <1 %	Any grade rash 6 % and hypothyroidism 5 % Grade 3–4 pneumonitis: 2 % ($n = 4$) Grade 3–4 arthralgia, neck pain, pneumonitis 2 % ($n = 4$)

Table 4 continued

Agent name	Setting	Phase	Dosing/description	Patients (*N*)	Treatment-related AEs	Grade 3/4 treatment-related AEs	Potential immune-related AEs[b]
Nivolumab [8]	Advanced or recurrent malignancies	I	0.1–10 mg/kg every 2 weeks, maximum of 12 cycles (4 doses per 8-week cycle)	296	70 % ($n = 207$), including fatigue 24 %, nausea 8 %, decreased appetite 8 %	14 % ($n = 41$), including fatigue 2 %, abdominal pain 1 %, hypophosphatemia 1 %, lymphopenia 1 %	All grades: 41 % ($n = 122$), including rash 12 %, diarrhea 11 %, pruritus 9 %, pneumonitis 3 %, vitiligo 3 %, infusion-related AEs 3 %, increased TSH 3 % Grade 3/4: 6 % ($n = 18$), including pneumonitis 1 %, diarrhea 1 %, increased AST 1 %, increased ALT 1 %, 3 deaths from pneumonitis 1 %
Nivolumab [28]	Advanced NSCLC (subset analysis)	I	1–10 mg/kg q2w maximum of 12 cycles (4 doses per 8-week cycle)	129	ND, including fatigue 24 %, decreased appetite 12 %, diarrhea 10 %	14 %	All grades: 41 % ($n = 53$) including skin 16 %, GI 12 %, pulmonary 7 %, endocrinopathies 6 % Grade 3/4: 5 % ($n = 6$), including pneumonitis, 3 % ($n = 4$), GI, hepatic, and infusion reaction 1 %, ($n = 1$, each); 3 deaths from pneumonitis
Nivolumab [38]	Advanced melanoma (subset analysis)	I	1–10 mg/kg q2w for a maximum of 12 cycles (4 doses per 8-week cycle)	107	84 % ($n = 90$), including fatigue 32 %, rash 23 %, diarrhea 18 %	22 %, including lymphopenia 3 %, fatigue 2 %, diarrhea 2 %, abdominal pain 2 %	Any grade: 54 % ($n = 58$), including skin 36 %, GI 18 %, endocrinopathies 13 %, hepatic 7 %, infusion reaction 6 %, pulmonary 4 %, and renal 2 % Grade 3/4: 5 % ($n = 5$), including GI and endocrinopathies 2 % each, and hepatic and renal 1 % each
Nivolumab [33]	mRCC	II	0.3, 2.0, or 10 mg/kg q3w	168	67–78 %[e], including fatigue 22–35 %, hypersensitivity 0–17 %, diarrhea 3–15 %, arthralgia 2–15 %, nausea 10–13%, rash 7–13%, pruritus 9–11%	5–17 %[e], including nausea, pruritus, arthralgia 0–2 % each	Any grade[e]: skin 22–28 %, hypersensitivity/infusion reaction 4–19 %, GI 5–15 %, endocrine 5–11 %, hepatic 3–7 %, pulmonary 4–7 %, renal 0–2 % Grade 3/4[e]: skin 0–4 %, endocrine 0–4 %, hepatic 0–4 %, GI 0–2 %
Pidilizumab [23]	Stage IV progressing melanoma	II	1.5 or 6.0 mg/kg q2w up to 54 weeks	107	68 %, including fatigue 31 %, diarrhea 16 %, arthralgia 13 %, anemia 11 %, nausea 10 %, hyperglycemia 2 %	4 %, including arthralgia 1 %, anemia 2 %	Grade 3 appendicitis, grade 3 arthritis, grade 4 hepatitis and grade 3 pneumonitis 1 % each

Table 4 continued

Agent name	Setting	Phase	Dosing/description	Patients (*N*)	Treatment-related AEs	Grade 3/4 treatment-related AEs	Potential immune-related AEs[b]
Anti-PD-L1 agents							
MPDL3280A [39]	Locally advanced or metastatic NSCLC (subset analysis)	I	Dose escalation, 1–20 mg/kg q3w up to 1 year	85	66 % ($n = 56$), including fatigue 20 %, nausea 14 %, decreased appetite 12 %, dyspnea 9 %, diarrhea 8 %	11 % ($n = 9$), including fatigue 2 %, nausea 1 %, dyspnea 1 %, vomiting 1 %	1 case of grade 3/4 diabetes mellitus 1 %
MPDL3280A [12]	Metastatic urothelial bladder cancer	I	15 mg/kg q3w up to 1 year	68	57 % ($n = 39$), including decreased appetite 12 %, fatigue 12 %, nausea 12 %, pyrexia 9 %, asthenia 7 %	4 % ($n = 3$), including asthenia 2 %	No cases were reported
BMS-936559 [13]	Advanced or recurrent solid tumors	I	Dose escalation, 0.3–10 mg/kg q2w in 6-week cycles up to 16 cycles	207	61 % ($n = 126$), including fatigue 16 %, infusion reactions 10 %, rash 9 %, diarrhea 9 %, arthralgia 7 %	9 % ($n = 19$), including fatigue 1 %, infusion-related AEs 1 %, lymphopenia 1 %	39 % ($n = 81$), including rash 7 %, hypothyroidism 3 %, hepatitis 1 %, one case each of sarcoidosis, endophthalmitis, diabetes mellitus, myasthenia gravis Grade 3/4: 5 % ($n = 10$), including infusion reaction <1 %, adrenal insufficiency <1 %
MEDI4736 [14]	Advanced solid tumors	I	10 mg/kg q2w up to 1 year	346	39 % ($n = 135$), including fatigue 13 %, rash/pruritus 9 %, vomiting 5 %, diarrhea 5 %	6 % ($n = 20$), including fatigue 1 %, elevated AST/ALT 1 %, vomiting, hypothyroidism, hyperglycemia, rash/pruritus, all <1 %	All grades: elevated AST/ALT 4 %, hypothyroidism 2 %, hyperthyroidism 1 %, pneumonitis 1 %, peripheral neuropathy 1 %

AE adverse event, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *CTLA-4* cytotoxic T-lymphocyte-associated antigen 4, *CTX* chemotherapy, *GI* gastrointestinal, *mCRPC* metastatic castration-resistant prostate cancer, *mRCC* metastatic renal cell carcinoma, *ND* no data, *NSCLC* non-small cell lung cancer, *PD-1* programmed death-1, *PD-L1* programmed death ligand 1, *qxd* every x days, *qxw* every x weeks, *TRD* treatment-related deaths, *TSH* thyroid stimulating hormone

[a] Monotherapy trials in solid tumors were included

[b] As determined by the investigator

[c] Treatment-related AEs not available

[d] At 15 mg/kg the following immune-related AEs were reported: Grade 3 colitis or diarrhea: 7 % ($n = 2$); Grade 3 Guillain-Barré-like peripheral neuropathy: 3 % ($n = 1$); Grade 4 increases in transaminases and pancreatic enzymes: 3 % ($n = 1$) [40]

[e] Depending on dose

2, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ—a process termed T-cell 'exhaustion' or 'tolerance'. In this state, tumor-infiltrating lymphocytes become tolerant and are less capable of carrying out antitumor immune responses as a result of chronic antigen exposure and prolonged negative immune regulation. While both CTLA-4 and PD-1 are immune checkpoint inhibitors, CTLA-4 is thought to act earlier in the process of T-cell activation, whereas PD-1 plays a role in attenuating T-cell responses later in the process, after T cells have migrated to the tumor microenvironment [44] (Fig. 2).

Given these observations, both PD-1 and PD-L1 antibody blockade may be a promising target for cancer immunotherapy (Fig. 2) [3, 45]. Anti-PD-1 antibodies are designed to inhibit PD-1 from engaging with any of its ligands, thereby preventing both PD-1:PD-L1 and PD-1:PD-L2 binding. In contrast, anti-PD-L1 agents prevent PD-1:PD-L1 binding, but not necessarily PD-1:PD-L2 binding. There is evidence that PD-L1 may bind CD80 (B7-1) on T cells, leading to a down-regulatory signal; hence, anti-PD-L1 could inhibit this interaction as well [3, 44]. Given the current understanding that PD-1:PD-L1 interactions are a predominant mechanism of tumor immune evasion, whether agents targeting PD-1 versus PD-L1 will have different clinical profiles is unknown.

To date, all data reported for PD-1 pathway inhibitors have been in phase I or II studies without control arms. The ongoing phase II and III trials that include control arms should provide data with greater context. Table 2 lists PFS and OS data from historical controls and studies where these data were available. However, as the studies differ greatly in terms of patient characteristics, study sizes, and length of follow-up, direct comparisons should be made with great caution.

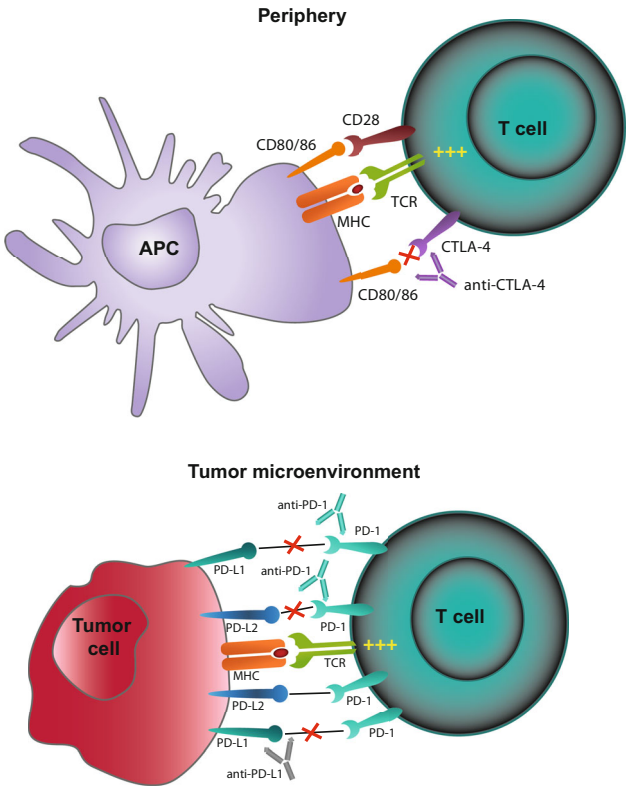

Fig. 2 Inhibiting the CTLA-4 and PD-1 immune checkpoint pathways to restore antitumor immune responses. In peripheral lymphoid organs and tissues, anti-CTLA-4 antibodies block CTLA-4 from binding CD80/86 on APCs and prevent T-cell inhibition. In the tumor microenvironment, PD-L1 and/or PD-L2 expression inhibits PD-1-expressing T cells. Interruption of PD-1:PD-L1 and PD-1:PD-L2 binding by anti-PD-1 antibodies or interruption of PD-1:PD-L1 binding by anti-PD-L1 antibodies restores T-cell immune responses. *APC* antigen-presenting cell, *CTLA-4* cytotoxic T-lymphocyte-associated antigen 4, *MHC* major histocompatibility complex, *PD-1* programmed death-1, *PD-L1* programmed death ligand 1, *PD-L2* programmed death ligand 2, *TCR* T-cell receptor

3.2 Efficacy with PD-1 Inhibitors

3.2.1 Pembrolizumab (MK-3475)

Pembrolizumab (MK-3475) is a humanized IgG4 monoclonal antibody against PD-1 [7] (Table 1 [4–16]). A phase I study, including expansion cohorts, evaluated pembrolizumab treatment in patients with advanced melanoma who had previously received or not received ipilimumab [7]. The overall response rate across all doses was 34 %; 88 % of responses were ongoing at the time of analysis and the median duration of response had not been reached. Prior treatment with immunotherapy, including ipilimumab and IL-2, did not preclude activity of pembrolizumab, nor were higher rates of AEs observed in patients who had received previous immunotherapy compared with those who had not. Preliminary survival data of pembrolizumab and other PD-1 pathway agents is listed in Table 2 [4, 5, 7, 18, 19, 21–33].

Pembrolizumab was also investigated in a phase I study in patients with previously treated NSCLC [25, 26]. Interim data analysis revealed that, in a cohort of 217 patients with NSCLC, the ORR was 18 % based on immune-related response criteria (irRC), and the median OS was 51 weeks. irRC have been used primarily to assess responses in patients with melanoma, and have not been validated in other cancers [46]. Responses were seen in patients with non-squamous and squamous histology, and in current/former and never smokers. [25, 26]. As a first-line therapy for NSCLC, pembrolizumab reported preliminary ORRs of 47 % by irRC. Median PFS was 27 weeks (Response Evaluation Criteria in Solid Tumors [RECIST]) and 37 weeks (irRC) [27].

Pembrolizumab also showed antitumor activity in patients with head and neck cancer in a phase I study [47]. Responses were seen in 20 % (11/56) of evaluable patients, which included human papillomavirus-positive and -negative tumors (Table 3 [16]).

3.2.2 Nivolumab

Nivolumab (BMS-936558) is a fully human IgG4 PD-1 immune checkpoint inhibitor [8] (Table 1 [4–16]). A phase I, dose-escalating study in multiple tumor types reported objective responses with nivolumab in a substantial portion of patients with melanoma, NSCLC, or renal cell carcinoma (RCC), but no objective responses in patients with colorectal cancer (CRC) or CRPC [8]. In patients with melanoma treated with nivolumab across all doses in the phase I trial, the ORR was 32 % and the median duration of response was 99 weeks. The median OS was 17.3 months and 1-, 2-, and 3-year survival rates were 63, 48, and 41 %, respectively [22] (Table 2 [4, 5, 7, 18, 19, 21–33]).

In the phase I trial among patients with NSCLC and across all doses, the ORR was 17 % (22/129), the estimated median response duration was 74 weeks, and overall 1- and 2-year survival rates were 42 and 24 %, respectively [28]. Ongoing trials are evaluating nivolumab alone or in combination with chemotherapy, erlotinib, or ipilimumab in patients with chemotherapy-naïve NSCLC, and have reported initial evidence of antitumor activity [29, 48–50]. With first-line nivolumab monotherapy in a phase I trial, the initial ORR was 30 %, median PFS was 36.1 weeks, and median OS was not reached (range 13.3–89.1+) in 20 evaluable patients. The 1-year OS rate was 75 % [29].

In phase II study in patients with previously-treated RCC, the overall response rate was 21 % (35/168), with the majority of responses lasting >1 year [33]. Across the evaluated doses, the PFS ranged from 2.7 to 4.2 months, and the median OS ranged from 18.2 to 24.7 months. The survival rate was approximately 35 % at 2.5 years [33] (Table 2 [4, 5, 7, 18, 19, 21–33]). Phase II and III trials of nivolumab are ongoing in melanoma, NSCLC, RCC, and squamous head and neck cancer (Table 3 [16]).

3.2.3 Pidilizumab (CT-011)

Pidilizumab (CT-011) is a humanized anti-PD-1 IgG1 monoclonal antibody [9] (Table 1 [4–16]). Pidilizumab was evaluated in patients with stage IV melanoma in a phase II open-label study. The ORR using irRC was 6 %, the median PFS was 1.9 months, and the 1-year survival rate was 65 % [23] (Table 2 [4, 5, 7, 18, 19, 21–33]).

Pidilizumab has also shown evidence of efficacy in patients with hematologic malignancies [51]. Separately, pidilizumab after autologous hematopoietic stem-cell transplantation (AHSCT) was evaluated in a phase II trial in patients with diffuse large B-cell lymphoma (DLBCL) or primary mediastinal large B-cell lymphoma [9]. A total of 66 patients completed all treatment cycles, and PFS at 16 months from the start of treatment was 72 %. This compares favorably with the PFS rate at 18 months of 52 % in a historical group of patients with DLBCL with similar characteristics (i.e., would have met the eligibility criteria for the study, including no progression or relapse within 2 months of AHSCT) who had received high-dose chemotherapy followed by AHSCT [9, 52]. Among the 35 patients with measurable disease after transplant, the overall response rate with pidilizumab treatment was 51 %, and the complete remission rate was 34 % [9].

3.2.4 AMP-224

AMP-224 is a recombinant fusion protein comprising the extracellular domain of PD-L2 and the Fc region of human IgG [10] (Table 1 [4–16]). This agent is designed to bind PD-1, and preclinical studies suggest its mechanism of action may differ from monoclonal antibody blockade. A phase I trial evaluating the safety of AMP-224 in patients with advanced cancer is ongoing (NCT01352884) [53]. Infusion reactions were common, occurring in 69 % of patients across dose cohorts. It is unclear whether this study is moving forward, as a high rate of infusion reactions and lack of efficacy have been observed compared with other PD-1 inhibitors.

3.3 Safety of PD-1 Inhibitors

While no head-to-head trials have been conducted, the safety profiles of the anti-PD-1 agents seem to be generally similar. In the largest and most mature studies of pembrolizumab and nivolumab, grade 3/4 treatment-related AEs were reported in 10–22 % of patients [7, 8, 26, 38]. The most common treatment-related AE was fatigue in all studies (20–36 % all grades, 2 % grade 3/4). Potential immune-related select AEs were also commonly reported: dermatologic toxicities (pruritus ≤24 % all grades, <1 % grade 3/4; rash 6–20 % all grades, <1 % grade 3/4; vitiligo ≤11 % all grades, 0 % grade 3/4), gastrointestinal toxicities (diarrhea 7–16 % all grades, ≤1 % grade 3/4; nausea 6–12 % all grades, <1 % grade 3/4), and endocrinopathies (hypothyroidism 2–8 % all grades, <1 % grade 3/4; hyperthyroidism 1–2 % all grades, <1 % grade 3/4). Arthralgia (all grades) was reported in 4–16 % of patients, and grade 3/4 in <1 %. Pneumonitis of all grades was noted in 3 % of pembrolizumab- or nivolumab-treated patients, with ≤1 % of patients developing grade 3/4 pneumonitis. Although rare, three deaths due to

pneumonitis in nivolumab-treated patients are concerning. Nivolumab or pembrolizumab in patients who had received prior ipilimumab showed similar safety profiles to those of ipilimumab-naïve patients, supporting the sequential use of these therapies [54, 55].

3.4 Efficacy with PD-L1 Inhibitors

3.4.1 MPDL3280A

MPDL3280A is a human anti-PD-L1 monoclonal antibody [11, 12] (Table 1 [4–16]). MPDL3280A is being assessed in a dose-ranging phase I study in patients with multiple tumor types. Interim results from 53 evaluable patients with NSCLC revealed an ORR of 23 % in patients across squamous and non-squamous histologies, including several patients with rapid tumor shrinkage [39]. Some patients (not included in the ORR) had delayed responses after apparent radiographic progression. Most responses were ongoing at the time of analysis. The ORR in patients with RCC was 13 %; durable responses were seen in patients with clear cell and non-clear cell disease [11]. In patients with urothelial bladder cancer, the ORR was 25 % (17/67) [12]. Phase II and III trials evaluating MPDL3280A in advanced lung cancer, advanced RCC, and urothelial bladder cancer are ongoing (Table 3 [16]).

3.4.2 BMS-936559

BMS-936559 is a fully human IgG4 monoclonal antibody directed against PD-L1 [13] (Table 1 [4–16]). The safety and activity of BMS-936559 was assessed in a phase I dose-escalating study in patients with advanced solid tumors [13]. Clinical activity was observed in patients with melanoma, NSCLC, RCC, CRC, ovarian, or pancreatic cancer, but not for patients with gastric or breast cancer. ORRs for patients with melanoma, NSCLC, RCC, and ovarian cancer were 17, 10, 12, and 6 %, respectively, and responses lasted for $\geq$1 year in 8 of 16 patients with at least 1 year of follow-up. No additional trials of BMS-936559 are currently listed in ClinicalTrials.gov [16].

3.4.3 MEDI4736

MEDI4736 is a human IgG1 monoclonal antibody that binds PD-L1 [14] (Table 1 [4–16]). MEDI4736 is being investigated in multiple tumor types, including melanoma, NSCLC, squamous head and neck cancer, and pancreatic cancer in a phase I trial. A preliminary analysis reported ORRs of 13 % (6/47) in patients with NSCLC and 14 % (3/22) in patients with squamous head and neck cancer, and evidence of activity against pancreatic and gastroesophageal cancer [14]. Numerous studies of MEDI4736 are planned or ongoing in patients with NSCLC or other malignancies (Table 3 [16]).

3.5 Safety of PD-L1 Inhibitors

The types of AEs reported with PD-L1 agents seem similar to those targeting PD-1, but the incidence of AEs appears to be lower. The reported rate of grade 3/4 treatment-related AEs across studies and tumor types ranged from 4 to 13 % [11–14, 39]. Fatigue (12–20 % all grades, 0–2 % grade 3/4), gastrointestinal AEs (diarrhea 5–9 % all grades, 0 % grade 3/4; nausea 6–14 % all grades, 0–1 % grade 3/4), dermatologic AEs (rash 7–9 % all grades, <1 % grade 3/4; pruritus 6 % all grades, <1 % grade 3/4), and endocrinopathies (hypothyroidism 2–3 % all grades, <1 % grade 3/4) were the most common.

BMS-936559 had a higher reported rate of infusion-related reactions (10 % all grades, $\leq$1 % grade 3/4) and arthralgia (7 % all grades, 0 % grade 3/4) than other PD-L1-targeting agents [13] (Table 4 [4, 7, 8, 12–14, 17, 21, 23, 26, 28, 36, 38–40]).

3.6 PD-L1 as a Predictive Biomarker

Emerging evidence suggests that PD-L1 expression on pre-treatment tumor specimens may be a predictive biomarker of efficacy with PD-1 pathway inhibitors. Across agents, studies, and tumor types, antitumor activity was generally higher against PD-L1-positive tumors versus tumors with low or negative staining for PD-L1 (Table 5 [11, 12, 14, 22, 28, 29, 39, 56–59]). However, the methodology, amount of staining (or 'cut-off') required to qualify as a PD-L1-positive tumor and timing of sample collection (archival or immediately pre-treatment) varied across studies. Thus, while these preliminary findings are encouraging, data on tumor PD-L1 expression as a potential predictive biomarker are evolving, and prospective validation will be needed.

4 Immunotherapy Clinical Response Patterns

Across the various immunotherapy clinical development programs, investigators have observed response patterns that sometimes differ from the conventional responses observed with cytotoxic agents. Standard response criteria may not capture or adequately describe the responses produced by novel immunotherapy; thus, clinical trials may not always capture the full clinical benefit to patients who receive immunotherapy. To better characterize this pattern of response, new irRC were created that accounted for antitumor response based on total measurable tumor burden as measured by the sum of index lesions and new

Table 5 PD-L1 expression and association with clinical activity [11, 12, 14, 22, 28, 29, 39, 56–59]

Agent	Setting	Cut-off for PD-L1^{+}[a]	ORR in pts with PD-L1^{+} tumors, % (*n*/*N*)	ORR in pts with PD-L1^{-}low/ negative tumors, % (*n*/*N*)	Median PFS in pts with PD-L1^{+} tumors	Median PFS in pts with PD-L1^{-} low/ negative tumors	Reference(s)
Pembrolizumab	Advanced melanoma	≥1 %[b]	51 (ND)	6 (ND)	12 months	3 months	[56]
Pembrolizumab	Advanced NSCLC	≥50 %[b]	37 (15/41)	11 (10/88)	14.0 weeks	9.3 weeks	[57]
Nivolumab	Previously-treated melanoma	≥5 %[b]	44 (8/18)	13 (3/23)	9 months	2 months	[22]
Nivolumab	Previously-treated NSCLC	≥5 %[b]	15 (5/33)	14 (5/35)	3.6 months	1.8 months	[28]
Nivolumab	CTX-naïve NSCLC	≥5 %[b]	50 (5/10)	0 (0/7)	45.6 weeks	36.1 weeks	[29]
Nivolumab	RCC	≥5 %[b]	22 (4/18)	8 (3/38)	ND	ND	[58]
MPDL3280A	Previously-treated NSCLC	Score = 3 (highly positive)[c]	83 (5/6)	20 (4/20)	ND	ND	[39, 59]
MPDL3280A	RCC	Positive staining[c]	20 (2/10)	10 (2/21)	ND	ND	[11, 59]
MPDL3280A	Urothelial bladder cancer	≥5 %[d]	43 (ND)	11 (ND)	ND	ND	[12]
MEDI4736	NSCLC	Undefined	39 (5/13)	5 (1/19)	ND	ND	[14]
MEDI4736	Head and neck cancer	Undefined	50 (2/4)	6 (1/16)	ND	ND	[14]

CTX chemotherapy, *ND* no data, *NSCLC* non-small cell lung cancer, *RCC* renal cell carcinoma, *PD-L1* programmed death ligand 1, *pts* patients

[a] Amount of staining required to qualify as a PD-L1^{+} tumor

[b] Membrane staining of tumor cells

[c] Staining of tumor-infiltrating immune cells; amount of staining to qualify as a PD-L1^{+} tumor was not defined

[d] Staining of tumor-infiltrating immune cells

measurable lesions [46]. Time point response assessment was also incorporated into the criteria, as two observations at least 4 weeks apart were necessary to help distinguish progression from 'pseudoprogression', in which there is an initial increase in tumor size followed by tumor shrinkage. The differences between World Health Organization (WHO) criteria and the new irRC are listed in Table 6 [46].

It has also been determined that some of these new response patterns to immune therapies are associated with a favorable survival outcome and may include the following:

- Stable disease, which in some cases may be followed by slow and steady decline of tumor burden. Durable, stable disease lasting months or even years has been observed in some patients.
- Reduction after an initial increase in tumor burden; this observation has been associated with T-cell infiltration into the tumor, giving the appearance of progressive disease.
- Reduction in total tumor burden during or after the appearance of new lesions, possibly due to the unique mechanism of action of immunotherapy, as the activated immune system may take some time to mount an effective antitumor response.

Time to response appears to be faster for agents that block PD-1 or PD-L1 compared with the many weeks it may take to observe tumor shrinkage in response to anti-CTLA-4 treatment [8, 13, 21, 38, 46]. Additionally, response rates in patients given anti-PD-1 or anti-PD-L1 treatment may be higher than in those receiving anti-CTLA-4, although head-to-head trial data are not yet available.

5 Other Immune Targets—Inhibitory and Stimulatory

In addition to CTLA-4 and PD-1, other classes of inhibitory and stimulatory molecules have potential to be used as anticancer immunotherapy. One inhibitory molecule that has drawn much attention recently is lymphocyte activation gene-3 (LAG-3), a CD4 homolog that binds to MHC class II molecules (Fig. 1). LAG-3 is expressed on activated CD4^{+} and CD8^{+} T cells, as well as B cells, natural killer cells, and tumor-infiltrating lymphocytes, and is thought to negatively regulate T-cell expansion by limiting T-cell activation. However, LAG-3 knockout mice do not develop overt autoimmunity, suggesting that it plays a more subtle role in regulating T-cell function than the checkpoint

Table 6 Comparison between WHO criteria and the irRC [46]

	WHO	irRC
New, measurable lesions (i.e., ≥5 × 5 mm)	Always represent PD	Incorporated into tumor burden
New, non-measurable lesions (i.e., <5 × 5 mm)	Always represent PD	Do not define progression (but preclude irCR)
Non-index lesions	Changes contribute to defining BOR of CR, PR, SD, and PD	Contribute to defining irCR (complete disappearance required)
CR	Disappearance of all lesions in two consecutive observations not less than 4 weeks apart	Disappearance of all lesions in two consecutive observations not less than 4 weeks apart
PR	≥50 % decrease in SPD of all index lesions vs. baseline in two observations at least 4 weeks apart, in absence of new lesions or unequivocal progression of non-index lesions	≥50 % decrease in tumor burden vs. baseline in two observations at least 4 weeks apart
SD	50 % decrease in SPD vs. baseline cannot be established nor 25 % increase vs. nadir, in absence of new lesions or unequivocal progression of non-index lesions	50 % decrease in tumor burden vs. baseline cannot be established nor 25 % increase vs. nadir
PD	At least 25 % increase in SPD vs. nadir and/or unequivocal progression of non-index lesions and/or appearance of new lesions (at any single time point)	At least 25 % increase in tumor burden vs. nadir (at any single time point) in two consecutive observations at least 4 weeks apart

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BOR best overall response, *CR* complete response, *irCR* immune-related complete response, *PD* progressive disease, *PR* partial response, *SD* stable disease, *SPD* sum of the products of the two largest perpendicular diameters, *WHO* World Health Organization

molecules CTLA-4 and PD-1 [3, 60]. While LAG-3 is another immune checkpoint that may be important in the immune response to cancer, blockade of this pathway has not been clinically evaluated to the same extent as the CTLA-4 and PD-1 pathways [3]. However, based on the success of other checkpoint molecules as targets for anticancer therapy, research is now ongoing to assess the possible clinical value of LAG-3 blockade. IMP321 is a soluble LAG-3 Ig fusion protein and MHC class II agonist (Table 1 [4–16]); it has been combined with gemcitabine in a phase I study in patients with advanced pancreatic adenocarcinoma [15]. IMP321 plus gemcitabine appears to be a well tolerated regimen that has not resulted in any serious AEs in patients to date; however, limited antitumor responses were attributed to the low doses of IMP321. A small study combining IMP321 and melanoma-associated antigen immunization in patients with melanoma is ongoing (NCT01308294).

A variety of other molecules that similarly regulate T-cell activation, tolerance/exhaustion, anergy, and even T-cell death are currently being assessed as potential targets for anticancer therapy. One of these inhibitory molecules is T-cell immunoglobulin and mucin domain 3 (TIM3), which is a member of the TIM family (Fig. 1). TIM3 is expressed by IFN-γ-secreting helper T (T_H1) cells, as well as dendritic cells, monocytes, and T cells [61]. When bound to its ligand, galectin-9, TIM3 induces T_H1 cell death [62]. Studies of TIM3-deficient mice suggest that the TIM3 pathway inhibits the expansion and effector functions of T_H1 cells and may be important for tolerance induction of T_H1 cells [63]. Administration of a TIM3 fusion protein resulted in hyperproliferation of T_H1 cells and inflammatory cytokine release, suggesting a ligand for TIM3 is also expressed by these cells.

Glucocorticoid-induced TNF receptor-related gene (GITR) can provide a co-stimulatory signal to both $CD4^+$- and $CD8^+$-naïve T-cells, particularly when T-cell receptor stimulation is weak [64] (Fig. 1). T cells that do not express GITR are more prone to activation-induced cell death, suggesting that GITR-mediated stimulation may enhance the survival of activated T cells. Also, preclinical studies showed that agonist anti-GITR antibodies could reverse regulatory T-cell suppression of effector T cells. Data from murine models suggest that GITR blockade may be most effective when combined with melanoma vaccination strategies and not as monotherapy [65]. Currently, a phase I dose-escalation trial of an anti-GITR monoclonal antibody (TRX518) is recruiting patients with unresectable stage III or IV melanoma or other solid tumor malignancies (NCT01239134).

6 Combination Strategies

Despite promising results as monotherapies, there remains a clear need to increase the number of patients with malignancies that can benefit from immune checkpoint inhibitors. The increasing arsenal of targeted and immune-

based therapies affords opportunities for sequencing and combination strategies to improve outcomes. Indeed, rapid and deep tumor regression was observed in a substantial number of patients when the CTLA-4 inhibitor, ipilimumab, was administered concurrently with nivolumab [24]. In this study, 42 % of patients had ≥80 % tumor reduction at 36 weeks, and 1- and 2-year survival rates were 85 and 79 %, respectively. Though the efficacy appeared to be increased as compared with either drug alone, so did the rate of AEs. The observed toxicities were similar to those reported with monotherapy, albeit with higher incidence, including 62 % of patients experiencing grade 3/4 AEs. Other anti-CTLA-4 plus anti-PD-1 trials are ongoing, as are trials exploring dual blockade of the PD-1 pathway (anti-PD-1 plus anti-PD-L1) [16]. Additional trials that are planned or have already entered the clinic include combinations of PD-1 pathway inhibitors with LAG-3, indolamine 2,3-dioxygenase 1 (IDO1), sipuleucel-T, or other vaccinations. Immune checkpoint inhibitor combinations with T-cell agonists (i.e., anti-CD40, anti-CD27, and anti-4-1BB) and with other immunostimulants (i.e., IFN, IL-21, and anti-killer immunoglobulin-like receptor [KIR]) are also underway.

It has been postulated that targeted agents and/or cytotoxic chemotherapy that effectively destroy tumor cells may increase circulating tumor antigens and, therefore, increase the immunogenic response and utility of checkpoint inhibitors [35]. In fact, inhibition of mitogen-activated protein kinase with BRAF and/or MEK inhibitors in melanoma cells has been shown to modulate the functions of immune cells in preclinical studies [66]. While a phase I/II study (NCT01400451) evaluating the safety and efficacy of combining vemurafenib with ipilimumab recently closed due to hepatic toxicity, a phase II study of vemurafenib followed by sequential ipilimumab in patients with V600 BRAF-mutated melanoma is still ongoing. Initial reports from phase I trials combining nivolumab with vascular endothelial growth factor (VEGF)-targeted therapies (sunitinib or pazopanib) are currently under study to determine the best dose and schedule [67, 68]. A combination regimen of pidilizumab (3 mg/kg) plus rituximab (375 mg/m^2) has also been investigated in a phase II trial in patients with relapsed follicular lymphoma. Of 29 patients evaluable for efficacy, 19 had an objective response (66 %), 15 had a complete response (52 %), and four had a partial response (14 %) [69].

Trials using ipilimumab combined with various cytotoxic chemotherapies, such as temozolomide plus doxycycline (NCT01590082) or fotemustine (NCT01654692) are underway in patients with metastatic melanoma, as are numerous trials of PD-1 pathway inhibitors plus chemotherapy or targeted agents [16]. Trials evaluating ipilimumab plus paclitaxel/carboplatin (NCT01165216) and pembrolizumab or nivolumab plus chemotherapy or targeted agents (NCT02039674; NCT01454102) have been started in patients with NSCLC. Details on ongoing phase II and III trials investigating combination regimens with immune checkpoint inhibitors are provided in Table 3 [16].

7 Conclusion

A better understanding of tumor immunology and immunotherapy, both at the bench and at the bedside, has led to a new and promising area of basic and clinical cancer research. Inhibitors of immune checkpoint regulators have in some cases led to deep and durable responses in patients with advanced malignancies. Many of the observed side effects are manageable and reversible following standard protocols.

Lessons learned from CTLA-4 and PD-1 blockade, in both the laboratory and the clinic, not only provide the foundation for a future era of superior immune checkpoint inhibition, but also provide a nidus of questions that remain unanswered. Furthering Virchow's initial observation and our understanding of the link between the immune system and malignancy, immune checkpoint inhibitors appear to offer new hope for cancer patients.

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