



Review

Checkpoint blocking antibodies in cancer immunotherapy

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ARTICLE INFO

Article history:

Received 17 September 2013

Revised 12 October 2013

Accepted 14 October 2013

Available online 23 October 2013

Edited by Wilhelm Just

Keywords:

Cytotoxic T-lymphocyte antigen 4

Programmed death-1 receptor

Immunotherapy

Checkpoint blocking antibodies

Malignancy

ABSTRACT

Cancers can be recognized by the immune system, and the immune system may regulate and even eliminate tumors. The development of checkpoint blocking antibodies, such as those directed against cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed death 1 receptor (PD-1), have demonstrated significant recent promise in the treatment of an expanding list of malignancies. While both CTLA-4 and PD-1 function as negative regulators, each plays a non-redundant role in modulating immune responses. CTLA-4 attenuates the early activation of naïve and memory T cells. In contrast, PD-1 is primarily involved in modulating T cell activity in peripheral tissues via interaction with its ligands, PD-L1 and PD-L2. Unfortunately, not all patients respond to these therapies, and evaluation of biomarkers associated with clinical outcomes is ongoing. This review will examine the efficacy, toxicities, and clinical development of checkpoint blocking antibodies, including agents already approved by the US Food and Drug Administration (anti-CTLA-4, ipilimumab) or in development (anti-PD-1, PD-L1). Future studies will likely uncover new promising immunologic checkpoints to target alone or in combination with other immunotherapeutic approaches, chemotherapy, radiotherapy, and small molecules.

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1. Introduction

The field of immune-oncology has evolved over more than 120 years with several key milestones providing increasing evidence for the role of the immune system in eradicating cancer. One of the most significant advances occurred in 1957 when Thomas and Burnett suggested that tumor cells could evoke an immune response and the concept of cancer immune surveillance was introduced [1]. Cancers can be recognized by the immune system, and under certain circumstances, the immune system may control or even eliminate tumors [2,3]. Both innate and adaptive immunity contribute to the recognition and rejection of malignant cells [4–6]. Subsequent evidence that the immune system is involved in tumor control [7] and the identification of key molecules that regulate cellular immune processes [8] have led to the development of novel immunotherapeutic approaches for cancer treatment.

Though a number of immunotherapeutic strategies have been shown to increase the immune system's ability to control cancer, immunomodulatory antibodies that directly enhance the function of T cells have been garnering significant recent attention. These agents are commonly called “checkpoint inhibitors” because they block normally negative regulators of T cell immunity such as

cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed death receptor-1 (PD-1).

The clinical development of checkpoint blocking antibodies such as ipilimumab (Bristol–Myers Squibb, Princeton, NJ), nivolumab (Bristol–Myers Squibb, Princeton, NJ), and MK-3475 (Merck, Whitehouse Junction, NJ) is built upon a foundation of basic research into the mechanisms that regulate T cell activation. In the “two signal” model of T cell activation, antigen-specific T cell activation requires both T cell receptor (TCR) engagement (signal 1) and a co-stimulatory signal B7-CD28 (signal 2) [9–13]. Subsequent studies have supported this two-signal model and added layers of complexity to its framework. It is now understood that a variety of immunomodulatory signals, both costimulatory and coinhibitory, are needed to orchestrate an optimal antigen-specific immune response. Blockade of coinhibitory molecules such as CTLA-4, PD-1, and lymphocyte-activation gene (LAG-3), or enhancement of co-stimulatory molecules, such as glucocorticoid-induced TNF receptor (GITR), OX40, and 4-1BB can amplify T cell responses against tumors (Fig. 1) [3,13].

This review will focus on the clinical development of antibodies that block the immunologic inhibitory molecules CTLA-4 and PD-1, including agents that are already approved by the FDA (ipilimumab) or in various stages of clinical development (anti-PD-1, anti-PD-L1) (Table 1). While both CTLA-4 and PD-1 function as negative regulators, each plays a non-redundant role in modulating immune responses. CTLA-4, through engagement with its

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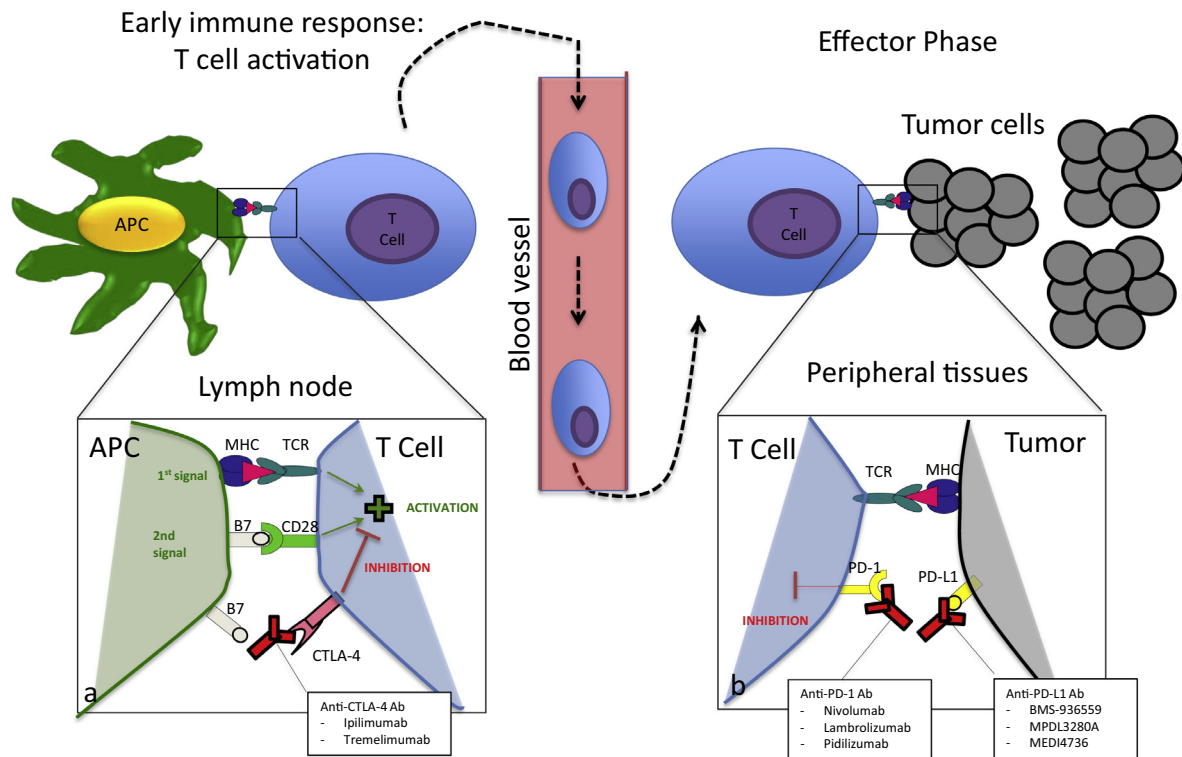


Fig. 1. Targeting immune checkpoints with CTLA-4 and PD-1 blocking antibodies in cancer immunotherapy. (a) The early immune response, “priming” phase, requires 2 signals for T cell activation, and occurs primarily within the lymph nodes. In the first signal, T cells recognize antigens presented by the major histocompatibility complex (MHC) on the surface of cancer cells through their T-cell receptor (TCR). A second signal is needed to complete T-cell activation via the B7 costimulatory molecules B7-1 and B7-2. Cytotoxic T-lymphocyte antigen 4 (CTLA-4) is up-regulated shortly after T-cell activation and through a variety of mechanisms negatively regulates T cells. Anti-CTLA-4 antibodies, such as ipilimumab and tremelimumab, block this inhibitory signal, and thereby enhance antitumor activity. (b) Programmed death 1 (PD-1) inhibitory receptor plays a more prominent role in modulating T cell activity in the peripheral tissues during the effector phase. PD-1 is expressed by T cells after antigen exposure, and its ligation with PD-L1 and PD-L2 results in negative regulation of T cells in the tumor microenvironment. Blockade with antibodies to PD-1 or PD-L1 (e.g., Nivolumab and MK-3475) results in the activation of T cells with specificity for the cancer. These mechanisms are additionally described in a recent review [91].

ligands CD80 and CD84, plays a pivotal role in attenuating the early activation of naïve and memory T cells. In contrast, PD-1 is primarily involved in modulating T cell activity in peripheral tissues via its interaction with PD-L1 and PD-L2. Lessons learned from treating patients with CTLA-4 and PD-1 pathway-blocking antibodies are likely to inform the clinical development of the next generation of antibodies which affect T cell regulation by targeting a diversity of coinhibitory and costimulatory molecules. Table 2 is a summary of the published or presented trials investigating these various checkpoint antibodies in cancer immunotherapy.

2. CTLA-4 blocking antibodies: ipilimumab and tremelimumab

Cytotoxic T-lymphocyte antigen 4 (CTLA-4) is a member of the CD28:B7 immunoglobulin superfamily and is normally expressed at low levels of the surface of naïve effector T-cells and regulatory T cells (Tregs). After stimulation of a naïve T cell through the T cell receptor (TCR), CTLA-4 localizes to the plasma membrane and competes with CD28 for B7, ultimately turning off T cell receptor signaling (Fig. 1) [14]. Antibodies that target CTLA-4 prevent the attenuating function of CTLA-4 and consequently enhance T cell function. CTLA-4 thereby serves as a physiologic “brake” on the activated immune system to maintain normal immune homeostasis [15].

Over 20 years of research have established the foundations for the therapeutic potential of therapies targeting CTLA-4. CTLA-4 was first described in 1987 as a member of the immunoglobulin superfamily [16], and subsequent studies continued to describe

its role in the inhibition of immune responses [14]. Studies in mice suggested that functional CTLA-4 is essential for viability as CTLA-4 (–/–) mice die one month after birth from diffuse lymphoproliferation and autoimmunity [17]. Based upon this improved understanding of the importance of CTLA-4 as an immunologic checkpoint, it was hypothesized that blocking CTLA-4 with monoclonal antibodies may be a strategy to enhance anti-tumor immunity. In 1996, the first preclinical report was published that demonstrated CTLA-4 blockade with monoclonal antibody therapy could result in tumor regressions in mice [18]. It appeared that efficacy of CTLA-4 blockade in preclinical experiments depended upon the particular tumor model. Mice bearing the B16 melanoma model were less sensitive to the effects of CTLA-4 blockade, and therapeutic efficacy was only achieved when CTLA-4 blockade was combined with the granulocyte/macrophage colony-stimulating factor (GM-CSF)-producing vaccine [19]. This preclinical finding may be related to early suggestions of enhanced clinical activity of CTLA-4 therapy in combination with GM-CSF [20].

Two antibodies that block CTLA-4, ipilimumab and tremelimumab, have been evaluated in clinical trials, and ipilimumab has been approved by the United States Food and Drug Administration for the treatment of advanced melanoma. Studies of these agents have shown that both can result in durable control of advanced cancer and, in the case of ipilimumab for patients with advanced melanoma, overall survival can be improved. As these agents prevent normal feedback inhibition of the immune response, tolerance to other host tissues can be lost, leading to adverse events which are often called immune-related adverse events (irAEs).

Table 1
Summary table of checkpoint blocking antibodies in cancer immunotherapy.

Drug	Antibody type	Approved for clinical use	Notable side effects	General stage of clinical development
<i>Anti-CTLA-4 antibodies</i>				
Ipilimumab	Fully human IgG1 isotype	Y	Diarrhea, colitis, fatigue, transaminitis, hypophysitis	FDA approved for melanoma, Phase II and Phase III trial ongoing for other multiple cancers
Tremelimumab	Fully human IgG2 isotype	N	Diarrhea, fatigue, nausea, vomiting, anorexia, rash	Phase II trials in mesothelioma and earlier phase studies for other multiple cancers
<i>Anti-PD-1 antibodies</i>				
Nivolumab	Fully human IgG4	N	Pneumonitis, lymphopenia, fatigue, diarrhea, hepatitis, renal insufficiency	Ongoing phase III trials in advanced melanoma and late stage trials in other malignancies, including renal cell carcinoma
MK-3475	Humanized IgG4-kappa isotype	N	Pneumonitis, fatigue, thyroid problems	Phase II/III trials in advanced melanoma and lung cancer
Pidilizumab	Humanized IgG1k isotype	N	Pneumonitis, fatigue, diarrhea	Phase II in hematologic malignancy, phase I/II in solid tumors
<i>Anti-PD-L1 antibodies</i>				
BMS-936559	Fully human IgG4 isotype	N	Fatigue, hyperglycemia, infusion reaction, endocrinopathies, adrenal insufficiency and myasthenia gravis	Phase I in multiple cancers
MEDI4736	Fully human IgG1-kappa	N	Still being evaluated	Phase I in multiple cancers
MPDL33280A	Fully human IgG4 isotype	N	Hyperglycemia, hypophysitis, pericardial effusion, fatigue	Phase I in multiple cancers

2.1. Ipilimumab

In two large phase III clinical trials of patients with advanced melanoma, ipilimumab was shown to significantly prolong overall survival [21,22]. The first phase III trial evaluated ipilimumab in previously treated patients with melanoma and the second evaluated ipilimumab for previously untreated patients.

2.1.1. Previously treated patients

676 Patients with unresectable stage III or stage IV melanoma were randomized to receive ipilimumab alone, ipilimumab plus a glycoprotein 100 (gp100) vaccine, or gp100 vaccine alone [21]. All patients had previously received systemic treatment for advanced melanoma. Ipilimumab (3 mg/kg) and/or gp100 vaccination were

given every three weeks for four doses. Overall survival was significantly increased in patients receiving ipilimumab (ipilimumab plus gp100 versus gp100, median overall survival 10.0 versus 6.4 months, HR for death 0.68; ipilimumab alone versus gp100 alone 10.1 versus 6.4 months, HR 0.66, $P < 0.001$).

2.1.2. Previously untreated patients

In a subsequent second phase III trial, 502 patients with metastatic melanoma previously untreated were randomly assigned to dacarbazine with ipilimumab at a dose of 10 mg/kg or dacarbazine with placebo [22]. All patients received dacarbazine (850 mg/m² intravenously) every three weeks for eight cycles in the absence of disease progression or significant toxicity. Overall survival was significantly increased in patients assigned to dacarbazine with

Table 2
Published or presented trials investigating various checkpoint modulators in solid tumors.

Study drug	Disease	n	Ph	Results	Refs.
<i>Anti-CTLA-4 antibodies</i>					
Ipilimumab	Melanoma	676	III	Ipi + gp100: 6% ORR; 14% S.D.; OS 10.0 vs 6.0 mo (gp100 arm)	[21]
	Melanoma	502	III	36.2% at 1 year 17.9% at 2 years 12.2% at 3 years	[22]
Tremelimumab	Prostate	14	I	14% pts with $\geq 50\%$ PSA decline	[80]
	RCC	61	II	10% ORR	[78]
Tremelimumab	CRC	47	II	ORR 2% (1 PR)	[32]
	Gastric/ GEJ	18	II	1 PR after 8 cycles, 4/18 S.D.	[31]
	Melanoma	655	III	10.7% ORR vs 9.8% (chemo arm) (ns); OS 12.6 vs 10.7 mo (ns)	[29]
	NSCLC	87	II	4.8% ORR vs 0% (supportive care); 21% 3 mo PFS vs 14% (ns)	[31]
<i>Anti-PD-1 antibodies</i>					
Nivolumab	Melanoma	107	I	31% ORR; median OS 16.8 mo; 1-year OS 61%	[59,92]
	NSCLC	127	I	ORR 16%; median OS 9.6 mo	[62,93]
	RCC	34	I	ORR 29%; median OS >22 mo, 1 year OS 70%	[92,94]
MK-3475	Melanoma	135	I	37% ORR; median PFS >7 mo; 77% had tumor shrinkage during study	[60]
Pidilizumab	Hematologic malignancies	17	I	33% ORR	[61]
<i>Anti-PD-L1 antibodies</i>					
BMS-936559	Melanoma	52	I	17% ORR; 27% S.D.	[62]
	NSCLC	49	I	10% ORR; 10% S.D.	
	Ovarian	17	I	6% ORR; 18% S.D.	
	RCC	17	I	12% ORR; 41% S.D.	
MPDL3280A	Melanoma	45	I	26% ORR	[63]

ipilimumab compared to dacarbazine with placebo (median 11.2 versus 9.1 months). Survival rates at one, two, and three years consistently favored treatment with ipilimumab (47% versus 36%, 29% versus 18%, and 21% versus 12%, respectively).

2.2. Duration of response

A unique effect of therapeutic CTLA-4 blockade is the possibility for remarkably durable tumor responses. Although only a minority of patients achieves complete tumor regression, such responses appear to be of prolonged duration in most cases. As an example, 177 patients were treated with ipilimumab between 2002 and 2005 in three early trials conducted by the National Institutes of Health [23]. Fifteen patients (nearly 9%) achieved a complete response (CR). All of these complete responses, except for one were ongoing at the time of publication, with the longest lasting 99+ months.

2.3. Dosing and schedule

Ipilimumab has been studied at different doses and schedules of administration. The approved dose of ipilimumab is 3 mg/kg by intravenous infusion given every three weeks for four doses which is based upon the results of the phase III trial in previously treated patients [21]. Nonetheless, whether this is the optimal dose of ipilimumab is still being studied.

Three dose levels of ipilimumab were compared in a double-blind phase II trial: 0.3, 3.0, and 10 mg/kg [24]. Patients were treated every three weeks for four doses, with provision for maintenance treatment every 12 weeks in patients with an objective response or stable disease. The objective response rate (complete plus partial response) increased progressively with dose (0%, 4.2%, and 11.1% in the 0.3, 3, and 10 mg/kg groups, $P = 0.002$ for trend). However, immune-related adverse events were also higher in the 10 mg/kg group, with 27% versus 10% of patients requiring treatment discontinuation in the 10 and 3 mg/kg arms, respectively.

In order to definitively determine the optimal ipilimumab dose, patients with advanced melanoma are being treated with 3 and 10 mg/kg of ipilimumab in an ongoing phase III trial which has completed accrual (NCT01515189). The primary endpoint of the trial is overall survival; results are pending. Additionally, it is unknown whether maintenance ipilimumab administered every 12 weeks offers any benefits beyond the initial ipilimumab induction course of four doses. Patients who experience clinical benefit from initial ipilimumab induction but who ultimately progress may achieve additional benefit from a repeat course of ipilimumab [25]. This is a treatment strategy that is widely used in clinical management of patients with advanced melanoma.

2.4. Toxicities of ipilimumab: immune-related adverse events (irAEs) and management

A wide range of immune-related adverse events (irAEs) have been observed following treatment with ipilimumab. The most common side effects include diarrhea, enterocolitis, hepatitis, dermatitis, and endocrinopathies. In the first phase III trial that demonstrated an increase in overall survival, irAEs occurred in approximately 60% of patients treated with ipilimumab; these typically did not occur until several weeks into therapy [21]. Overall, severe or life-threatening (grade 3 or 4) toxicity was seen in 10–15% of ipilimumab-treated patients, compared to 3% in those receiving only gp100. The phase III trial used a dose of 3 mg/kg of ipilimumab every three weeks. A somewhat higher incidence of side effects was observed with an ipilimumab dose of 10 mg/kg every three weeks in the randomized phase II trial that assessed

the effects of dose on activity and toxicity [24]. Treatment requires interruption of ipilimumab and the use of corticosteroids, mycophenolate mofetil, or tumor necrosis factor (TNF)-alpha blockade [26] and is based upon the severity of the observed toxicity. Algorithms have been developed to aid in the management of these side effects, but it is generally believed that prompt recognition and early use of immunosuppressant therapy leads to resolution without long-term sequelae.

2.5. Additional clinical consideration: opportunistic infections in patients treated for irAEs

In refractory or severe irAEs, patients require immunosuppression with ongoing steroids or TNF-alpha antagonists, which places this emerging patient population at higher risk for opportunistic infections. In our recent clinical experience, we have observed cases of rare or opportunistic infections in patients treated with novel immunotherapies who received immunosuppression for immune-related toxicities, including pulmonary aspergillosis, zygomycosis, *Pneumocystis carinii* pneumonia, Fournier's gangrene, and cytomegalovirus viremia. Optimal management requires a multidisciplinary approach with a high index of suspicion to improve clinical outcomes. Whether irAEs could be managed equally effectively with alternative immunosuppression or whether prophylactic antiviral and antibacterial therapies are beneficial in this unique population needs to be studied prospectively.

2.6. Ipilimumab in the adjuvant setting

Ipilimumab has been studied extensively in advanced melanoma, but the role of ipilimumab in the adjuvant setting after complete surgical resection of high-risk melanoma, is currently unclear. The European Organization for Research and Treatment of Cancer (EORTC) is conducting a phase III trial in patients with high-risk stage III disease (EORTC 18071, NCT00636168). In this trial, following complete resection of high risk melanoma, patients are randomly assigned to ipilimumab (dose 10 mg/kg every three weeks for four cycles then every 12 weeks for a total of three years treatment) or to placebo to determine whether ipilimumab prevents disease recurrence. The trial has completed accrual and results are pending.

In addition, the Eastern Cooperative Oncology Group (ECOG) is also evaluating ipilimumab in the adjuvant setting. Patients with resected stage IIIB, IIIC, or IV disease are able to receive 3 or 10 mg/kg of ipilimumab versus standard high dose interferon-alpha. This study is currently recruiting patients (NCT01274338).

2.7. Tremelimumab

Tremelimumab (formerly Pfizer, New York, NY and currently licensed to MedImmune, Gaithersburg, MD) is another monoclonal antibody directed against CTLA-4. Similarly to ipilimumab, tremelimumab showed activity with durable responses in phase I and II clinical studies [27,28]. Based upon these promising early results, a phase III trial was conducted in which previously untreated patients were randomly assigned to either tremelimumab or chemotherapy [29]. Although there was a prolongation in response duration among those treated with tremelimumab, the difference in overall survival was not statistically significant. It is possible that the lack of a statistically significant overall survival difference was due to subsequent ipilimumab use in patients randomized to initial chemotherapy in this trial. Further, this trial was the only phase III trial comparing a CTLA-4 blocking antibody alone to chemotherapy for patients with untreated advanced melanoma. Tremelimumab has also been studied in phase II trials of refractory patients with metastatic colorectal, gastric and esophageal cancers,

and NSCLC [30–32] and additional investigations of tremelimumab in combination with other anticancer therapies is expected.

3. Programmed death-1 antibodies: nivolumab, MK-3475, and pidilizumab

Programmed death-1 (PD-1) is another key immune checkpoint receptor expressed by activated T cells and is a promising therapeutic target [33]. Although CTLA-4 and PD-1 both function as negative regulators, they play unique non-redundant roles in the regulatory pathway of activated T cells. PD-1 appears to play a more prominent role in modulating T cell activity in peripheral tissues via interaction with its ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC). The expression pattern of PD-L1, expressed broadly on hematopoietic and non-hematopoietic tissues, and PD-L2, expressed on dendritic cells, macrophages, mast cells, and B cells, supports this notion.

Like CTLA-4, PD-1 is a type 1 transmembrane protein of the Ig superfamily. PD-1 was identified originally as a gene induced by a T cell hybridoma undergoing apoptotic cell death [34]. When PD-1 binds to its ligand, which in the case of PD-L1, is expressed on tumor cells, T cell activity is attenuated which prevents these T cells from rejecting the tumor. Since monoclonal antibodies can block the interaction between PD-1 and PD-L1, they have been evaluated as a strategy to augment the antitumor immune response.

The expression of PD-1 by tumor-infiltrating lymphocytes [35] along with the expression of PD-L1 by numerous tumor types [36] suggest that the pathway may be involved in immune evasion by human cancers (Fig. 1). In particular, PD-L1 and to a lesser extent, PD-L2 are expressed on many human tumors, including urothelial, ovarian, breast, cervical, colon, pancreatic, gastric cancers, as well as melanoma glioblastoma and NSCLC [37–46]. In addition, these molecules have also been detected on hematologic malignancies, including Hodgkin lymphoma, primary mediastinal B cell lymphoma, angioimmunoblastic T cell lymphoma, multiple myeloma, acute myeloid leukemia, chronic lymphocytic leukemia, and adult T cell leukemia/lymphoma [46–50]. Expression of PD-L1 has been correlated with prognosis in many of these malignancies, supporting the hypothesis that PD-L1 expression may be a mechanism for tumor immune evasion [42,51–53]. Together, these findings suggest that interrupting the PD-1:PD-L1/PD-L2 interaction could be an effective anticancer therapy by blunting inhibition of immune responses in the tumor microenvironment [54,55].

Preclinical murine models have also demonstrated the therapeutic potential of blocking the PD-1 axis [56]. Mice with a genetic deficiency of PD-1 manifest enhanced immunity with phenotypes characterized by autoimmune cardiomyopathy and a lupus-like syndrome [57,58]. The less severe phenotype of PD-1 deficient compared to CTLA-4 deficient mice parallels clinical results from trials where preliminarily, PD-1 blockade appears to result in few significant clinical toxicities.

Based upon the preclinical rationale, several antibodies that inhibit the PD-1 pathway by blocking PD-1 or PD-L1 have been developed for clinical use. This area is evolving rapidly, and though we mention a number of agents in clinical development, we focus on the agents for which clinical data are the most mature.

3.1. Nivolumab

Nivolumab (previously, BMS-936558) is a fully human monoclonal antibody that targets the PD-1 protein. This monoclonal antibody was evaluated in a phase I/II study in 296 patients with a variety of heavily pretreated malignancies including non-small cell lung cancer (NSCLC), prostate cancer, renal cell carcinoma (RCC), and colorectal cancer (CRC), including 107 patients with advanced melanoma [33].

At the 2013 American Society of Clinical Oncology (ASCO) meeting, updated efficacy data from this study were presented with one year of additional follow-up for the cohort of 107 patients with advanced melanoma [59]. The overall objective response rate was 31% for the entire melanoma cohort and an additional 7% had stable disease; there was no evidence for a dose response relationship, although the dose selected for further clinical development as monotherapy appears to be 3 mg/kg. The median duration of response was 24 months. The median overall survival in this heavily pretreated population was 17 months, and 43% of patients were alive at two years.

In comparison to CTLA-4 blockade, rates of previously defined irAEs appeared to be less frequent. Nonetheless, grade 3 or 4 treatment related adverse events were observed in 32 of 296 patients (14%) enrolled in the study and appear to be somewhat distinct from irAEs associated with CTLA-4 blockade. In particular, several patients died with inflammatory pneumonitis, which has been much less frequently described with CTLA-4 blockade. The exact mechanism of pneumonitis induced by PD-1 blockade is unclear.

Three phase III trials are being conducted to evaluate the role of nivolumab in patients with advanced melanoma. In NCT01721746, patients who have progressed following ipilimumab and a RAF inhibitor (if BRAF mutant) are being randomly assigned to receive nivolumab or chemotherapy with either dacarbazine or carboplatin plus paclitaxel. In another trial, previously untreated patients will be randomly assigned to nivolumab or dacarbazine (NCT01721772). Finally, in a third ongoing phase III trial, previously untreated patients are being randomized to receive either ipilimumab, nivolumab, or the ipilimumab and nivolumab (NCT01844505). The high durable response rate of this agent has demonstrated early promise, but its effects on overall survival in comparison to standard approaches will be known when these ongoing phase III trials are completed.

3.2. MK-3475

MK-3475 is humanized IgG4 anti-PD-1 monoclonal antibody that has been extensively evaluated in both ipilimumab naïve and previously treated patients. In a prospective non-randomized large phase I study, 135 patients with advanced melanoma were treated with MK-3475 on one of three dose schedules (10 mg/kg every two weeks, 10 mg/kg every three weeks, or 2 mg/kg every three weeks) [60]. No dose-limiting toxicity was identified within this range, and promising clinical activity was noted.

The overall response rate at the initial reported analysis of the 135 patients was 38% using the standard response evaluation criteria in solid tumors (RECIST) version 1.1 in the 117 patients with measurable disease. The 10 mg/kg every two-week schedule appeared most active with a 50% response rate, but this did not differ significantly from the other dosing regimens tested. Over 80% of responses were ongoing at the time of analysis, and the median progression-free survival was greater than 7 months. There was no difference in response rate among those patients who had or had not received prior ipilimumab. These data suggest that the PD-1 blocking antibodies have activity in patients with advanced melanoma who are refractory to treatment with ipilimumab.

Two randomized trials for patients with advanced melanoma are accruing patients. One compares MK-3475 to chemotherapy (NCT01704287) in patients who have previously received ipilimumab, and the other compares MK-3475 to ipilimumab (NCT01866319) in treatment naïve patients.

3.3. Pidilizumab

Pidilizumab (previously CT-011, CureTech, Yavne Israel) is a humanized IgG1 antibody initially tested in a phase I dose-escalation, single-dose (0.2–6 mg/kg) study of 17 patients with

hematological malignancies [61]. The maximum tolerated dose (MTD) was not achieved, and the doses tested were well-tolerated. Patient who achieved a response or a sustained stable disease totaled 33% of the study population, and one patient with follicular lymphoma had a complete response. Based on these promising results, additional clinical investigation of pidilizumab is being conducted in a number of malignancies.

4. Programmed death ligand-1 antibodies

In addition to antibodies targeting PD-1, clinical activity has been observed with several different anti-PD1-L1 monoclonal antibodies.

4.1. BMS-936559

BMS-936559 is a fully human, PD-L1 specific IgG4 mAb. It binds to PD1-L1, thus preventing its interaction with PD-1. BMS-936559 was tested in a dose escalation phase I/II study in pretreated patients with a variety of malignancies including NSCLC, melanoma, CRC, RCC, ovarian cancer, pancreatic cancer, and breast cancer [62]. The trial included 207 patients. Overall, potentially irAEs were seen in 39% of patients. There were nine objective responses among the 52 evaluable melanoma patients (17%). Five of these had an objective response lasting at least one year, and six additional patients had stable disease that was maintained for at least 24 weeks. As with nivolumab, irAEs appeared less common with PD-L1 blockade than with CTLA-4 blockade. In contrast to nivolumab, no cases of pneumonitis were reported with BMS-936559. This antibody was the first published experience demonstrating the clinical efficacy of PD-L1 blockade.

4.2. MPDL3280A

MPDL3280A (Genentech, San Francisco, CA) is another monoclonal antibody that binds to PD1-L1. Initial results of a phase I dose escalation study were presented at the 2013 ASCO meeting [63]. The dose escalation component of that study included 45 patients with advanced melanoma. An overall response rate of 29% was reported, and 43% of patients were progression-free at 24 weeks. Additional clinical experience is being gained through a dose expansion cohort in patients with advanced melanoma and other malignancies. The Fc domain of this antibody has been engineered to prevent antibody dependent cytotoxicity, but whether this unique feature of this specific antibody is relevant to its clinical activity requires ongoing clinical investigation.

4.3. MEDI3740

MEDI4736 (MedImmune, Gaithersburg, MD) is another PD-L1 antibody that is undergoing phase I clinical evaluation in a variety of malignancies. As trials of this agent have only started more recently, results of its safety and efficacy are expected.

5. Understanding efficacy of checkpoint blockade

5.1. Atypical patterns of radiographic response

Standard criteria for evaluating responses to anticancer agents have been developed to promote objectivity and to facilitate comparisons across trials. One commonly used response criteria is the RECIST criteria, which was developed based upon patterns of responses to standard chemotherapeutic agents. According to RECIST and the closely related modified World Health Organization (mWHO) criteria, an increase in tumor size and/or development

of new lesions are defined as progressive disease, typically implicating that a change in therapy is warranted.

The patterns of radiographic response to treatment with the checkpoint blocking antibodies described in this review, however, may differ from patterns of response seen with standard chemotherapeutic agents in several important respects. First, when patients are treated with these immunomodulatory antibodies, they may have an apparent transient worsening of disease, manifested either by progression of known lesions or appearance of new lesions, before ultimate disease stabilization or tumor regression. Secondly, responses can take an appreciably long time to become apparent. The average time to achieve a complete response from ipilimumab in one long-term study was 30 months [23]. Third, some patients who do not meet criteria for objective response can have prolonged periods of stable disease, and this is believed to contribute to the beneficial effects of ipilimumab on overall survival.

Based on these observations, alternative response criteria, termed the immune-related response criteria (irRC) were proposed to capture these atypical responses. As an initial investigation of this radiographic assessment approach, 487 patients treated on three multicenter phase II clinical trials of ipilimumab were analyzed retrospectively [64]. In this analysis, it appeared that the irRC better distinguished patients who had long-term survival than the traditionally used mWHO response criteria. The concept of the irRC is continuing to be evaluated and refined [65], but prospective evaluation in the context of a randomized controlled trial will be necessary. Anecdotal immune-related responses have also been described for patients treated with PD-1 and PD-L1 antibodies.

5.2. Biomarkers associated with clinical outcome

Studies have shown improved overall survival for patients with advanced melanoma treated with ipilimumab and durable responses with the other agents, but unfortunately only a subset of patients benefit from treatment. Identifying patients most likely to respond and/or develop side effects will be essential to optimizing use of these antibodies. Since these antibodies target the immune system, biomarker investigations have largely focused on investigating various immunologic parameters through monitoring assays. The majority of putative biomarkers has been identified in small, retrospective analyses, and prospective validation is an area of active research.

5.3. Absolute lymphocyte count

Several studies suggest that the absolute lymphocyte count (ALC) may be a pharmacodynamic marker for ipilimumab. In a dose-finding phase II study of ipilimumab, patients receiving 10 mg/kg of ipilimumab seemed to have a greater rise in ALC than patients receiving 3 mg/kg [24]. Small, single-center retrospective analyses have also shown associations between ALC values at various timepoints and clinical outcomes following ipilimumab therapy [66,67]. The association between ALC and clinical outcomes was also found in patients with uveal melanoma treated with ipilimumab [68]. ALC increases while on ipilimumab therapy are an intriguing possible biomarker because the ALC is readily available from clinical laboratories. Further, since CTLA-4 blockade is associated with increased lymphocyte proliferation, an increase in ALC is consistent with one of the mechanisms of action of CTLA-4 blockade.

5.4. Antigen-specific immune responses

Antigen-specific immune responses during CTLA-4 blockade have been evaluated for a number of cancer-related antigens, such

as NY-ESO-1, MAGE, Melan-A, gp-100, tyrosinase, prostate-specific antigen, and prostate acid phosphatase. Most extensively, immune responses to the cancer-testis antigen NY-ESO-1 have been characterized to correlate with clinical activity. In a retrospective study of 144 ipilimumab-treated patients with melanoma, patients with detectable titers of antibodies against NY-ESO-1 were more likely to have achieved clinical activity with ipilimumab than patients with no detectable NY-ESO-1 antibody titers [69]. Patients with additional CD8+ T cell reactivity against NY-ESO-1 were shown to be even more likely to achieve clinical activity. Whether an immune response to NY-ESO-1 is just a surrogate of a functional anti-tumor immune system or a direct mediator of the antitumor activity of ipilimumab therapy remains unknown. Likely many additional mediators are involved. Whether NY-ESO-1 vaccination in combination with ipilimumab is a safe and efficacious therapeutic strategy for patients whose tumors express NY-ESO-1 is being evaluated (NCT01810016).

5.5. Inducible co-stimulator

Inducible co-stimulator (ICOS) is a costimulatory molecule expressed on the surface of T cells and is thought to play an especially important role in T cell survival, proliferation, and generation of memory [70]. In bladder cancer patients treated with neoadjuvant ipilimumab, ICOS expression was correlated to clinical activity, as it was upregulated on tumor-infiltrating T cells. In a small retrospective analysis of melanoma patients treated with ipilimumab, increased frequency of CD4+ ICOS high T cells, sustained over a period of 12 weeks correlated positively with increased overall survival [71].

5.6. Future directions of biomarker analyses

Ongoing investigations into biomarkers associated with clinical benefit to immune checkpoint inhibitors may ultimately enable clinicians to select the most appropriate immunotherapeutic strategy for an individual patient. At present, however, no biomarker associated with clinical outcomes described herein has yet been developed to a sufficient degree to enable physicians to select particular patients for one specific immunotherapeutic strategy. Prospective investigation of several of these biomarkers is ongoing. In particular, analysis of PD-L1 as a pre-treatment biomarker for PD-1 outcomes is being evaluated in ongoing phase II and III studies (NCT01927419 and NCT01844505) to see whether it correlates with clinical outcomes to anti-PD-1 antibody therapy and other immunotherapy strategies. It is quite possible that immunologic biomarkers will not be used for selecting patients at baseline most likely to respond to immunotherapy but instead may serve as ongoing measures of the expected pharmacodynamic effects of therapy which may be used to inform ongoing dosing.

6. Checkpoint blockade in other tumor types

While evaluations of immunotherapy have largely focused on melanoma (because of durable responses noted historically with immunotherapy, such as interleukin-2) [72], there is now growing evidence that this approach is also applicable to other malignancies, including prostate, lung, pancreatic, hepatocellular carcinoma and hematologic malignancies [33,73–78]. In a phase I study evaluating BMS-936559, evidence of clinical activity was seen in patients with NSCLC, RCC, and melanoma, and one patient with ovarian cancer also had a response [62]. Patients with prostate cancer have also been reported to have objective radiographic and biochemical responses with ipilimumab [79,80]. Future studies are ongoing and necessary to elucidate the diversity of responses in

the spectrum of tumor types, and evaluate whether checkpoint blocking antibodies have additional clinical benefits.

7. Future directions: combination therapies

7.1. Combined anti-CTLA plus PD-1 blockade

The combined administration of anti-CTLA-4 immunotherapy with ipilimumab plus anti-PD-1 immunotherapy with nivolumab was recently described to have an acceptable safety profile and a preliminarily high response rate in the small group of patients evaluated [81]. Out of all patients treated with both ipilimumab and nivolumab, 40% had an objective response. Though grade 3 or 4 irAEs were described in 53% of patients, many were asymptomatic laboratory abnormalities of unclear significance and most resolved without significant sequelae.

Additional clinical experience and longer follow-up will be required to determine whether combination immune checkpoint inhibition offers significant benefits over sequential use of each checkpoint antibody alone. A phase III trial is studying the combination of nivolumab plus ipilimumab compared with each of these checkpoint inhibitors as a single agent (NCT01844505). Sequential therapy with each agent used individually is also being investigated in a phase II trial (NCT01783938). Additional investigation of this combination approach is expected in multiple additional malignancies.

7.2. Ipilimumab plus granulocyte–macrophage colony-stimulating factor

The addition of granulocyte–macrophage colony-stimulating factor (GM-CSF) to ipilimumab was studied in a phase II trial conducted by Eastern Cooperative Oncology Group (ECOG). In this trial, 245 patients with advanced melanoma were randomly assigned to ipilimumab plus GM-CSF or ipilimumab alone [20]. Ipilimumab was given at a dose of 10 mg/kg every three weeks for four cycles, followed by maintenance every 12 weeks. GM-CSF (250 micrograms/day subcutaneously) was given on days 1–14 of each 21 day cycle. At a median follow-up of 13 months, there was no difference in the objective response rate with or without GM-CSF (15.5% and 14.8%, respectively) nor was there a significant difference in progression-free survival (34.0% versus 29.6% at 6 months, hazard ratio [HR] 0.92, 95% CI 0.69–1.23). However, overall survival was significantly improved by the addition of GM-CSF (median 17.5 versus 12.7 months, one-year survival rates 69 versus 53%, HR 0.64, $P = 0.014$). The addition of GM-CSF also resulted in a significant reduction in the incidence of high grade adverse events, particularly related to pulmonary and gastrointestinal toxicity. It is important to consider that this trial used a higher dose of ipilimumab (10 mg/kg) than is currently approved for commercial use (3 mg/kg) and that maintenance therapy was also included. The clinical implications of these results will require further study and confirmation but is consistent with preclinical evidence showing efficacy of combining CTLA-4 blockade with GM-CSF [19].

7.3. Ipilimumab plus radiation therapy

The abscopal effect describes the phenomenon of tumor regression at sites distant from the primary site of radiotherapy. This effect could be caused by radiation-induced antigen and cytokine release, which subsequently potentiates a systemic immune response against the tumor. Case studies have provided anecdotal evidence of the abscopal effect, along with immunologic correlates [82,83]. In-depth immune monitoring of one patient treated with

radiotherapy and ipilimumab revealed a diversification of antibody responses to various antigens, as well as a decrease in an immunosuppressive cellular population during an abscopal effect [82].

The combination of radiotherapy plus immunotherapy is currently being prospectively evaluated (NCT01703507, NCT01497808, NCT01565837, NCT01689974). Combinations with alternative modalities of local control – for example with cryoablation or electrochemotherapy – are also being investigated (NCT01502592, NCT01642290) [84].

7.4. Ipilimumab plus small molecule, targeted therapy

Significant preclinical data suggest that small molecule therapies directed against oncogenic signaling pathways such as those that interact with the mitogen activated protein kinase pathway in melanoma (BRAF mutant melanoma) have important immunologic effects [85–89]. Investigating whether these targeted therapies enhance the effects of immunotherapy, therefore, has been an area of great interest. Unfortunately, a phase I trial combining the BRAF inhibitor, vemurafenib (Roche, Basel, Switzerland) with ipilimumab was closed early due to hepatotoxicity [90]. Careful consideration into the safest and most thoughtful way of further clinically evaluating the combination of small molecule cancer therapies with immunotherapy will be necessary. Ongoing trials will continue to shed light on this therapeutic strategy (NCT01767454).

8. Conclusions

Immunologic checkpoints are an essential component of the immune system, and manipulation of the effects of these natural checkpoints with therapeutic antibodies may control or even eliminate tumors. Antibodies that block inhibitory pathways, such as those directed against CTLA-4 and PD-1 have been developed for clinical use. These checkpoint-blocking antibodies are revitalizing interest in solid tumor immunotherapy and have resulted in promising clinical outcomes, exemplified by the FDA approval of ipilimumab in 2011. Lessons learned from studies of CTLA-4 and PD-1 blockade provide a foundation for the further development of checkpoint blocking antibodies. Continued research into the understanding of biomarkers associated with response and the unique adverse effect profiles of these agents will ultimately lead to improved patient care. While evaluations of immunotherapy have largely focused on melanoma, there is now growing evidence that this approach is also applicable to other malignancies. Future studies should likely uncover new promising immunologic checkpoints, which can be targeted alone or in combination with other immunotherapeutic approaches, chemotherapy, radiotherapy, and small molecules that target oncogenic pathways.

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