Immune Modulation in Cancer with Antibodies

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Abstract

Ipilimumab is the prototypical immunomodulatory antibody, approved by the FDA in 2011 for advanced melanoma on the basis of survival benefit. Since that time, we have made significant strides in optimizing this therapy: we have characterized the spectrum of immune-related adverse events and learned how to mitigate them with treatment algorithms, discovered potential biomarkers of activity, and identified the potential synergy between checkpoint modulation and other therapeutic modalities. Recent phase I trials have established the efficacy and safety of next-generation checkpoint agents, including PD-1 and PD-L1 inhibitors, across multiple tumor types. Much work lies ahead in developing these next-generation checkpoint agents, testing them in combination, and determining how to integrate them into the treatment paradigms of various tumor types.

INTRODUCTION

Cytotoxic T lymphocyte antigen 4 (CTLA-4): coinhibitory molecule expressed acutely on activated T cells; competes with costimulator CD28 in binding to B7 ligand

Programmed cell death protein 1 (PD-1): coinhibitory molecule expressed on chronically stimulated T cells; regulates T cell function when bound to PD-L1 ligand

Programmed cell death 1 ligand 1 (PD-L1): natural ligand to PD-1, expressed in many tumor types; PD-1/PD-L1 interactions inhibit T cell–mediated tumor clearance

A novel strategy of immunotherapy called checkpoint inhibition is poised to dramatically reshape the treatment of a broad spectrum of malignancies. Checkpoint inhibitors function by modulating the immune system's endogenous mechanisms of T cell regulation. Ipilimumab (Yervoy™, Bristol-Meyers Squibb, New York, NY)—the first such checkpoint inhibitor to be approved by the US Food and Drug Administration (FDA)—has become standard treatment for metastatic melanoma (1, 2). Ipilimumab binds and blocks inhibitory signaling mediated by the T cell surface coinhibitory molecule cytotoxic T lymphocyte antigen 4 (CTLA-4). Because the mechanism of action is not specific to one tumor type, and because a wealth of preclinical data supports the role of tumor immune surveillance across multiple malignancies (3, 4), ipilimumab is being investigated as a treatment for patients with prostate, lung, renal, and breast cancer, among other tumor types.

Second-generation checkpoint inhibitors capitalize on a similar concept of blocking T cell regulatory pathways. Both programmed cell death protein 1 (PD-1) and programmed cell death 1 ligand 1 (PD-L1) inhibitors have shown clinical activity and safety in phase I trials (5–7). This review describes the existing checkpoint modulatory agents, highlights key lessons learned from our collective clinical experience with ipilimumab, and comments on future avenues of investigation utilizing combination therapy with this class of agents.

THE SPECTRUM OF CHECKPOINT MODULATORS

Targeting CTLA-4: Ipilimumab and Tremelimumab

CTLA-4 is a homolog of the coactivation receptor CD28 that, when bound to B7 ligands (CD80 and CD86), inhibits T cell function by both intrinsic and extrinsic mechanisms (8). CTLA-4 induces inhibitory downstream T cell receptor signaling but also leads to upregulation of CTLA-4 expression and competitive inhibition of CD28-mediated coactivation. CTLA-4 is also highly expressed on $CD25^{+}FOXP3^{+}$ T regulatory cells (T regs) and is instrumental in T reg function. CTLA-4 blockade has been shown to promote T cell activation, as well as depletion of intratumoral T regs (9). Ipilimumab (IgG1) and tremelimumab (IgG2) are the two human anti-CTLA-4 antibodies that have undergone clinical evaluation.

In 2010, a landmark phase III trial for previously treated patients evaluated ipilimumab 3 mg/kg with or without gp100 peptide vaccine versus gp100 peptide vaccine alone. Given the improvement in overall survival (OS) in patients who received ipilimumab compared to the gp100 vaccine, this trial led to the approval of ipilimumab for patients with unresectable melanoma (1). Median OS in the ipilimumab and ipilimumab + gp100 cohorts was 10.1 and 10.0 months, respectively, compared to 6.4 months for the gp100 control arm [hazard ratio (HR) 0.68, $p < 0.001$] (**Table 1**). The subsequent first-line trial comparing dacarbazine +/− ipilimumab 10 mg/kg reported improved OS with the combination but also increased liver toxicity, presumably due to enhancement of known single-agent hepatotoxicity for both drugs (**Table 2**) (2).

The optimal dose and scheduling of ipilimumab are still unknown. A phase II study compared doses of 0.3 mg/kg to 10 mg/kg and revealed a higher best overall response rate (BORR) in the 10-mg/kg arm [11.1% versus 4.2% (3 mg/kg) versus 0% (0.3 mg/kg), $p = 0.0015$] (10). However, immune-related adverse events and treatment discontinuation rates were also higher in the 10-mg/kg group. A phase III randomized trial comparing the two doses (10 mg/kg versus 3 mg/kg) is awaiting interim analysis with an OS endpoint (NCT01515189). Similarly, the relative benefit of reinduction versus maintenance therapy is uncertain. In the ipilimumab + gp100 trial, responding patients who subsequently progressed were eligible for reinduction ipilimumab. Of these patients, 19% (6/31) achieved objective response (OR), in addition

Table 1 Published trials investigating checkpoint modulators in solid tumors

Study drug	Cancer	N	Phase	Results	Treatment-related adverse events (≥GIII)	Reference
BMS-936559 (anti-PD-L1)	melanoma	52	I	17% ORR; 27% SD	9%: 1% fatigue; 1% vomiting; 1% hyperglycemia; 1% infusion reaction; 1% sarcoid; 1% adrenal insufficiency; 1% endophthalmitis; 1% myasthenia gravis; 1% lymphopenia	6
	NSCLC	49	I	10% ORR; 10% SD		
	ovarian	17	I	6% ORR; 18% SD		
	RCC	17	I	12% ORR; 41% SD		
CP-870,893 (anti-CD40)	solid tumor	29	I	27% ORR in melanoma (4/15); 24% SD in melanoma	38% lymphopenia; 7% ↑LFTs; 3% venous thromboembolism; 3% headache	87
Ipilimumab (anti-CTLA-4)	melanoma	676	III	Ipi + gp100: 6% ORR; 14% SD; OS 10.0 mo v. 6.0 mo (gp100 arm)	17% (ipi+gp100): 5% diarrhea; 5% fatigue; 3% colitis; 1% endocrinopathy; 1% ↑LFTs	1
	prostate	14	I	14% patients with ≥50% PSA decline	7% rash/pruritus	92
	RCC	61	II	10% ORR	33%: 21% diarrhea; 5% hypophysitis	93
MK-3475 (anti-PD-1)	melanoma	135	I	37% ORR; median PFS >7 mo; 77% had tumor shrinkage during study	13%: 2% rash, 1% ↑LFTs, 1% pruritus, 1% diarrhea, 1% hypothyroidism	7
MPDL3280A (anti-PD-L1)	melanoma	45	I	26% ORR	33% (regardless of attribution): 7% hyperglycemia, 7% ↑ALT, 7% ↑AST	22
	NSCLC	37	I	24% ORR	34% (regardless of attribution): 6% pericardial effusion, 4% dehydration, 4% dyspnea, 4% fatigue	94
	RCC	53	I	50% 24-wk PFS	13%: 4% hypophos.; 4% fatigue; 4% dyspnea; 4% hyperglycemia	95
Nivolumab (anti-PD-1)	melanoma	107	I	31% ORR; median OS 16.8 mo; 1-yr OS 61%	21%: 3% lymphopenia; 3% fatigue; 2% ↑lipase; 2% diarrhea; 2% endocrinopathy; 1% hepatitis	17, 96
	NSCLC	127	I	16% ORR; median OS 9.6 mo	2% fatigue; 2% pneumonitis; 2% ↑LFTs	97
	RCC	34	I	29% ORR; median OS >22 mo, 70% 1-yr OS	21%: 6% hyperphos.; 5% respiratory disorders	17, 98
Tremelimumab (anti-CTLA-4)	colorectal	47	II	2% ORR (1 PR)	11% diarrhea; 2% colitis; 2% fatigue	99
	gastric	18	II	1 PR after 8 cycles, 4/18 SD	6% diarrhea (resulting in death from perforation); 6% ↑LFTs	100
	melanoma	655	III	10.7% ORR v. 9.8% (chemo arm) (ns); OS 12.6 mo v. 10.7 mo (ns)	52%: 18% diarrhea; 6% fatigue; 4% nausea; 4% vomiting; 4% anorexia; 2% rash	14
	NSCLC	87	II	4.8% ORR v. 0% (supportive care); 21% 3-mo PFS v. 14% (ns)	20.5%: 9% diarrhea; 9% colitis	101

Abbreviations: RCC, renal cell carcinoma; NSCLC, non–small cell lung cancer; ORR, objective response rate; PFS, progression-free survival; OS, overall survival; SD, stable disease; PSA, prostate-specific antigen; PR, partial response; mo, month; wk, week; yr, year; ns, not significant; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LFT, liver function test; v., versus; Ipi, ipilimumab.

Table 2 Examples of published combination trials in solid tumors

Cancer	Study drug	N	Phase	Results	Treatment-related adverse events (≥GIII)	Reference
Breast	tremelimumab + exemestane	26	I	0% ORR; 42% SD ≥12 wk	27%: 15% diarrhea; 4% dyspnea; 4% rash; 4% ↑lipase	102
Small-cell lung cancer	taxol/carbo +/− ipilimumab	130	II	irPFS HR = 0.64 (p = 0.03) phased ipilimumab v. control; median OS 12.9 v. 9.9 mo (ns)	17% phased; 21% concurrent	103
Melanoma	adjuvant nivolumab + vaccine	33	I	21% relapsed at median 14 mo; nonrelapsing patients had greater increase in T regs at 12 wk	12%: 9% diarrhea/colitis; 3% rash	104
	decarbazine +/− ipilimumab	502	III	OS 11.2 mo v. 9.1 mo, 3-yr OS 20.8% v. 12.2%	42% immune-related: 21% ↑ALT; 17% ↑AST; 6% diarrhea/colitis; 2% pruritus	2
	ipilimumab + peginterferon alpha-2b	17	IB	3/10 ORR; 1/10 SD	0% (however 41% required dose reduction)	105
	nivolumab + ipilimumab	53	I	40% ORR (31% with ≥80% tumor reduction at 12 wk)	53%: 15% ↑LFTs; 6% diarrhea; 4% colitis; 4% rash; 4% renal failure	59
	tremelimumab + peginterferon alpha-2b	37	II	24% ORR; 38% SD; OS 21 mo	16% neutropenia; 11% diarrhea; 11% ↑LFTs; 11% depression/anxiety; 8% ↑CPK	106
	tremelimumab + PF-3512676 (TLR-9 agonist)	17	I	12% ORR; 25% SD	33%: 18% diarrhea; 12% colitis; 12% rash/pruritus; 12% nausea/vomiting	107
NSCLC	chemo + ipilimumab (phased versus concurrent)	204	II	32% (phased) ORR v. 21% (concurrent) v. 18% (chemo); irPFS HR = 0.72 (p = 0.05) phased v. control; OS 12.2 mo v. 9.7 mo v. 8.3 mo (ns)	phased—39%: 6% anemia; 3% thrombocytopenia; 5% fatigue; 5% diarrhea; 3% rash; 3% neuropathy	108
	nivolumab + platinum doublet chemotherapy	43	I	ORR 31–43%	49%: 7% pneumonitis; 5% rash; 2% colitis	109
Prostate	ipilimumab + PSA-TRICOM vaccine + GM-CSF	30	I	58% PSA decline; 25% ≥50% PSA decline; OS 31.8 mo v. 18.5 mo predicted	27%: 10% colitis; 7% hypophysitis; 7% ↑LFTs; 7% adrenal insuffic.; 3% hypothyroid; 3% neutropenia	110

(*Continued*)

Table 2 (*Continued*)

Cancer	Study drug	N	Phase	Results	Treatment-related adverse events (≥GIII)	Reference
	ipilimumab +/− ADT	43	II	55% undetectable PSA at 3 months versus 38% (monotherapy)	4.5% colitis; 4.5% diarrhea	111
	ipilimumab +/− docetaxel	43	II	7% ≥50% PSA decline (across both arms)	5 "severe" adverse events: adrenal (1); colitis (2); diarrhea (1); melena (1)	112
	ipilimumab +/− radiation	50	I/II	16% ≥50% PSA decline(across both arms)	16% colitis; 10% hepatitis; 8% diarrhea	83
	tremelimumab + ADT	11	I	3/11 with improvement in PSA doubling time	3/11: diarrhea, colitis, rash	113
RCC	tremelimumab + sunitinib	28	I	43% ORR; + 33% SD	61% (regardless of attribution): includes renal failure (3); sudden death (1); colitis (1); perforation (1)	114

Abbreviations: NSCLC, non–small cell lung cancer; GM-CSF, granulocyte macrophage colony-stimulating factor; ADT, androgen deprivation therapy; RCC, renal carcinoma; ORR, objective response rate; OS, overall survival; SD, stable disease; PSA, prostate-specific antigen; PR, partial response; mo, month; wk, week; yr, year; ns, not significant; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LFT, liver function test; irPFS, immune related progression-free survival; HR, hazard ratio; CPK, creatine phosphokinase.

to 48% (15/31) who achieved stable disease (1, 11). Reinduction had a similar toxicity profile compared to initial treatment (12); efficacy will be prospectively evaluated in a trial comparing reinduction versus physician's choice chemotherapy (NCT00495066).

Another CTLA-4-blocking antibody, tremelimumab, continues to be investigated in clinical trials and has also demonstrated durable responses in patients with melanoma (13–14). In a phase III trial of tremelimumab 15 mg/kg versus dacarbazine/temozolomide, the endpoint of improved OS was not reached despite a proportion of subjects experiencing durable response to therapy. It is possible that the lack of an OS benefit was due to exclusion of patients with elevated lactate dehydrogenase and crossover to ipilimumab in patients in the control arm. For this reason, tremelimumab is continuing to be investigated in other malignancies and in combination regimens.

Targeting PD-1: Nivolumab and Others

Whereas CTLA-4 serves to regulate early T cell activation, PD-1 signaling functions in part to regulate T cell activation in peripheral tissues. PD-1 is expressed on a number of cell types, including activated T cells, T regs, activated B cells, and natural killer (NK) cells. PD-1's endogenous ligands, PD-L1 and PD-L2, are expressed in activated immune cells as well as nonhematopoietic cells, including tumor cells. Tumors have been demonstrated to escape immune surveillance by expressing PD-L1/L2, thereby suppressing tumor-infiltrating lymphocytes via PD-1/PD-L1,2 interactions (15). Inhibition of these interactions with therapeutic antibodies has been shown to enhance T cell response and stimulate antitumor activity (16).

The first anti-PD-1 inhibitor to be evaluated was nivolumab (BMS-936558), a fully human IgG4 blocking monoclonal antibody against PD-1 (5). In the phase I dose escalation trial, nivolumab was safe, and objective responses were 16–31% across tumor types (**Table 1**), with most responses being durable for >1 year (17). As with ipilimumab, some patients experienced apparent progression or stable disease before ultimately responding to therapy, and responses have been observed with reinduction therapy (18). With ipilimumab, both

adverse effects and efficacy appear to be dose dependent (10). However, with nivolumab, this correlation was not observed, which may be explained by high receptor-antibody occupancy even at lower doses. The most common adverse event is fatigue; however, some rare events have been serious or life threatening, including interstitial nephritis or pneumonitis. Additional anti-PD-1 agents such as MK-3475, AMP-224, and CT-011 are being evaluated and show promising preliminary evidence of clinical activity (7, 19, 20).

Immune-related response criteria: novel set of response criteria for immunomodulatory agents that allows for transient disease progression before response, or decreasing tumor burden despite new lesions

Targeting PD-L1: MPDL3280A and Others

Two anti-PD-L1 inhibitory antibodies, MPDL3280A (Genentech, South San Francisco, CA) and BMS-936559 (Bristol Meyers Squibb, New York, NY), have undergone clinical investigation. Like nivolumab and MK-3475, these antibodies are thought to function principally by blocking PD-1/PD-L1 signaling. Unlike PD-1 antibodies, PD-L1 antibodies spare potential interactions between PD-L2 and PD-1, but additionally block interactions between PD-L1 and CD80 (21). The therapeutic significance of these interactions remains to be determined. MPDL3280A has been evaluated in multiple tumor types, with safety and preliminary efficacy identified in melanoma, renal cell carcinoma, non–small cell lung carcinoma (NSCLC), colorectal, gastric, and head/neck squamous cell carcinoma (22–24). Similarly, BMS-936559 was shown to be safe and clinically active across multiple tumor types in a phase I trial. MEDI-4736 is another PD-L1-blocking antibody currently in clinical development (NCT01693562).

Next-Generation Checkpoint Agents: Lirilumab and Others

In addition to CTLA-4 and PD-1/PD-L1, numerous other immunomodulatory targets have been identified preclinically, many with corresponding therapeutic antibodies that are being investigated in clinical trials (**Figure 1**). The majority of these targets are T cell surface receptors, but targets in other immunologic cell populations are being investigated. For example, NK cells express killer immunoglobulin-like receptors (KIRs), which bind HLA class I molecules on target cells, thereby delivering an inhibitory signal preventing NK cell–mediated cytotoxicity (25). Anti-KIR antibodies may release these inhibitory KIR-mediated signals, thereby enabling tumor cytotoxicity and immune clearance.

THE MELANOMA EXPERIENCE WITH IPILIMUMAB

The "Tail of the Curve": Unique Kinetics of Response

Traditional measures such as overall response rate may not accurately reflect the clinical benefit derived from immunomodulatory therapy. First, responses may be delayed, with some patients progressing before ultimately responding. This might reflect the underlying immune mechanism whereby activated T cells produces a tumor "flare" prior to tumor shrinkage. Additionally, patients with stable disease often have durable clinical benefit, sometimes with slow tumor regression and conversion to partial response. Thus, the immune-related response criteria require confirmation of progression with interval imaging at least four weeks apart (26); correspondingly, in clinical protocols, patients with clinically insignificant progression of disease are often allowed to continue therapy until confirmed progression.

The Kaplan-Meier survival curves in the phase III ipilimumab trials reveal two points. First, the survival curves overlap until approximately four months, after which the ipilimumab arm diverges (1, 2). This suggests that clinical benefit takes time to develop. Second, the survival curve for ipilimumab reaches a plateau, indicating that some patients experience durable survival. Long-term follow-up of patients treated on earlier phase I–II trials confirms the durability

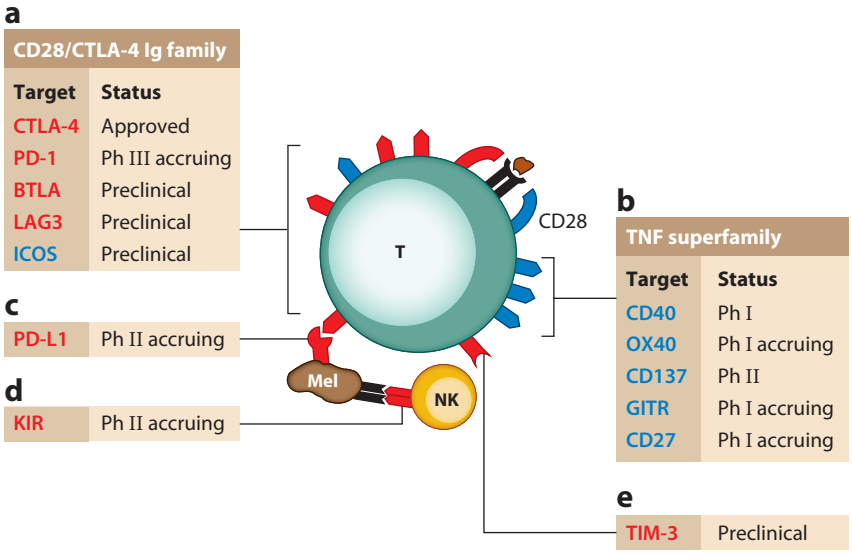

Figure 1

Targets of antibody immune modulators. (*a*) Targetable members of the CD28/CTLA-4 immunoglobulin superfamily include cytotoxic T lymphocyte antigen 4 (CTLA-4) (1), programmed cell death protein 1 (PD-1) (5, 7), B and T cell attenuator (BTLA) (84), lymphocyte activation gene 3 (LAG3) (85), and inducible T cell costimulator (ICOS) (86). (*b*) Targetable members of the tumor necrosis factor (TNF) superfamily include CD40 (87, 88), OX40 (89), CD137/4-1BB (90), glucocorticoid-induced TNFR-related protein (GITR) (91), and CD27. (*c*) Programmed cell death 1 ligand 1 (PD-L1). Mel: melanoma. (*d*) Killer inhibitory receptor (KIR). (*e*) T cell Ig and mucin-containing domain 3 (TIM3).

of response: 14 of 15 complete responders exhibited durability of response ongoing at 54+ to 99+ months (27). Five-year OS in these early trials ranges from 13.8% to 49.5% at various doses of ipilimumab (28). Even more impressively, in this long-term follow-up analysis, some patients whose best radiographic outcome to ipilimumab was classified as progressive disease nonetheless had long-term survival. This reinforces the message that, indeed, a proportion of patients achieve durable disease control and patients can experience benefit that may not be evident upon first radiographic evaluation.

Toxicities of Therapy: Notable But Manageable

Ipilimumab has been associated with a novel spectrum of adverse events called immune-related adverse events (irAE) (29). The most common irAEs following ipilimumab include rash, diarrhea, colitis, hepatotoxicity, and endocrinopathies. Analyses of sera and biopsies indicate that irAEs are mediated by infiltration of highly-activated CD4 and CD8 T cells, as well as increased serum inflammatory cytokines (30, 31). In the first phase III ipilimumab + gp100 trial, 14 patients (2.1%) died of treatment-related toxicities, whereas in the phase III ipilimumab + dacarbazine trial, there were no treatment-related deaths. With accumulating experience, we and others have learned to successfully manage these toxicities utilizing mechanism-based algorithms. For example, with diarrhea/colitis, grade I diarrhea can be managed with oral hydration, the American Dietary Association colitis diet, and loperamide; more significant diarrhea can be managed successively with oral budesonide, oral steroids, intravenous methylprednisolone, and intravenous infliximab.

Immune-related adverse events (irAEs): adverse effects thought to be related to therapy-associated cytokine release and T cell–mediated organ infiltration

In managing irAEs that are refractory to conservative measures, clinicians should not delay corticosteroid use. Early administration of systemic corticosteroids is associated with effective and rapid reversal of symptoms in the majority of patients. For example, in a post hoc review of patients with grade ≥3 colitis, 90% of subjects received corticosteroids and only 14% required the addition of infliximab (32). Median time until resolution of symptoms was 2 weeks; however, subjects initiating steroids within 5 days of symptom onset recovered rapidly (33). Colitis-associated mortality was associated with delays in reporting of symptoms, not holding ipilimumab when symptomatic, and non-compliance with antidiarrheal regimen (32). Although rare, some subjects will require infliximab for enterocolitis. Infliximab is generally administered at 5mg/kg as a single dose. In a series of 15 reported cases requiring infliximab, the treatment was effective in resolving colitis within 3 days in the majority of subjects (34). Despite the theoretical concern that corticosteroids or immunomodulators may blunt the antitumor effect of therapy, corticosteroids have not been associated with changes in survival or duration of response (35).

Utilizing these management strategies, life-threatening complications have been minimized, with bowel perforations occurring in <1% of patients (29). Despite these toxicities, ipilimumab has not been associated with detriment in quality of life, as measured by the validated QLQ-C30 questionnaire (36).

Antibodies targeting the PD-1/PD-L1 axis have a favorable toxicity profile in preliminary trials, with a grade III/IV adverse event rate of 13% in patients receiving MK-3475, 9% in patients receiving BMS-936559, and 14% in patients receiving nivolumab (5–7). One unique and potentially life-threatening toxicity for these agents is pneumonitis, of which three patients (1%) died in the phase I nivolumab trial despite treatment with corticosteroids, infliximab, or mycophenolate (5). Algorithms are being developed to mitigate progression to life-threatening pneumonitis.

Response Heterogeneity and Appropriate Sequencing of Therapies

With the advent of molecular profiling and targeted therapy, we must determine how to optimize the sequencing of targeted therapies with immune checkpoint inhibitors. Melanoma is an excellent example: vemurafenib, dabrafenib, and trametinib have all been recently FDA-approved for the treatment of melanoma in patients harboring a sensitizing BRAF kinase mutation. Ipilimumab is efficacious in both BRAF-wildtype and BRAF-mutant melanoma. Whether it is preferable to treat with a RAF kinase inhibitor before or after immunotherapy is also unclear and has been the subject of retrospective analyses (37, 38). It is currently assumed that use of vemurafenib or dabrafenib first in patients with BRAF-mutated tumors who have symptomatic or high tumor burden is logical, given the greater likelihood of a rapid response with RAF kinase inhibition (39). Finally, the potential relationship between molecular profile and response to therapy remains an active area of investigation (40–42).

In melanoma and other malignancies, histologic or anatomic stratification of disease reveals heterogeneous molecular derangements and response to therapy. For example, both uveal and mucosal melanomas are believed to be more resistant to classical melanoma chemotherapy regimens. Thus, we cannot assume that immunomodulatory agents will have the same response profile in these melanoma subtypes as in cutaneous melanoma. In uveal melanoma, a single-institution retrospective analysis demonstrated the possibility of ipilimumab producing durable responses with acceptable toxicity (43). In mucosal melanoma, two independent retrospective analyses showed activity with ipilimumab, with an OR rate of 6% and disease control rate of 23.1–26.7% (44, 45). This indicates that ipilimumab is a reasonable choice for mucosal and uveal melanoma; however, this must be confirmed with prospective trials that are currently accruing (NCT01585194, NCT01355120). These data are also important in determining proper

sequencing of therapy with emerging targeted therapies, such as imatinib/dasatinib for c-KIT-mutant mucosal melanoma or selumetinib for gnaq/Gna11 mutant uveal melanoma (46).

Crossing the Blood–Brain Barrier: CNS Metastases

Central nervous system (CNS) metastases occur frequently in malignancies that are potentially amenable to immunotherapy, such as melanoma, lung cancer, and renal cancer, with inferior survival compared to matched controls (47). Although antibodies are not thought to cross the blood–brain barrier, activated T cells are capable of trafficking to the CNS (48). Therefore, by enhancing antitumor immunity, immunomodulatory therapy may be effective in the treatment of CNS lesions. In melanoma, a recent phase II trial of patients with CNS lesions receiving ipilimumab 10 mg/kg demonstrated an 18% disease control rate in asymptomatic patients versus a 5% disease control rate in symptomatic patients who required steroids (49). Disease control rates within the CNS and outside the CNS were concordant. The reduced efficacy in the corticosteroid group was likely due to the overall poorer health of this study arm; however, there may also be a detrimental effect of ongoing corticosteroid use blunting the immune response. Other studies have evaluated ipilimumab in conjunction with chemotherapies that cross the blood–brain barrier, for example with fotemustine (OR rate 29%) (50) and temozolomide (OR rate 28%) (51, 52). These results are encouraging, with tolerable toxicity and evidence of activity in CNS lesions.

POTENTIAL BIOMARKERS FOR RESPONSE

Efficacy in checkpoint modulation is associated with certain immunologic changes, raising the hope that biomarkers for response may be identified. Only a minority of patients experience long-term survival with ipilimumab; therefore, considerable efforts are ongoing to discover predictors of response.

Clinical Correlates

One of the first and most thoroughly described potential biomarkers is the absolute lymphocyte count (ALC). Multiple parameters have been associated with increased OS, for example, the ALC at seven weeks ($\geq$1000 cells/μL) (53, 54) and the magnitude of ALC increase with therapy (55, 56). Additionally, baseline absolute eosinophil count and relative eosinophil count have been associated with improved survival (57). These findings may not be applicable, however, to more potent immunomodulatory regimens: a significant relationship between ALC and OR was not observed in patients treated with the combination ipilimumab + nivolumab (58, 59).

PD-L1 Expression in the Tumor

Based on the premise that anti-PD-1 therapy functions by blocking interactions between PD-1 and PD-L1, tumoral PD-L1 expression is being evaluated as a potential biomarker for response. In the original nivolumab phase I trial, 101 patients with pretreatment biopsies were evaluated for PD-L1 expression by immunohistochemistry. Patients with tumors expressing PD-L1 had an OR rate of 44% versus 17% among PD-L1-negative patients (5, 60). However, analysis of response by PD-L1 expression in a recent ipilimumab + nivolumab trial revealed similar response in both cohorts (OR rate 8/17 in PD-L1 negative versus 4/10 in PD-L1 positive) (58, 59). One potential explanation is that PD-L1/PD-1 expression is dynamic and heterogeneous, with baseline PD-L1 expression being modifiable by clinical factors, such as potential induction after anti-CTLA-4-mediated local IFN-γ production. PD-L1 expression is being prospectively evaluated as a biomarker in a phase III trial comparing nivolumab versus chemotherapy in melanoma (NCT01721746). If validated, the PD-L1 marker might serve to personalize therapeutic decision making, for example in deciding whether to treat with nivolumab or an alternative agent.

NY-ESO-1: cancer-testis antigen expressed in melanoma and other malignancies; elicits spontaneous antibody and T cell responses and is being investigated therapeutically

Immune Monitoring and Novel Assays

Immune and tumor response to therapy has been monitored by utilizing a variety of laboratory techniques (**Figure 2**), and numerous correlates of response have been retrospectively identified. For example, response has been associated with antibody and T cell antigen-specific responses to NY-ESO-1, the percentage of $CD4^+$ cells expressing the inducible

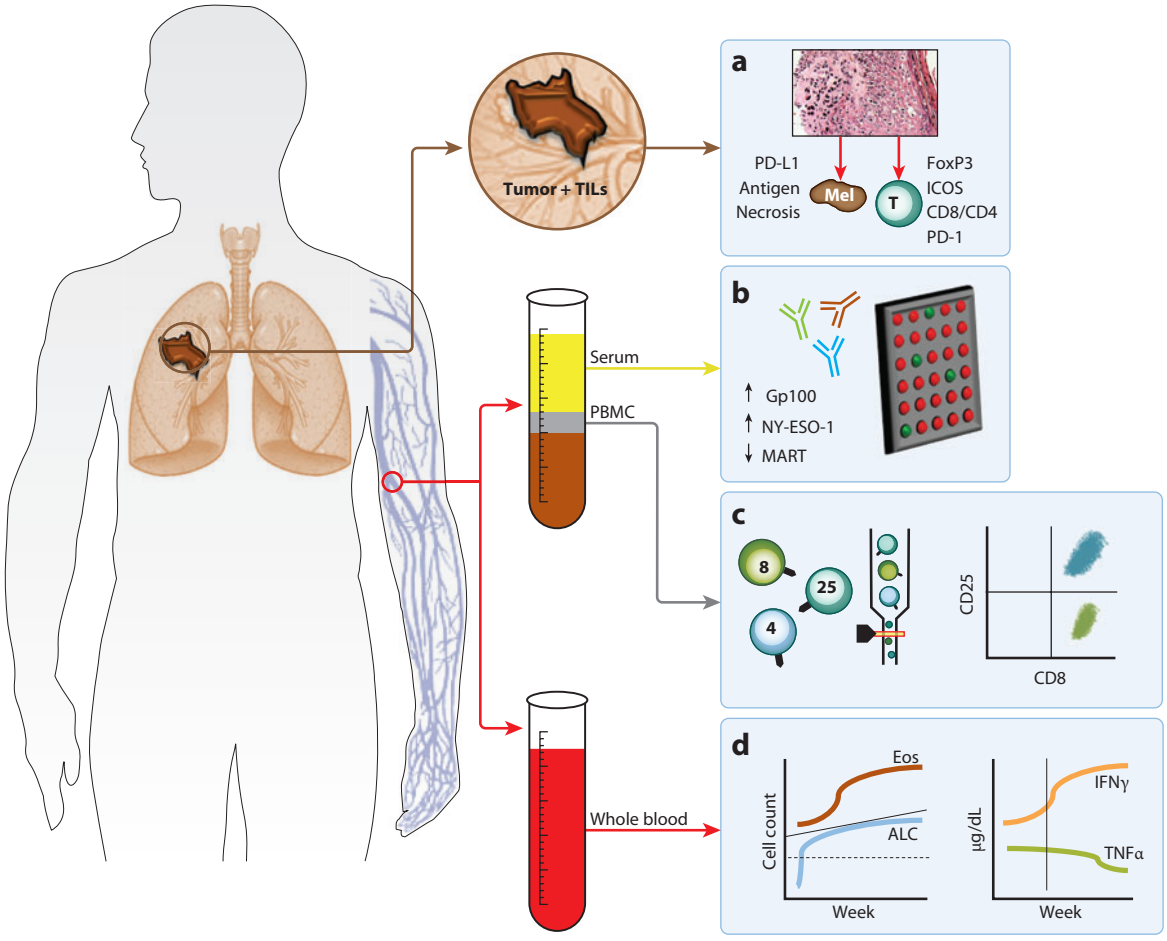

Figure 2

Strategies for immune monitoring in patients receiving checkpoint agents. (*a*) Surgical specimens may be analyzed using immunohistochemical or immunofluorescence techniques to evaluate tumor antigen expression, T cell infiltrate, tumor necrosis, or expression of surface markers such as PD-L1. Similarly, the tumor microenvironment can be dissected histopathologically in order to characterize spatial relationships between tumor and immune infiltrate, and transcriptional profiling assays can evaluate changes in gene expression in both the tumor and in lymphocytes. Mel: melanoma. (*b*) Using enzyme-linked immunoabsorbent assays or protein arrays, treatment-related production of tumor-specific antibodies can be detected in the serum. (*c*) Flow cytometric analysis of tumor-infiltrating lymphocytes (TILs) and peripheral blood mononuclear cells (PBMCs) can quantitate the effect of therapy on immune subsets such as $CD25^+$ T regs, activated $CD8^+$ T cells, or myeloid-derived suppressor cells. Using polychromatic flow cytometry, multiple surface and intracellular markers can be detected, allowing in-depth characterization of T cell phenotype and activation state. Antigen-loaded soluble MHC tetramer stains can be used to analyze tumor-specific T cell subpopulations. (*d*) Whole blood can be used to evaluate changes in cell count with therapy or changes in cytokine levels. Deep sequencing techniques enable quantification of changes in individual T cell clonotypes.

costimulator (ICOS) activation marker, expression of genes involved in immune response, post-treatment increases in tumor-infiltrating lymphocytes, and low baseline levels of myeloid-derived suppressor cells (MDSCs) (58, 61–67). Gene expression analysis is also being evaluated as a predictor of immune-related gastrointestinal toxicities, with expression of CD77 and CEACAM1 strongly associated with gastrointestinal toxicity (68). Going forward, we anticipate prospective evaluation of these findings, as well as development of novel immune monitoring techniques, including analyses of tumor exome gene/microRNA, T cell receptor diversity, and T cell activation markers. In the future, immune monitoring may facilitate personalization of immune-based therapies, for example by detecting intervenable resistance mechanisms.

MOVING FORWARD: COMBINATION THERAPIES

Combination Therapy with Multiple Checkpoint Agents

Concurrent blockade of immunologic checkpoints with multiple inhibitory molecules may enhance efficacy. For example, the ipilimumab monotherapy ORR was 6% and OS was 10 months (1, 2); in the phase I nivolumab trial, the ORR was 31% and OS was 17 months (17). However, the combination of ipilimumab + nivolumab was recently evaluated in a phase I trial, which demonstrated impressive preliminary evidence of improved benefit; the response rate was 53% at the maximum tolerated dose, and all responding subjects experienced a ≥80% decline in tumor burden at 12 weeks (59). The combination was safe, but there were more frequent (53%) grade III/IV adverse events. These were manageable with treatment algorithms. This combination, and other monoclonal antibody combinations, must be confirmed in prospective phase III randomized trials, which are currently accruing (NCT01844505).

Supplemental Material

Combination Therapy with Other Agents

Multiple other viable combination strategies are being explored in early-phase trials, each with a compelling preclinical rationale (see **Supplemental Table 1**; follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**). For example, checkpoint modulation might be enhanced in combination with alternative immunotherapies such as vaccines, cytokine therapy, and adoptive cellular therapy (70–72). Another strategy is to combine checkpoint agents with radiotherapy (see sidebar Checkpoint Modulation with

Myeloid-derived suppressor cells (MDSCs): a heterogeneous population of myeloid-derived cells that accumulate in the tumor microenvironment and promote T cell suppression and tumor escape

CHECKPOINT MODULATION WITH RADIATION THERAPY: THE ABSCOPAL EFFECT

In radiotherapy, the abscopal effect describes the phenomenon of tumor regression outside the irradiated field. This effect could be caused by radiation-induced antigen and cytokine release, which subsequently potentiates a systemic immune response against the tumor. Several provocative anecdotes have been published recently describing the abscopal effect with ipilimumab. For example, a female with metastatic melanoma was treated with palliative radiotherapy following ipilimumab to a subcutaneous mass; she later experienced disease regression within the irradiated field, but more impressively, regression also occurred in areas outside the irradiated field that had initially progressed following ipilimumab (79). In-depth immune monitoring of the patient revealed a pronounced antibody response to ten tumor-associated antigens, as well as a decrease in the immunosuppressive MDSC population.

On the basis of such anecdotes and supporting preclinical data (10, 80, 81), the combination of radiation plus immunotherapy is being prospectively evaluated as a means to improve upon monotherapy (NCT01703507, NCT01497808, NCT01565837, NCT01689974). Similarly, combinations with alternative modalities of local control are being investigated, for example with cryoablation or electrochemotherapy (NCT01502592, NCT01642290) (82). Ipilimumab plus radiation has also been evaluated in a phase I/II study in prostate cancer, with demonstrated clinical activity and tolerable adverse effects (83).

Radiation Therapy: the Abscopal Effect), or with cytotoxic or targeted therapies such as BRAF or VEGF inhibitors, capable of generating immune-stimulating pleiotropic effects such as inducing antigen expression (73, 74) or inhibiting MDSC maturation (75). Unfortunately, the combination of vemurafenib and ipilimumab produced significant hepatic toxicity requiring termination of the trial (76). A phase I trial of bevacizumab + ipilimumab revealed an impressive number of clinical responses with manageable toxicity (77). A recent study also reported that adding granulocyte macrophage colony-stimulating factor (GM-CSF) to ipilimumab 10 mg/kg improved OS compared to ipilimumab 10 mg/kg alone (78). Patients who received the combination also had reduced side effects compared to patients who received ipilimumab alone.

CONCLUSION

Ipilimumab has ushered in a new era of oncology research, paving the way for next-generation immune checkpoint antibodies such as nivolumab and MK-3475. Despite decades of skepticism, immunotherapy now has proven itself as a viable therapeutic strategy, both in melanoma and in other malignancies. We must continue to investigate this new class of therapeutic agents and incorporate them into clinical practice in combinations with established and novel therapies. We anticipate that continued investigation will reaffirm the clinical utility of anti-PD-1 and anti-PD-L1 therapy, setting the stage for ultimate FDA approval. Additionally, clinical trials combining multiple checkpoint agents will come to fruition, allowing novel subsequent combination approaches.

SUMMARY POINTS

1. Ipilimumab is an FDA-approved anti-CTLA-4 antibody therapy for metastatic melanoma, producing durable 5-year survival in 12.3–49.5% of patients.
2. Tremelimumab is another CLTA-4 antibody with clinical activity. It is being developed for treatment of malignancies such as mesothelioma and hepatocellular carcinoma, as well as in combination regimens.
3. Nivolumab and MK-3475 are anti-PD-1 antibodies with highly promising single-agent activity and safety across multiple tumor subtypes.
4. For patients with melanoma, the combination of nivolumab + ipilimumab was safe and dramatically reduced tumor bulk in a significant percentage of patients in a phase I trial.
5. Although early immunomodulatory antibody trials have focused primarily on the treatment of melanoma, activity has been demonstrated in renal cell carcinoma, NSCLC, and prostate cancer, among other tumor types.

FUTURE ISSUES

1. Prospective clinical trials will be required to determine how best to sequence these novel immunotherapies with targeted agents such as vemurafenib for BRAF-mutant melanoma, or erlotinib for EGFR-mutant NSCLC.
2. Combination regimens show superiority in murine models. Early phase I trials also demonstrate promise. Future trials will investigate whether combination therapy is superior to monotherapy.

3. Next-generation antibodies will target novel receptors on T cells as well as other immune cells such as NK cells.
4. Immune monitoring techniques will allow in-depth characterization of the immune and tumor response in individual patients, paving the way for identification of novel biomarkers of response.

DISCLOSURE STATEMENT

Drs. Callahan and Wolchok serve as consultants to Bristol-Myers Squibb and GlaxoSmithKline, and Dr. Wolchok additionally serves as a consultant to Merck. Drs. Callahan and Postow receive research support from Bristol-Myers Squibb.

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2. Phase III study showing improved overall survival with ipilimumab + dacarbazine compared to a previous standard of care, dacarbazine.

5. Sentinel phase I trial demonstrating safety and clinical activity for PD-1 blockade.

6. Sentinel phase I trial demonstrating safety and clinical activity for PD-L1 blockade.

7. Phase I trial demonstrating safety and clinical activity for MK-3475, a second PD-1 antibody.

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