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Reorienting the immune system in the treatment of cancer by using anti-PD-1 and anti-PD-L1 antibodies

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Physiologically, the programmed death 1 (PD-1) pathway is involved in limiting the killing of bystander cells during an infection and controlling autoimmunity. However, cancers exploit this system to avoid immune killing, and PD-1 ligand 1 and 2 (PD-L1 and PD-L2) expression on tumor cells, as well as PD-1 expression on tumor-infiltrating lymphocytes, have shown to be negative prognostic factors. Promising clinical results have been obtained by PD-1 pathway blockade in a range of cancers while still maintaining a manageable toxicity profile, and two anti-PD-1 antibodies are now approved by the US Food and Drug Administration (FDA) for the treatment of metastatic melanoma. As already shown with nivolumab and ipilimumab, the combination of PD-1 pathway blockade with other anticancer agents holds promise in the form of additive synergistic anticancer effects.

Introduction

Classically, the initiation of a T cell immune response is thought to rely entirely on stimulation through the T cell receptor (TCR) when bound to the major histocompatibility complex on the antigen-presenting cell (APC) and co-stimulation through CD28 (T cell)-B7 (APC) interaction [1]. However, this model turned out to be simplifying the complex balance between stimulation and suppression of adaptive immune responses. If it was this simple, the immune system would be overactivated and destroy its host through killing of bystander cells during a systemic infection or through autoimmunity. Thus, an 'immune checkpoint' system is involved in maintaining homeostasis of the immune system by controlling T cell activation. Indeed, a plethora of immune-inhibitory pathways has been discovered, as reviewed by Freeman and Sharpe [2].

Under physiological conditions, these immune checkpoints work as molecular brakes to suppress hyperactivation of T cells to prevent autoimmunity and serious damage to the host. The first of these molecules to be successfully targeted by monoclonal antibodies was cytotoxic T lymphocyte-associated protein 4 (CTLA-4 or CD152) located on the surface of T cells. Upon T cell stimulation, CTLA-4 is transported to the membrane and binds to B7 with a higher affinity than CD28, thus exerting its inhibiting role by competing with CD28 for B7 binding and by sending inhibitory signals to the naïve T cell in the priming phase. During early functional characterization of this molecule, it was reported that blocking CTLA-4 could shift the immune system balance toward T cell activation and lead to anticancer effects, initially shown in animal models [3] and later confirmed in human clinical trials. This led to the first-in-class FDA approval in 2011 of the anti-CTLA-4 antibody, ipilimumab, for the treatment of metastatic melanoma (MM) on the basis of a phase III trial showing improved survival for the first time [4,5].

In this review, we highlight an additional group of receptors in the B7:CD28 family, PD-1 and its ligands PD-L1 (B7-H1) and PD-L2 (B7-DC), which are also promising targets in the treatment of cancer.

PD-1 receptor structure, expression and function

The PD-1 receptor was discovered in 1992 because the *pdcd1* gene was upregulated during apoptotic cell death in lymphocytes [6]. Structurally, it is a 288-amino acid (aa) type I transmembrane receptor and belongs to the immunoglobulin (Ig) superfamily. In addition to the Ig domain, it comprises a stalk, a transmembrane

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http://dx.doi.org/10.1016/j.drudis.2015.07.003

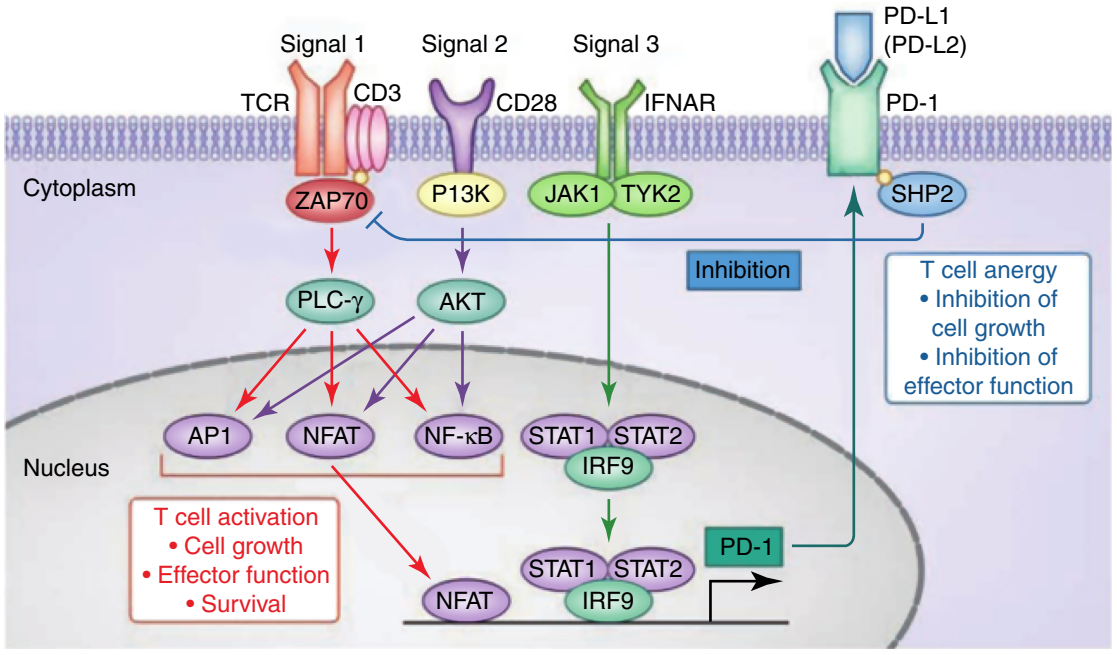

FIGURE 1

Intracellular signaling following programmed death 1 (PD-1) ligation. Upon T cell–antigen-presenting cell (APC) interaction, PD-1 is most likely relocated to the immune synapse. Src homology 2-containing tyrosine phosphatase (SHP-2) is recruited to the immunoreceptor tyrosine-based switch motif (ITSM) and dephosphorylates T cell receptor (TCR)-associated CD-3ζ, leading to inactivation of zeta chain-associated protein kinase 70 (Zap70), resulting in downstream inhibition and simultaneously blocking of phosphoinositide 3-kinase (PI3K) and Akt activity and, consequently, disruption of glucose metabolism and interleukin (IL)-2 secretion. Reprinted by permission from Macmillan Publishers Ltd: Nature Immunology [82], Nature Publishing Group 2013. *Abbreviations*: AP-1, activator protein 1; IFNAR, interferon-α/β receptor; IRF9, interferon regulatory factor 9; JAK, Janus kinase; NF-κB, nuclear factor κB; NFAT, nuclear factor of activated T cells; STAT, signal transducer and activator of transcription; TYK2, tyrosine kinase 2.

domain, and an intracellular domain containing an immunoreceptor tyrosine-based inhibitory motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Unlike other CD28-family receptors, PD-1 is a monomeric glycoprotein [7].

PD-1 is expressed on T cells, B cells, natural killer (NK) T cells, monocytes, and dendritic cells (DC) [6,8]. At least on T cells, activation induces PD-1 expression and persistent expression results in T cell exhaustion, as reviewed by Wherry [9].

In 1999, PD-1 was found to have a negative regulatory function in autoimmune disease because *pdcd1*$^{-/-}$ mice developed systemic lupus erythematosus (SLE) on the basis of PD-1 dysfunction. With the discovery of the two ligands of PD-1, PD-L1 and PD-L2 in 1999 and 2001, respectively [10,11], knowledge of the function of the receptor has increased markedly. Physiologically, PD-1 is involved in protecting the host from autoimmunity and from bystander killing of healthy cells in an immune reaction against foreign pathogens [12,13].

PD-1 is distributed uniformly on the cell surface [14] and is most likely relocated to the immune synapse upon T cell–APC interactions because inhibition of T cell function is mediated through phosphorylation of the ITIM and ITSM upon binding of PD-L1 or PD-L2. A Src homology 2-containing tyrosine phosphatase (SHP-2) is recruited to the ITSM and dephosphorylates TCR-associated CD-3ζ, leading to inactivation of zeta chain-associated protein kinase 70 (Zap70). The result is inhibition of downstream signaling and simultaneously blocking of phosphoinositide 3-kinase (PI3K) and Akt activity and, consequently, disruption of glucose metabolism and interleukin (IL)-2 secretion [15]. Figure 1 provides a graphical presentation of the intracellular signaling following PD-1 ligation.

PD-L1 and PD-L2 receptor structure, expression and function

PD-L1 (B7-H1) was discovered in 1999 as a ligand for PD-1 and is a 290-aa type I transmembrane protein encoded by *CD274*. The extracellular part comprises IgV- and IgC-like domains and the intracellular part is a 30-aa tail [10].

PD-L1 is not only constitutively expressed on lymphoid cells, such as monocytes, DCs, and T cells, but is also present on nonhematopoietic cells, such as endothelial and epithelial cells [16–18]. The receptor can be upregulated by type I and II interferons (IFNs) through IFN regulatory factor 1 (IRF-1) seemingly in a Janus kinase/signal transducer and activator of transcription (JAK/STAT)-dependent matter [19].

PD-L1 is a critical negative regulator of self-reactive T cells during both the induction and effector phases of autoimmune disease [20] and exerts its inhibitory function in multiple ways. Besides being a ligand for PD-1, PD-L1 additionally binds B7-1 (CD80) preventing B7-1 co-stimulation. IL-10 is produced upon ligation of PD-L1 [10], possibly augmenting the apoptosis of activated T cells [21]. In addition, it has a pivotal role in the conversion of naïve T cells to regulatory T cells (Tregs) by inhibiting Akt-mammalian target of rapamycin (mTOR) signaling and, thus, increasing phosphatase and tensin homolog (PTEN) activity [22].

PD-L2 (B7-DC) was discovered in 2000 as another ligand for PD-1 [11]. It is a transmembrane protein encoded by *pdcd1lg2* and is structurally similar to PD-L1. PD-L2 expression is largely restricted to DCs [23], but can additionally be induced on macrophages and nonhematopoietic cells [17,24]. PD-L2 is induced by IFN-γ, granulocyte macrophage colony-stimulating factor (GM-CSF), and especially IL-4 [24] through the JAK/STAT6 pathway [25].

Being a ligand to PD-1, PD-L2 dampens T cell effector functions [11] and probably has a role in preventing autoimmunity and immune-mediated killing of host cells. Recently, PD-L2 has been shown to bind repulsive guidance molecule b, which is also associated with the control of autoimmunity [26].

Rationale for targeting PD-1 and its ligands in cancer

The immune system is continuously surveying the host for foreign pathogens and irregular cells, such as cancer cells. Consequently, for the cancer to continue to grow, it needs to hide or escape from the immune system to avoid getting killed, as put into theory by Vesely *et al.* [27]. As described previously, PD-1 and its ligands have a central role in maintaining peripheral tolerance and preventing autoimmunity, and the cancer cells can exploit this system to create a suppressing microenvironment, thus protecting them from immune-mediated killing. Indeed, PD-L1 and PD-L2 expression has been found to be high in multiple cancers [28,29] and PD-L1 expression was first described as an indicator of tumor aggressiveness in renal cell carcinoma [30]. In addition, PD-L1 expression on tumor cells has been suggested as a prognostic factor in several solid cancers, including ovarian cancer [31] and pancreatic cancer [32].

Additionally, PD-1 expression of tumor-infiltrating lymphocytes (TILs) has also been shown to be high and to be a negative prognostic factor [33] that is thought to be due, at least in part, to T cell exhaustion and dysfunction [34]. Multiple reports on anti-PD-1 and anti-PD-L1 blockade have shown the restoration of T cell effector function and proliferation, increased infiltration of tumors by cytotoxic T lymphocytes (CTLs) altering the CTL:Treg ratio and, ultimately, the killing of tumor cells [32,35,36].

Strategies for targeting PD-1 and its ligands in cancer

The huge promise of blocking the PD-1 pathway has resulted in great commercial interest and intensive competition among drug companies to develop agents targeting PD-1 or PD-L1. Multiple agents have already been developed (Table 1). Anti-PD-1 antibodies block the PD-1:PD-L1 and PD-1:PD-L2 interactions, whereas the anti-PD-L1 antibodies block the PD-1:PD-L1 and PD-L1:CD80 interactions. This difference results in slightly different modes of action, and different adverse events and response patterns.

AMP-224 is a recombinant B7-DC-Fc fusion protein that has a unique mode of action by depletion of PD-1 high-expressing T cells, representing exhausted CD8+ T cells, and inhibition of the proliferation of Tregs, thus making room for a more vigorous anticancer response [37].

Recently, it was shown that the immune system itself appears to have established a counteractive mechanism by the discovery of PD-L1-specific effector T cells. These naturally occurring PD-L1-specific T cells recognize both PD-L1-expressing immune cells and malignant cells [38,39]. The activation of PD-L1-specific T cells enhanced additional T cell responses, both directly and indirectly [40,41]. Hence, PD-L1 peptide-based vaccination is an easily applicable and attractive option to target cancer cells, in addition to boosting the clinical effect of any anticancer immunotherapy.

Clinical experience with anti-PD-1 antibodies

Pembrolizumab

Pembrolizumab (previously known as lambrolizumab) was the first anti-PD-1 antibody to achieve FDA approval (September 2014). It was approved for treatment of unresectable stage III or stage IV metastatic melanoma as a second-line treatment after progression on ipilimumab and a BRAF inhibitor, in cases of BRAFV600 mutation, at a dose level of 2 mg/kg every 3 weeks (Q3W). Phase I results were reported by Hamid *et al.* [42], but approval was based on the results of a subsequent multicenter, randomized, and dose-comparative phase II trial [43]. In total, 173 patients were treated with 2 mg/kg Q3W or 10 mg/kg Q3W until progression. Of these, 157 patients were evaluable and the overall response rate (ORR) per response evaluation criteria in solid tumors (RECIST) was 26% in both groups [complete response (CR) 1%; partial response (PR) 25%] with an additional 25% (2 mg/kg) and 24% (10 mg/kg) achieving disease stabilization. Pembrolizumab was generally well tolerated, with 12% patients experiencing grade 3 or 4 events, and only 3% of patients discontinuing treatment because of adverse events (AEs). Later, pembrolizumab was compared with ipilimumab head to head in a randomized phase III and showed superior

TABLE 1

Anti-PD-1 and PD-L1 antibodies

Drug	Class	Target	Company	Refs or clinicaltrials.gov ID
Pidilizumab (CT-011)	Humanized IgG1	PD-1	CureTech	[63]
Pembrolizumab (MK-3475)	Humanized IgG4	PD-1	Merck (MSD)	[42]
Nivolumab (BMS-936558, MDX1106, ONO-4538)	Human IgG4	PD-1	Bristol-Meyers Squibb	[49]
AMP-224	PD-L2-IgG2a fusion protein	PD-1	Amplimmune/GSK	[37]
MEDI0680 (AMP-514)	Humanized IgG4	PD-1	Amplimmune/AstraZeneca	NCT02013804
BMS-936559	Human IgG4	PD-L1	Bristol-Meyers Squibb	[67]
MEDI4736	Humanized IgG1	PD-L1	AstraZeneca	[83]
MPDL3280A (RG7446)	Human IgG1	PD-L1	Roche	[28]
MSB0010718C (Avelumab)	Human IgG1	PD-L1	Merck KGaA/Pfizer	[84]

TABLE 2

Published and select ongoing trials

Study drug	Indication	*n*	Phase	Refs or clinicaltrials.gov ID
Published trials investigating PD-1 antibodies				
Nivolumab	Multiple solid cancers	39	I	[49]
	Melanoma	296	I	[50]
	RCC	186	II	[53]
Nivolumab (published, but ongoing)	Hodgkin's lymphoma	23 (accrual continues)	I	[52]
Nivolumab (+ vaccine for monitoring)	Melanoma	90	I	[51]
Nivolumab or dacarbazine	Melanoma	418	III	[55]
Nivolumab + ipilimumab	Melanoma	86	I	[58]
Pembrolizumab	Melanoma	135	I	[42]
		173	II	[43]
Pidilizumab	Advanced hematological malignancies	17	I	[63]
	Diffuse large B cell lymphoma	66	II	[64]
Pidilizumab + rituximab	Follicular lymphoma	32	II	[65]
Published trials investigating PD-L-1 antibodies				
BMS-936559	Multiple solid cancers	207	I	[67]
Select ongoing trials investigating PD-1 antibodies				
AMP-224	Solid tumors or cutaneous T cell lymphoma	44 (accrual completed)	I	NCT01352884
Pembrolizumab	Melanoma, NSCLC, other solid tumors	Estimated final accrual 1137	I	NCT01295827
Nivolumab	Melanoma	390 (accrual completed)	III	NCT01721746; [54]
	NSCLC	Estimated final accrual 495	III	NCT02041533
	RCC	822 (accrual completed)	III	NCT01668784
	Hepatocellular carcinoma	Estimated final accrual 90	I	NCT01658878
MEDI0680	Solid tumors	Estimated final accrual 48	I	NCT02013804
Select ongoing trials investigating PD-L-1 antibodies				
AMP-224 + low-dose cyclophosphamide	Solid tumors	44 (accrual completed)	I	NCT01352884
Pidilizumab	Melanoma	103 (accrual completed)	II	NCT01435369; [66]
MPDL3280A	Multiple malignancies	Estimated final accrual 344	I	NCT01375842; [28,68]
	NSCLC	Estimated final accrual 850	III	NCT02008227
MSB0010718C	Multiple solid tumors	Estimated final accrual 825	I	NCT01772004
MEDI4736	Multiple solid tumors	Estimated final accrual 760	I/II	NCT01693562
MEDI4736	Squamous cell carcinoma of the head and neck	Estimated final accrual 112	II	NCT02207530

clinical efficacy with improved ORR, median overall survival (OS) and less toxicity [44].

Recently, pembrolizumab showed promising activity in gastric [45], urothelial [46], head and neck [47], and non-small cell lung (NSCLC) [48] cancers and is now being investigated in several trials alone or in combination with other drugs (Tables 2 and 3).

Nivolumab

Nivolumab was the second anti-PD-1 antibody to achieve FDA approval (December 2014). Similar to pembrolizumab, it was approved for the treatment of unresectable stage III or IV metastatic melanoma as a second-line treatment after ipilimumab and a BRAF inhibitor, in cases of BRAFV600 mutation, at a dose level of 3 mg/kg every 2 weeks (Q2W). Later, nivolumab was also FDA approved for the treatment of metastatic squamous NSCLC after progression on platinum-based chemotherapy (March 2015). The first results of a trial including multiple solid cancers were reported by Brahmer *et al.* in 2010 [49]. On the basis of an acceptable toxicity profile and preliminary evidence of clinical activity, a large, multicenter, dose-escalation phase I study treating 296 patients with advanced melanoma, NSCLC, castration-resistant prostate cancer (CRPC), renal cell (RCC), or colorectal cancer (CRC) was conducted [50]. It showed an acceptable toxicity profile with grade 3 or 4 events in 14% of patients, with no significant difference among cancer subtypes. Antitumor responses were observed at all dose levels tested and ORR across all dosing regimens was 28% in melanoma, 18% in NSCLC, and 27% in RCC. No objective responses were observed in CRPC or CRC. Interestingly, 3 mg/kg was superior to 10 mg/kg in melanoma, with ORR being 41% versus 20%, respectively. Also notable was the response rate in NSCLC, given that 55% of patients had received more than three previous lines of therapy. Another phase I trial treating only melanoma was reported by Weber *et al.* [51]; a phase I trial treating relapsed or refractory Hodgkin's lymphoma was reported by Ansell *et al.* [52] and a phase II trial treating RCC was reported by Motzer *et al.* [53] (Table 2). Clinical activity was demonstrated in all these studies.

FDA approval for melanoma was granted upon the results from the first 120 patients treated in a multicenter, randomized phase III trial enrolling unresectable stage III or stage IV melanoma who had progressed after treatment with ipilimumab and, if BRAF mutation positive, with a BRAF inhibitor [54]. This study reported a similar toxicity profile compared with earlier studies, and an ORR of 32%. Responses were seen in both patients with or without *BRAF* mutation. In melanoma, nivolumab has also shown superiority to dacarbazine in a first-line setting [55]. FDA approval for NSCLC was granted upon the results of a randomized phase III trial showing improved ORR, median OS, and less toxicity on

TABLE 3

Select ongoing combination trials[a]

Study drugs	Indication	Phase	Refs and/or Clinicaltrials.gov ID
PD-1 antibodies			
MEDI0680 + MEDI551[b]	B cell lymphoma	Ib/II	NCT02271945
MEDI0680 + **MEDI4736**	Advanced malignancies	I	NCT02118337
AMP-224 + SBRT[c] + CTX[d]	CRC	I	NCT02298946
Pidilizumab + lenalidomide	Multiple myeloma	I/II	NCT02077959
Pembrolizumab + INCB024360[e]	Various solid tumors	I/II	NCT02178722
Pembrolizumab + pazopanib	RCC	I/II	NCT02014636
Pembrolizumab + PF-05082566[f]	Solid tumors	I	NCT02179918
Pembrolizumab + peginterferon	Melanoma	I	NCT02112032
Nivolumab + ipilimumab	Colon cancer	II	NCT02060188
Nivolumab + ipilimumab or lirilumab[g]	Multiple myeloma, Hodgkin's and non-Hodgkin's lymphoma	I	NCT01592370
Nivolumab + ipilimumab, erlotinib, chemotherapy or bevacizumab	NSCLC	I	NCT01454102; [62]
Nivolumab *or* **nivolumab** + bevacizumab or **nivolumab** + ipilimumab	RCC	II	NCT02210117; [61]
GVAX[h] + CTX + CRS-207[i] with or without **nivolumab**	Pancreatic cancer	II	NCT02243371
BMS-986016[j] with or without **nivolumab**	Solid tumors	I	NCT01968109
PD-L1 antibodies			
MEDI4736 + dabrafenib + trametinib	Melanoma	I/II	NCT02027961
MEDI4736 + tremelimumab	Solid tumors	I	NCT02261220
MPDL3280A + bevacizumab versus sunitinib	RCC	III	NCT02420821

[a] PD-1 and PD-L1 antibodies are highlighted in bold.
[b] MEDI551, anti-CD19 mAB.
[c] SBRT, stereotactic body radiation therapy.
[d] CTX, cyclophosphamide.
[e] INCB024360, IDO inhibitor.
[f] PF-05082566, anti-4-1BB monoclonal antibodies.
[g] Lirilumab, anti-KIR monoclonal antibodies.
[h] GVAX, GM-CSF stimulating agent.
[i] CRS-207, live, attenuated strain of *Listeria monocytogenes* aimed to induce immunologic response against mesothelin.
[j] BMS-986016, anti-LAG-3 monoclonal antibodies.

treatment with nivolumab compared with docetaxel as a second-line treatment after platinum-based chemotherapy [56]. Nivolumab has been further investigated in several trials alone or in combination with other therapies (Tables 2 and 3).

Combination of nivolumab and ipilimumab

The promise of the combination of nivolumab and ipilimumab was shown for the first time in a preclinical mouse model, in which the antibodies showed synergistic effects [57]. On the basis of this finding, a multiple-dosing regimen phase I trial was conducted treating a total of 86 unresectable stage III or stage IV melanoma [58]. In total, 53 patients received concurrent therapy with ipilimumab and nivolumab in different dose cohorts. Toxicity was significantly more pronounced than either monotherapies, with 53% of patients experiencing grade 3 or 4 treatment-related AEs, of which 37% were biochemical elevation of liver enzymes or lipase and 9% were gastrointestinal events. Across all dosing regimens, ORR was 40% (CR 9.6%, PR 30.8%), of which 76% had a reduction of tumor volume of 80% or more at a 12-week evaluation, showing that responses occurred fast and were deep. Another 25% achieved disease stabilization. A more recent analysis of the first 53 patients who received concurrent therapy showed an impressive 2-year survival rate of 75% [59].

The combination therapy was further investigated in a double-blind, randomized phase III study treating 945 patients with previously untreated metastatic melanoma with nivolumab or ipilimumab alone or in combination [60]. The median progression-free survival (PFS) was significantly improved from 2.9 months for ipilimumab alone to 11.5 months for the combination. Grade 3 or 4 treatment-related AEs also increased, from 16.3% (nivolumab alone) and 27.3% (ipilimumab alone) to 55% (combination); however, AEs were reported manageable with established treatment guidelines.

The combination has also been investigated in several other malignancies (Table 3) and has shown promising results in RCC [61] and NSCLC [62].

Pidilizumab

The first anti-PD-1 antibody to enter clinical trials was pidilizumab, which has mainly been tested in hematological malignancies (Table 2). After a phase I trial [63] in patients with advanced hematological malignancies that showed a favorable safety profile and early evidence of clinical activity, a phase II trial evaluating pidilizumab following autologous hematopoietic stem-cell transplantation (AHSCT) for diffuse large B cell lymphoma was conducted [64]. In total, 66 patients received at least three rounds of 1.5 mg/kg pidilizumab every 6 weeks (Q6W). Of these, 35 patients had measurable disease after AHSCT and of those the ORR was 51% (34% with CR and 17% with PR), whereas 72% of the patients had not progressed at 6 months. The most frequently reported grade 3 or 4 AEs were neutropenia (19%) and thrombocytopenia (8%).

In another phase II trial, 32 patients with relapsed follicular lymphoma received pidilizumab 3 mg/kg Q4W and, in addition, rituximab 375 mg/m^2 Q1W for 4 weeks. Of these, 29 patients were evaluable; 15 (52%) had a CR and four (14%) had a PR. Median PFS

for all patients was 18.8 months. No grade 3 or 4 events were reported [65].

At ASCO 2014, preliminary results from a phase II study treating 103 patients with stage IV melanoma were presented. Patients were randomized to receive either 1.5 mg/kg or 6 mg/kg Q2W. Using immune-related response criteria (irRC), ORR was 5.9% across doses with a 1-year survival of 64.5% independent of dose, previous, or later treatment [66].

AMP-224 and MEDI0860

Clinical trials investigating the anti-PD-1 antibodies AMP-224 and MEDI0680 (AMP-514) are ongoing (Clinicaltrials.gov IDs NCT01352884 and NCT02013804 respectively; Table 2).

Clinical experience with anti-PD-L1 antibodies

BMS-936559

BMS-936559 was the first anti-PD-L1 antibody to reach clinical trials and results from a multicenter, dose-escalation phase I trial were reported by Brahmer *et al.* [67]. A total of 207 patients with various solid tumors were enrolled. Grade 3 or 4 AEs were reported in 9% of patients and a maximum tolerated dose was not reached. Clinical activity was observed at all doses of 1 mg/kg or higher. In melanoma, ORR was 17% (CR 5.8%, PR 11.5%) and another 27% had stable disease (SD). In NSCLC, ORR was 10% and 10% had SD. In ovarian cancer, ORR was 6% and 18% had SD. In RCC, ORR was 12% and 41% had SD. No objective responses were seen in CRC and pancreatic cancer.

Although promising, to our knowledge, this drug is not currently being investigated in clinical cancer trials.

MPDL3280A

MPDL3280A is currently being investigated in a phase I trial including patients with multiple solid tumors. Preliminary and promising results have already been reported [28,68]. Especially promising are the results in the subgroup of heavily pretreated patients with urothelial bladder cancer (UBC). In that group of patients, ORR was 26% across histology profiles and responses were durable; 4% had grade 3 AEs but no grade 4 or 5 AEs were reported.

MEDI4736 and MSB0010718C

MEDI4736 is currently being investigated in a dose-escalation phase I/II trial including patients with a variety of solid cancers (clinicaltrials.gov ID NCT01693562). MSB0010718C (Avelumab) is currently under investigation in a phase I trial recruiting patients with various solid tumors (clinicaltrials.gov ID NCT01772004; Table 2).

Predictive biomarkers in PD-1 pathway blockade

A lot of work has been put into finding predictive biomarkers for response. PD-L1 expression on tumors was suggested as a candidate for predicting response to anti-PD-1 treatment by Topalian *et al.* [50], who reported an ORR of 36% in patients with PD-L1-positive and 0% in PD-L1-negative tumors. Even though Weber *et al.* [51] also found a positive correlation between tumor PD-L1 expression and response to anti-PD-1 therapy, they concluded that patients with PD-L1-negative tumors could not be excluded from therapy because of an ORR of 19% in this group. Naturally, PD-L1 expression as a predictive biomarker has also been investigated in the context of response to anti-PD-L1 treatment. Recently, Herbst *et al.* [28] and Powles *et al.* [68] showed that PD-L1 expression on tumor-infiltrating immune cells correlated better with clinical response than did PD-L1 expression on the tumor. Despite the clear correlation, some patients with no PD-L1 expression on either immune cells or tumor cells had response and, consequently, should not be excluded from therapy. PD-L1 expression as a biomarker has been reviewed in more detail elsewhere [69].

Interestingly, it was recently published that four out of ten patients with mismatch repair-deficient CRC responded to treatment with pembrolizumab, whereas zero out of ten patients with mismatch repair-proficient CRC responded [70], suggesting 'that the evaluation of tumor genomes can help guide immunotherapy' [70].

Rationale for combining anti-PD-1 and anti-PD-L1 antibodies with other therapies

Single-agent antibodies blocking the PD-1 pathway have already shown their worth. However, many patients still do not respond to immunotherapy. As reviewed by Spranger and Gajewski [71], this could be because of a lack of immune cell infiltration in the tumor, lack of immune activation, a dense immunosuppressive stroma, or the exploitation of multiple inhibiting pathways or molecules. By treating with multiple therapies with mechanistically different modes of action, a more powerful anticancer effect might be obtained. This is a major focus of the field and a search on clinicaltrials.gov for trials combining PD-1 pathway blockade with other therapies reveals a plethora of ongoing trials, some of which are listed in Table 3.

Early clinical results of simultaneous CTLA-4 and PD-1 blockade are already available (see above), suggesting that combinations of immune checkpoint inhibitors will result in not only increased rates of efficacy, but also significantly increased toxicities. With the emergence of novel treatments targeting immune inhibitory pathways, such as IDO (ClinicalTrials.gov ID NCT02048709) or LAG-3 (ClinicalTrials.gov ID NCT01968109), or costimulatory receptors, such as 4-1BB (ClinicalTrials.gov ID NCT02179918) or CD40 (ClinicalTrials.gov ID NCT02376699), the possibilities for combining different antibodies will be enormous.

Another rational approach would be to combine PD-1 pathway blockade with a vaccination strategy, because vaccines have been shown to be able to recruit immune effector cells into the tumor microenvironment [72]. In a similar framework, it has been suggested to use specific T cells targeting, for example, IDO or PD-L1, to target immune suppression [38,73]. The boosting of such T cells could directly modulate immune regulation and alter tumor tolerance. The combination of vaccination with PD-1 pathway blockade would be easily implementable and most likely highly synergistic [74].

Adoptive T cell transfer with TILs is another promising novel approach to target melanoma [75,76]. In this strategy, TILs from a patient's own tumor material are expanded *in vitro* for 4–6 weeks. Importantly, tumor-reactive TILs are reported to express PD-1 [77]. In a mouse model, combination of PD-1 blockade and ACT with TILs showed increased efficacy compared with either treatment alone [35]. Thus, an exciting strategy would be to add PD-1 antibodies to the ongoing clinical adoptive T cell trials.

Other therapies of interest to combine with PD-1 pathway blockade could be conventional chemotherapy, radiation therapy,

targeted agents, and unspecific immunostimulatory agents, as reviewed elsewhere [71,78–80]. However, as was seen when combining nivolumab and ipilimumab [58,60] or ipilimumab and vemurafenib [81], significant toxicities could be the result of combination therapies and trials should be carefully designed and carried out with caution.

Concluding remarks

Both anti-PD-1 and anti-PD-L1 antibodies have shown promising clinical activity, even in cancers not usually considered immunogenic and in heavily pretreated patients. Currently, clinical activity has been consistently demonstrated in metastatic melanoma, NSCLC, bladder urothelial cancer, and RCC. Two anti-PD-1 blocking antibodies, pembrolizumab and nivolumab, have already been FDA approved for the treatment of metastatic melanoma on the basis of phase II and ongoing phase III trials, respectively [43,54]. It is likely that both anti-PD-1 and anti-PD-L1 antibodies will be approved for use against a multitude of cancers within a few years, further broadening the indication of PD-1 pathway blockade and immunotherapy. Although a direct comparison has not been carried out, in general response rates seem to be higher for anti-PD-1-blocking agents compared with anti-PD-L1. Both classes of agent are generally well tolerated, with grade 3 and 4 responses in approximately 15% of patients for anti-PD-1 antibodies and seemingly even lower for anti-PD-L1 antibodies. Toxicity is usually manageable with treatment interruption or corticosteroids.

Again, although not directly compared, it seems logical to suggest that biological differences among different antibodies are responsible for the different response rates observed with different agents even of the same class (e.g. nivolumab/pembrolizumab and pidilizumab). In addition, different schedules of administration have been used, potentially complicating a tentative comparison even more. Interestingly, different dosing regimens have not shown a dose–response relation.

Despite early reports of seemingly comparable efficacy rates and toxicity in melanoma among the most advanced competitors (i.e. nivolumab and pembrolizumab), more data could still change that picture. Also, differences in schedule of administration exist: for example, Q2W and Q3W for nivolumab and pembrolizumab, respectively. This might result in approximately 26 versus 17 drug infusions/year, with potential impacts on costs of clinical care and quality of life.

The mode of action of PD-1 blockade holds promise for additive or synergistic effects with a broad range of immunotherapies, chemotherapies, radiation therapy, and targeted agents. Indeed, the combination of the anti-PD-1 antibody nivolumab with the anti-CTLA-4 antibody ipilimumab resulted in unprecedented response rates and deep, durable responses. However, toxicity was also increased significantly.

Thus, new combination therapies should be designed to target mechanistically different modes of action to obtain even more powerful anticancer effects with minimized toxicity.

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