

Promising immunotherapeutic approaches to the treatment of metastatic melanoma: modulation of the immune response

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Durable remissions of advanced unresectable melanoma can be achieved in a small fraction of patients with immune-based therapy, such as high-dose interleukin-2 (IL-2). Based on an increased understanding of normal and pathologic immune responses at the molecular level and the specific interactions between growing tumors and the host immune response, new approaches to cancer immunotherapy are emerging. Optimism for advances in the immunotherapy of melanoma comes from new agents and approaches that specifically target and modify the regulators of T-cell proliferation, survival and effector function, and/or block tumor-related immunosuppressive mechanisms. One of these agents, anti-CTLA4 (anti-cytotoxic T-lymphocyte-associated antigen 4), has shown the ability to produce durable responses in patients with metastatic melanoma, including some previously unresponsive to IL-2. Preclinical studies indicate that even stronger antitumor immune responses can evolve when agents are combined rationally.

Results from animal studies examining the mechanism of antitumor immune responses indicate that T lymphocytes have a key role in mediating tumor regression. Thus, the suboptimal efficacy of immune therapies is likely related to inadequate activation and expansion of tumor-specific T-cell responses, suboptimal T-cell function (eg, inability to traffic to tumor; low affinity for target antigens; tumor microenvironmental interactions that decrease their activity), or loss of recognition molecules by the tumor.

New approaches to cancer immunotherapy are emerging based on an evolving understanding of normal and pathologic immune responses at the molecular level and specific interactions that occur between growing tumors and the host immune response (Table 1). The control of T-lymphocyte activation and regulation is better defined. This knowledge has allowed the development of new agents and approaches to broaden, expand, and increase the effectiveness of T-cell immune responses against cancer. In part, this new knowledge forms the basis for development of more sophisticated cancer vaccines to induce tumor-specific T-cell responses. There is an increasing consensus that cancer vac-

cines alone will not be sufficient to treat melanoma in most patients, including those with residual micrometastatic disease after surgery. This finding is due to natural regulatory mechanisms that limit the magnitude of induced T-cell responses and tumor-related dampening of T-cell effector function.

The greatest optimism for advances in the immunotherapy of melanoma comes from the introduction of new agents and approaches that block or bypass the aforementioned regulatory mechanisms and tumor-related immunosuppressive mechanisms. Preclinical studies indicate that antitumor immune responses can be more powerful when agents are combined rationally. This article reviews the rationale and data supporting selected promising new immunotherapeutic approaches for the treatment of metastatic melanoma. Potential combination strategies are discussed, including combinations of distinct agents as well as adoptive

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TABLE 1

Investigational immunotherapeutic approaches to the treatment of metastatic melanoma

Target	Drug	Class	Developmental phase
<i>Blockade of natural regulation or tumor-induced suppression of T-cell effector function</i>			
CTLA4	Ipilimumab (MDX-010)	Fully human IgG1 mAb	Phase III
	Tremilimumab (CP-675,206)	Fully human IgG2 mAb	Phase III
PD1	MDX-1106	Fully human mAb	Phase I
TGF- β	GC1008	Fully human mAb	Phase I
TGF- β 2	AP12009	Antisense oligonucleotide	Phase I
Indoleamine 2,3-dioxygenase	D-1-methyl-tryptophan (1-MT)	Indoleamine 2,3-dioxygenase inhibitor	IND filing
Arginine	BCT-100	Recombinant arginase	Preclinical
TLR regulation of T _{reg} cells	CpG 7909 (Promune, PF-3512676)	Synthetic oligonucleotide	Phase I
<i>New cytokines and other T-cell-activation signals</i>			
CD137	BMS-663513	mAb	Phase I
Cytokines	Interleukin-21	Recombinant human molecule	Phase II
<i>Dendritic cell-activating signals</i>			
CD40	CP-870,893	Fully human mAb	Phase I
TLR	PF-3512676	TLR9 agonist	Phase II
<i>Delivery of cytokines to tumor</i>			
Immunocytokines	EMD 273063	Humanized anti-GD2 mAb linked to IL-2	Phase II
Nanoparticles	Aurimune (CYT-6091)	TNF covalently linked to pegylated colloidal gold	Phase I

CTLA4 = cytotoxic T-lymphocyte-associated antigen 4; IgG = immunoglobulin G; IL-2 = interleukin-2; IND = investigational new drug; mAb = monoclonal antibody; PD = programmed death; TGF = transforming growth factor; TLR = Toll-like receptor; T_{reg} = regulatory T cell; TNF = tumor necrosis factor

immunotherapy with tumor antigen-specific T cells.

Because of the complexity of the immune system and the presence of the molecular immunologic target on multiple cell types (with potentially different downstream effects that are cell-type specific), the predominant mechanism for an individual agent's antitumor activity often cannot be determined with certainty. For example, the ligand for a T-cell receptor that is targeted by an antibody may be present on both tumor and antigen-presenting dendritic cells (DCs). Blocking the receptor may affect both T-cell activation and interactions between tumor and infiltrating effector

T cells. No attempt is made to group agents by specific mechanisms in this article because of the potentially broad actions of most agents.

Anti-CTLA4 (ipilimumab, tremilimumab)

CTLA4 (cytotoxic T-lymphocyte-associated antigen 4) is a homolog of the T-cell surface molecule CD28. Binding of CD28 to ligands from the B7 family expressed on the surface of antigen-presenting cells (APCs) is one of two signals essential for T-cell activation and differentiation to effector function.^{1,2} In contrast, binding of CTLA4 to the same ligands inhibits or dampens the T-cell response.

Critical evidence pointing to the key role of CTLA4 in maintaining tolerance was provided by studies in which CTLA4 knockout mice developed a lethal lymphoproliferative disorder, characterized by progressive accumulation of activated T-cell blasts in critical organs.³ Leach et al first demonstrated that in vivo administration of anti-CTLA4 antibodies led to rejection of tumors in mouse models of colon cancer and other tumors.⁴ Other investigators reported similar findings using anti-CTLA4 blockade alone or in combination with other treatments in experimental models of mammary carcinoma,⁵ melanoma,⁶ and prostate cancer.^{7,8}

The findings that CTLA4 blockade boosts antitumor responses in vivo stimulated a keen interest in targeting this molecule with antibodies to improve the antitumor immune response in patients.^{4,9} Ipilimumab and tremilimumab are fully human anti-CTLA4 monoclonal antibodies in late-stage clinical development.

Ipilimumab

Ipilimumab (formerly, MDX-010) is a fully human immunoglobulin G (IgG) 1 κ monoclonal antibody that has been shown to cause cancer regression and autoimmunity in patients with metastatic melanoma¹⁰ and other cancers, including hormone-refractory prostate cancer (HRPC).¹¹ Small clinical studies have examined the safety and efficacy of ipilimumab as monotherapy and in combination with other agents for the treatment of patients with metastatic melanoma (Table 2).¹²⁻¹⁶ Durable complete responses (CRs) and partial responses (PRs) have been observed; some patients had prolonged stable disease (SD), but the clinical significance of disease stabilization within the context of these single-arm trials remains to be verified in randomized phase III studies. The majority of objective responses to ipilimumab have a slow onset and may

TABLE 2

Clinical trial experience with ipilimumab in patients with unresectable stage III or metastatic melanoma

Study	Number of patients	Dosing schedule	Efficacy	Safety
Attia et al ¹²	56	Ipilimumab 3 mg/kg Q3W or 3 mg/kg as initial dose, then 1 mg/kg Q3 W	2 CRs (ongoing at 31 and 30 months); 5 PRs (ongoing at 34, 26, and 25 months); 3% ORR; no differences between schedules	14 patients (25%) had grade 3/4 autoimmune toxicities (ie, colitis, dermatitis, uveitis, enterocolitis, hepatitis, hypophysitis); no differences between schedules
Weber et al ¹³	25 (resected stage III or IV)	Ipilimumab 3 mg/kg IV Q8W for 12 months + multi-peptide vaccine	12-month median follow-up: no deaths and 6 relapses	12 patients had grade 2/3 colitis, rash, or hypophysitis
Maker et al ¹⁴	36	Ipilimumab dose escalation (0.1, 0.3, 1.0, 2.0, 3.0 mg/kg Q3W) + IL-2	3 CRs; 5 PRs; 22% RR; 6 of these 8 patients had ongoing responses at 11–19 months	5 patients (14%) had grade 3/4 autoimmune toxicities (ie, enterocolitis, arthritis, uveitis)
Fischkoff et al ¹⁵	Monotherapy, 37; combination therapy, 35	Ipilimumab 3 mg/kg Q1M × 4 ± dacarbazine 250 mg/m ² for 5 days each month × 4	Monotherapy arm: 2 PRs (ongoing at 16–18 months); 5% RR Combination therapy arm: 2 CRs; 4 PRs; 17% RR	Adverse events due to immune activation were managed with drug discontinuation and corticosteroids
Weber et al ¹⁶	Cohort A: 34 Cohort B: 30 Cohort C: 24	Cohort A: 2.8, 3, or 5 mg/kg on days 1, 57, and 85 Cohort B: single dose of 7.5, 10, 15, or 20 mg/kg Cohort C: 10 mg/kg Q3W on days 1, 22, 43, and 64	Cohort A: 1 PR Cohort B: 1 PR Cohort C: 1 CR; 1 PR	Overall, 72% had an adverse event, mostly grade 1 or 2, that was reversible without sequelae; 1 patient had a perforated bowel, grade 4 diarrhea, grade 4 colitis

CR = complete response; IL = interleukin; ORR = overall response rate; PR = partial response; Q3W = every 3 weeks; Q8W = every 8 weeks; Q1M = every month; RR = response rate

be preceded by disease progression, mixed response, or months of SD. This finding suggests a slower time course for evaluation of responses to anti-CTLA4 compared with conventional therapy.^{17,18} The slow evolution of response and occasional reports of initial disease progression or mixed response followed by tumor regression complicate the clinical management of these patients, in particular the critical decision to continue anti-CTLA4 treatment/observation versus administration of a new therapy after an initial treatment induction period. The reason for slow response to treatment is unclear but may be related to the relative balance and kinetics of expansion of regulatory and effector cells over time.¹⁹

Most adverse events linked to ipilimumab stem from presumed autoimmune reactions, referred to as autoimmune breakthrough events (ABEs) or immune-related adverse events (irAEs). The most common grade 3/4 events are enterocolitis (~20%) and hypophysitis (~10%), but other

organs can be affected.²⁰ Hepatitis, dermatitis (with or without pruritus), alveolitis, uveitis, nephritis, and arthralgias have been reported in ipilimumab-treated patients. Patients may develop multiple irAEs after single or multiple doses of the drug. The time course to evolution of an irAE is variable.

Treatment of most grade 3/4 irAEs requires the use of steroids. Algorithms have been developed for detecting and managing the more common events, such as enterocolitis and hypophysitis. Because of the small risk of bowel perforation related to enterocolitis, immediate use of high-dose steroids with tapering over at least 1 month is recommended for patients with grade 3/4 diarrhea or persistent grade 2 diarrhea.²¹ Enterocolitis refractory to steroids can be treated effectively with a single dose of infliximab (Remicade).

Analysis of data from the ipilimumab trials suggests a strong correlation between irAEs and antitumor response.^{17,20} For example, 22% of 36

patients with grade 1/2 irAEs and 28% of 50 patients with grade 3/4 irAEs had objective responses, compared with only 2% of 53 patients who had no autoimmune toxicity ($P = 0.0004$).²⁰ In their study, Weber et al noted that all patients who had an objective response to ipilimumab experienced an ipilimumab-related adverse event.¹⁶

Several clinical trials are actively recruiting patients with melanoma to further evaluate ipilimumab monotherapy and combination studies. Opened in June 2006, a phase III placebo-controlled trial is actively recruiting patients with untreated, unresectable stage III or IV melanoma for randomization to dacarbazine with or without ipilimumab (10 mg/kg). The primary endpoint of this study is progression-free survival (PFS), and the planned enrollment is 500 patients.

Tremilimumab

Tremilimumab (formerly, CP-675,206) is an IgG2 monoclonal antibody directed against CTLA4. Be-

cause it is an IgG2 antibody, it would be expected to induce minimal complement activation and antibody-dependent cell-mediated cytotoxicity (ADCC).²² In an initial phase I dose-escalation trial of a single dose of tremilimumab in 39 patients with solid tumors (including 34 patients with melanoma), autoimmune events including diarrhea, dermatitis, vitiligo, panhypopituitarism, and hyperthyroidism were observed (Table 3).²³ Among the 29 patients with measurable melanoma, 2 had CRs maintained for > 34 and > 25 months, respectively; 2 had PRs lasting > 26 and > 25 months, respectively. The maximum tolerated dose was 15 mg/kg.

These results led to a larger, multi-dose phase I/II trial conducted in 119 patients with unresectable or stage IV recurrent melanoma.²⁴ The phase I, open-label portion of the study tested doses of 3, 6, or 10 mg/kg every month (Q1M) for up to 1 year, with an expansion cohort for HLA (human leukocyte antigen)-A2-positive patients to receive 10 mg/kg Q1M for immune monitoring. The phase II portion of the study randomized patients to treatment with 10 mg/kg Q1M or 15 mg/kg every 3 months (Q3M).²⁵ The primary endpoint of the phase II part of the study was objective response. Most patients enrolled in the phase II study had received one or two prior chemotherapy

or immunotherapy regimens.

There were 1 CR and 3 PRs among 44 patients in the monthly dosing group and 2 CRs and 1 PR among 46 patients in the Q3M dosing group.²⁵ The median time to best response was 219 days (range, 51–396 days) for the monthly dosing group and 125 days (range, 49–845 days) for the Q3M group. Responses were durable, with CRs and almost all PRs lasting > 2 years. The 1-year survival was 32% for the monthly dosing group and 46% for the Q3M dosing group; the median overall survival (OS) was 10.2 months and 11.5 months, respectively. The survival data, although promising, require confirmation in a randomized trial.

Grade 3/4 adverse events were experienced by 27% of patients in the 10 mg/kg dosing group and 13% of patients in the 15 mg/kg dosing group. The most common drug-related grade 3/4 adverse events were diarrhea and colitis, which occurred in 28% of patients in the monthly administration arm and 13% of patients treated with the Q3M regimen. Toxicity was dose-dependent.

Based on these results, which suggested comparable antitumor activity and less toxicity, the 15 mg/kg Q3M regimen was selected for further clinical testing. Several trials are open for enrollment of patients with melanoma and other solid tumors. An ongoing

phase III, open-label randomized study, with a planned enrollment of 630 patients, is comparing tremilimumab with either dacarbazine or temozolomide (Temodar; investigator choice) in patients with advanced melanoma not treated with previous systemic therapy, except adjuvant cytokines or vaccines after initial complete resection. The primary endpoint of this study is OS.

Anti-TGF-β

Tumor secretion of the potent immunosuppressive cytokine TGF-β (transforming growth factor-beta) is believed to mediate an important mechanism by which tumors can evade the immune response.²⁶

A phase I trial is evaluating the fully human, anti-TGF-β monoclonal antibody GC1008 in patients with unresectable, locally advanced or metastatic renal cell carcinoma or malignant melanoma. A small-molecule TGF-β receptor also is in clinical trials, and an antisense phosphorothioate oligonucleotide (AP12009) directed against TGF-β2 mRNA was evaluated in a phase I/II study in patients with pancreatic cancer, melanoma, and colorectal cancer.^{27–29} Preliminary results showed a good safety profile in 17 patients. One patient with metastatic melanoma was alive 64 weeks after the start of treatment, and another with pancreatic

TABLE 3

Clinical trial experience with tremilimumab in patients with unresectable stage III or metastatic melanoma

Study	Number of patients	Dosing schedule	Efficacy	Safety
Ribas et al ²³	34 (29 with measurable disease)	Tremilimumab dose escalation (0.01 mg/kg and up); single infusion	2 CRs (ongoing at 34+ and 25+ months); 2 PRs (ongoing at 26+ and 25+ months)	Dose-limiting toxicities were dermatitis and diarrhea
Gomez-Navarro et al ²⁴ ; Ribas et al ²⁵	119	Phase I: 3, 6, and 10 mg/kg, followed by 10 mg/kg Q1M (expansion cohort) Phase II: 10 mg/kg Q1M (n = 44) vs 15 mg/kg Q3M (n = 46)	Phase II: 1 CR (ongoing at 33+ months) and 3 PRs (11 months and ongoing at 34+ and 25+ months) at 10 mg/kg Q1M; 2 CRs (ongoing at 33+ and 29+ months) and 1 PR (ongoing at 29+ months) at 15 mg/kg Q3M	Grade 3/4 diarrhea/colitis 27.5% at 10 mg/kg Q1M and 13% at 15 mg/kg Q3M

CR = complete response; PR = partial response; Q1M = every month; Q3M = every 3 months

cancer had CR and is still alive after 72 weeks. An additional study is planned.

Anti-PD1 and anti-CD137

The biology of PD1 and CD137 is explained in detail in the article by Dr. Wong in this supplement. Agents targeting PD1 and CD137 are in phase I trials, and clinical data have not been reported. Data suggest that PD1 and its ligand (PDL1) play an important role in allowing potentially immunogenic tumors to evade the immune response and provide a compelling rationale for blocking the ligand or the receptor as a stand-alone or combination immune therapy strategy.^{30,31}

The fully human anti-PD1 monoclonal antibody MDX-1106 is currently being tested in a phase I dose-escalation trial in patients with recurrent or treatment-refractory solid malignancies, including non-small cell lung cancer, colorectal cancer, melanoma, renal cell cancer, and HRPC. The agonistic anti-CD137 monoclonal antibody BMS-663513 is being investigated as monotherapy and in combination therapy in patients with solid tumors.

Anti-CD40

Activated T cells upregulate expression of CD40 ligand. T-lymphocyte engagement with CD40, which is a cell-surface molecule expressed by various immunologic cell types (eg, DCs, B lymphocytes, and monocytes), activates APCs and stimulates an immune response. CD40 is also expressed by a variety of tumor cells.³² The fully human, anti-CD40 monoclonal antibody CP-870,893 induced DC maturation and promoted autologous antitumor T-cell responses in vitro and in vivo.³³⁻³⁵

A phase I trial in 29 patients with advanced solid tumors showed that a single dose of CP-870,893 was well tolerated and resulted in 4 PRs in patients with melanoma (corresponding to 14% of all patients and 27%

of patients with melanoma).³⁶ The most common adverse event was a grade 1/2 cytokine-release syndrome, which included chills, rigors, and fever. Transient changes in lymphocyte, monocyte, and platelet counts and laboratory abnormalities in D-dimer and liver function tests were observed 24-48 hours post infusion. A multi-dose phase I trial is ongoing.

Anti-interleukin-10

Melanoma cells appear to be the main producers of interleukin-10 (IL-10) in metastatic lesions, where IL-10 may allow tumors to evade or suppress an immune system response.^{37,38} Experiments with cultured human melanoma cells from primary melanomas, locoregional lymph nodes, and metastases suggest that IL-10 downregulates immunerecognition molecules, such as HLA class I, HLA class II, and intercellular adhesion molecule (ICAM)-1.³⁹ In a mouse model of melanoma, IL-10 promoted melanoma growth by inhibiting macrophage functions and inducing proliferation of tumor and vascular cells.⁴⁰ Investigators have also determined that secretion of IL-10 by metastatic, but not primary, melanoma cells caused downregulation of CD1 molecules on infiltrating DCs, leading to a suppressed immune response.⁴¹ This suppressive effect was blocked by a neutralizing anti-IL-10 antibody. IL-10 is also produced by a subset of T-regulatory (T_{reg}) cells. Although the cumulative data support clinical evaluation of anti-IL-10 alone or in combination in patients with metastatic melanoma, IL-10 has also been shown to promote T-cell activation. Thus, the net antitumor effect of neutralizing this cytokine in patients remains to be determined.

Interleukin-21

The pleiotropic class I cytokine interleukin-21 (IL-21) has been shown to activate CD8⁺ T cells and natural killer (NK) cells.^{42,43} In experimental

tumor models, IL-21 therapy inhibited tumor growth and induced antitumor immunity.⁴⁴⁻⁴⁶ Unlike IL-2, which stimulates effector and T_{reg} cells, IL-21 may not support T_{reg}-cell survival or expansion.

In a phase I trial of a recombinant human IL-21 in patients with metastatic melanoma, the dose-limiting toxicities included an increase in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, neutropenia, fatigue, and thrombocytopenia.⁴⁷ In one patient, a PR was observed after 6 weeks of treatment and then was converted to a CR after 3 additional months.

Based on this study, a phase II trial was initiated using a regimen of IL-21 (30 µg/kg/d × 5) followed by 9 days of rest in patients with previously untreated metastatic melanoma. Early results indicate that 1 of 14 patients initially treated achieved a CR (subsequently progressing in the central nervous system), and 9 others had SD.⁴⁸ This study is continuing with recruitment of a second patient cohort. A National Cancer Institute of Canada phase II trial also will evaluate the safety and activity of recombinant human IL-21 in previously untreated metastatic melanoma.

TLR agonists

At least nine known Toll-like receptors (TLRs), which act as pattern-recognition receptors for innate immune responses, are known to exist. In preclinical tumor models, stimulation of TLR9 by natural or synthetic molecules directly activates DCs and indirectly induces an innate immune response.⁴⁹ The clinical and immunologic effects of weekly injections of the synthetic oligonucleotide TLR9 agonist PF-3512676 (also called CpG 7909) were tested in a phase II trial in 20 patients with unresectable stage IIIb/c or stage IV melanoma.⁵⁰ Few adverse events were observed; overall treatment was well tolerated. Two patients had a confirmed PR,

one of which was ongoing at 140+ weeks; three patients had SD. Evidence of immune activation included induction of an activated phenotype of plasmacytoid DCs, elevation of serum levels of 2',5'-oligoadenylate (a surrogate marker of type I interferon production), and stimulation of NK cytotoxicity. Additional clinical evaluation of PF-3512676 in combination with other immune modulators is warranted. Other TLR agonists may prove useful as activators of anti-tumor immune responses.

Strategies to inhibit T_{reg} cells

Studies suggest that tumor cells can recruit T_{reg} cells to locally inhibit the antitumor immune response, creating localized tumor-specific immune tolerance, a major barrier to successful anticancer immunotherapy.⁵¹ The phenotype of lymphocytes with T_{reg} cell function can vary (although it is generally characterized as expressing CD4⁺, CD25^{high}, CTLA4, GITR, and Foxp3), and no markers specific for human T_{reg} cells have been defined that would allow specific elimination of T_{reg} cells or suppression of their function. Clinical strategies currently used include low-dose cyclophosphamide and denileukin diftitox (Ontak; CD25 immunotoxin). Although the approaches are perhaps partially and transiently effective in diminishing CD25^{high} T_{reg} cells, better strategies are needed. Controversy exists over the role of anti-CTLA4 in suppressing T_{reg} cell function; the majority of preclinical and clinical data suggest, however, that the major antitumor effects of anti-CTLA4 are unrelated to T_{reg}-cell inhibition.

Potential combination strategies

Individual agents such as IL-2 and anti-CTLA4 can produce impressive durable CRs in only a small fraction of patients. The complex regulatory checkpoints to immune cell activation and multiple tumor-induced immune-

suppressive mechanisms suggest that no single agent will significantly impact the disease course in most patients. Inevitably, combinations will be needed to produce optimal antitumor activity. The right combination for each patient will depend on the individual's tumor biology and host factors; however, at this time, identification of the relevant biology and host factors that would predict response to an individual agent or combination is not possible.

Nevertheless, preclinical data provide a compelling rationale for a first generation of combinations to test in clinical trials. For example, preclinical data strongly support combinations of vaccines or other methods to release antigen to APCs (such as chemotherapy or irradiation) with anti-CTLA4 or anti-CD137. In mouse tumor models, combinations of anti-CD137 with anti-PDL1 produced particularly impressive tumor regression despite minimal or no activity of these agents when used alone. Similar results could be expected with combinations of anti-CTLA4 and anti-PD1 (or anti-PDL1). Combinations of anti-CTLA4 with anti-CD137 produced enhanced antitumor effects. In animal tumor models, T_{reg} inhibition increased the activity of anti-CTLA4 and vaccines.⁵² In the future, it may be possible to identify the various strategies used by an individual's melanoma to blunt T-cell activation and effector responses. Because evidence exists that many patients with melanoma already have activated tumor-specific T cells at the time of presentation, excellent antitumor activity may be observed by administering a rational combination of agents that break down the tumor's immune defenses.

Adoptive immunotherapy

Adoptive immunotherapy is an antitumor treatment that involves the isolation, *in vitro* expansion, and activation of antigen-specific cells,

followed by autologous administration.⁵³ Early clinical studies of adoptive immunotherapy with infusion of activated autologous tumor-specific T lymphocytes in metastatic melanoma demonstrated modest activity. In the largest of these studies, autologous tumor-infiltrating lymphocytes plus high-dose bolus IL-2 with or without concomitant cyclophosphamide resulted in objective responses in 34% of 86 patients with metastatic melanoma.⁵⁴

Several factors may have limited the activity of adoptive immunotherapy. For example, infused cells did not persist *in vivo*, and the activity of the infused cells may have been blunted by preexisting T_{reg} cells. Basic studies in animal models showed that adoptively transferred T cells demonstrated increased proliferation and survival if the host was first depleted of existing lymphocytes. This finding probably occurred because depletion of existing lymphocytes caused a rise in circulating cytokines, such as IL-7 and IL-15, which were necessary for the proliferation and survival of the adoptively transferred cells.⁵⁵⁻⁵⁹ In addition, lymphodepletion prior to adoptive transfer of tumor-specific T cells theoretically removes preexisting T_{reg} cells.

Following animal models that confirmed the potential increased efficacy of tumor antigen-specific T cells administered following lymphodepletion in tumor-bearing mice, the National Cancer Institute Surgery Branch began a series of protocols to assess the activity of tumor-infiltrating lymphocytes (TILs) and IL-2 in patients with metastatic melanoma who were prepared with a lymphodepleting but nonmyeloablative regimen of cyclophosphamide and fludarabine. Among 35 patients, all but 1 of whom had progressed following high-dose IL-2, 51% demonstrated a PR or CR.⁶⁰ Subsequent studies are focusing on myeloablative preparative regimens prior to TIL transfer and the transfer of T cells genetically modi-

fied to express melanoma-specific T-cell receptors.

Conclusion

Immunotherapy is the only treatment that offers the possibility of durable remissions in patients with metastatic melanoma. For patients able to tolerate IL-2–related toxicity, IL-2 remains the treatment of choice. Anti-CTLA4 also has been shown to produce durable remission in a small fraction of patients, including patients previously unresponsive to IL-2. Newer agents that release regulatory checkpoints to T-cell activation or provide additional T-cell activation signals, as well as agents that reverse or blunt tumor-related defenses blocking effector T-cell function, offer substantial promise for improving antitumor immune responses and overall outcome. Many of these agents are in phase I or II clinical trials. Inevitably, optimal therapy will require rational combinations of these agents with each other or with other targeted therapy, chemotherapy, or radiotherapy. However, substantial challenges exist. Although significant progress has been made, strategies for choosing the appropriate therapeutic regimen from the large number of possible permutations is difficult. In addition, the optimization of combination regimens, dosing, scheduling, and overcoming potentially increased toxicity related to induction of autoimmunity are concerns that need to be addressed.

Roundtable discussion

The roundtable discussion on key issues in immunotherapeutic approaches to the treatment of metastatic melanoma included Dr. Mario Sznol and Dr. Michael B. Atkins.

What is the overall state of immunotherapy for melanoma?

Dr. Sznol: Most of the effort to date has been in the development of cancer vaccines, but the results have

been disappointing. High-dose IL-2 can be effective, but durable responses are observed in only ~5% of patients. We believe one reason for failure with this approach has been the regulatory checkpoints that limit immune activation. In addition, we should remember that even when a good immune response occurs, the tumor creates a hostile microenvironment for the immune effector cells and may block their function.

Another concern is that when we try to induce immune responses, for example, when giving a vaccine or IL-2, we may also be inducing regulatory immunosuppressive mechanisms, such as T_{reg} cells. We don't know how to control or negate the effects of increased T_{reg} lymphocytes, which are increased by IL-2 and vaccines.

New drugs that address some of these limitations are under development. These drugs may be active alone, ie, without vaccines or specific immunization against tumor antigens, or through broad immune activation that may release regulatory checkpoints or block tumor immunosuppressive factors. These new drugs may be used to increase the antitumor activity of vaccines. I believe they may be necessary for successful development of anticancer vaccines. Therefore, combination treatment strategies that affect the immune system at various points will probably be important.

What is the most promising pathway under investigation for melanoma therapy?

Dr. Sznol: Anti-CTLA4 antibodies are probably the best example of an approach targeted to release a regulatory checkpoint for T-cell activation. The kinetics of tumor response to anti-CTLA4 agents is interesting and unique. Early disease progression or long-term stable disease sometimes occurs before a later response. As with IL-2, we've also observed mixed responses, which, in some cases, appear to have provided overall benefit for

the individual patient. An observation that is not fully understood is a fairly strong correlation between autoimmune events and tumor response.

Although the anti-CTLA4 monoclonal antibodies are well tolerated overall, toxicities do occur, usually with the second or subsequent infusions. The side effects are immune related and can include a pruritic rash, pituitary dysfunction, hepatitis, and colitis (rarely leading to bowel perforation). Algorithms for appropriate early recognition and management will help to assure that any potential side effects are addressed in a timely and effective fashion.

What other immunotherapeutic approaches show promise?

Dr. Sznol: In addition to anti-CTLA-4, several other agents are promising, including antibodies to TGF- β , CD40, CD137, and PD1 and newer cytokines such as IL-7 and IL-21. They work by various mechanisms; for some agents, multiple parts of the immunologic response are affected, enhancing antigen presentation, increasing T-cell proliferation, or blocking tumor defenses. A promising approach is the use of adoptive transfer of melanoma-specific T cells after lymphodepleting chemotherapy, as reported by the National Cancer Institute. In the future, we may use genetically modified lymphocytes to recognize melanoma specifically and to improve their function within the tumor.

Dr. Atkins: Although still in phase I development, anti-PD1 antibodies may prove important. I believe that since the PD1 pathway negatively regulates T-cell activation and immune responses in a broad sense, it may counteract immunomodulatory therapies by providing tumors with an additional means of immune escape. It is a major obstacle to treatment with activated tumor-specific lymphocytes and may be a reason why activated tumor-specific lymphocytes that traffic into tumors are unable to kill them.

What are appropriate endpoints for melanoma trials of immunotherapy?

Dr. Szabolcsovics: Tumor regression is the best clinical endpoint for such trials.

Dr. Atkins: As opposed to delay in progression-free survival without shrinkage?

Dr. Szabolcsovics: I have not seen any evidence in immunotherapy trials that stable disease without any shrinkage is beneficial for the patient or correlates with any improved outcome. That doesn't mean it won't happen, but we will have to wait for the phase III trials to know whether stable disease translates into improved overall survival.

Dr. Atkins: How important is median survival?

Dr. Szabolcsovics: Clearly, it's important. However, because we are currently only affecting a small subpopulation of patients within any group, it's a mistake to look at only median survival as the measure of drug benefit.

Dr. Atkins: I agree completely.

What are the implications of these new approaches for the community oncologist?

Dr. Szabolcsovics: Unlike high-dose IL-2, which requires administration in the hospital setting and relatively intensive attention from the treating physician or in-hospital medical personnel, community oncologists will be more inclined to use these agents, probably before chemotherapy, because of the potential for durable responses.

Dr. Atkins: I believe community oncologists will be comfortable using these agents because, for the most part, they're easy to administer. Anti-CTLA4 antibodies are being designed as outpatient treatments. Community oncologists are familiar with administration of antibodies and outpatient therapies. We have not seen many problems with these antibodies during the early period after administration; many patients have no problems at all. Unfortunately, these are the pa-

tients who for the most part don't appear to respond to therapy either.

Dr. Szabolcsovics: I agree but would add that for safe administration of some of these agents, anti-CTLA4 in particular, it will be important for community oncologists to become familiar with the presentation and management of immune breakthrough events. Academic oncologists need to understand that anti-CTLA4 will be used first line in patients in the community. We need clinical trials that take advantage of the fact that anti-CTLA4, even in nonresponders, may affect response or toxicity from subsequent therapies.

References

- Allison JP, Krummel MF. The Yin and Yang of T cell costimulation. *Science* 1995;270:932-933.
- Kapadia D, Fong L. CTLA-4 blockade: autoimmunity as treatment. *J Clin Oncol* 2005;23:8926-8928.
- Waterhouse P, Penninger JM, Timms E, et al. Lymphoproliferative disorders with early lethality in mice deficient in Ctl4. *Science* 1995;270:985-988.
- Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. *Science* 1996;271:1734-1736.
- Hurwitz AA, Yu TF, Leach DR, Allison JP. CTLA-4 blockade synergizes with tumor-derived granulocyte-macrophage colony-stimulating factor for treatment of an experimental mammary carcinoma. *Proc Natl Acad Sci U S A* 1998;95:10067-10071.
- van Elsas A, Hurwitz AA, Allison JP. Combination immunotherapy of B16 melanoma using anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and granulocyte/macrophage colony-stimulating factor (GM-CSF)-producing vaccines induces rejection of subcutaneous and metastatic tumors accompanied by autoimmune depigmentation. *J Exp Med* 1999;190:355-366.
- Kwon ED, Foster BA, Hurwitz AA, et al. Elimination of residual metastatic prostate cancer after surgery and adjunctive cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) blockade immunotherapy. *Proc Natl Acad Sci U S A* 1999;96:15074-15079.
- Kwon ED, Hurwitz AA, Foster BA, et al. Manipulation of T cell costimulatory and inhibitory signals for immunotherapy of prostate cancer. *Proc Natl Acad Sci U S A* 1997;94:8099-8103.
- Peggs KS, Quezada SA, Korman AJ, Allison JP. Principles and use of anti-CTLA4 antibody in human cancer immunotherapy. *Curr Opin Immunol* 2006;18:206-213.
- Phan GQ, Yang JC, Sherry RM, et al. Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. *Proc Natl Acad Sci U S A* 2003;100:8372-8377.
- Small EJ, Tchekmedyian NS, Rini BI, Fong L, Lowy I, Allison JP. A pilot trial of CTLA-4 blockade with human anti-CTLA-4 in patients with hormone-refractory prostate cancer. *Clin Cancer Res* 2007;13:1810-1815.
- Attia P, Phan GQ, Maker AV, et al. Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti-cytotoxic T-lymphocyte antigen-4. *J Clin Oncol* 2005;23:6043-6053.
- Weber JS, Targan S, Scotland R, et al. Phase II trial of extended dose anti-CTLA-4 antibody ipilimumab (formerly MDX-010) with a multi-peptide vaccine for resected stages IIIc and IV melanoma. *J Clin Oncol* 2006;24(18S):2510.
- Maker AV, Phan GQ, Attia P, et al. Tumor regression and autoimmunity in patients treated with cytotoxic T lymphocyte-associated antigen 4 blockade and interleukin 2: a phase I/II study. *Ann Surg Oncol* 2005;12:1005-1016.
- Fischkoff SA, Hersh E, Weber J, et al. Durable responses and long-term progression-free survival observed in a phase II study of MDX-010 alone or in combination with dacarbazine (DTIC) in metastatic melanoma. *J Clin Oncol* 2005;23(16S):7525.
- Weber JS, Hersh EM, Yellin M, et al. The efficacy and safety of ipilimumab (MDX-010) in patients with unresectable stage III or stage IV malignant melanoma. *J Clin Oncol* 2007;25(18S):8523.
- Weber J. Review: anti-CTLA-4 antibody ipilimumab: case studies of clinical response and immune-related adverse events. *Oncologist* 2007;12:864-872.
- Hamid O, Urban WJ, Yellin M, et al. Kinetics of response to ipilimumab (MDX-010) in patients with stage III/IV melanoma. *J Clin Oncol* 2007;25(18S):8525.
- Fong L, Kavanagh B, Hou Y, et al. Combination immunotherapy with GM-CSF and CTLA-4 blockade for hormone refractory prostate cancer: balancing the expansion of activated effector and regulatory T cells. *J Clin Oncol* 2007;25(18S):3001.
- Downey SG, Klapper JA, Smith FO, et al. Prognostic factors related to clinical response in patients with metastatic melanoma treated by CTL-associated antigen-4 blockade. *Clin Cancer Res* 2007;13:6681-6688.
- Beck KE, Blansfield JA, Tran KQ, et al. Enterocolitis in patients with cancer after antibody blockade of cytotoxic T-lymphocyte-associated antigen 4. *J Clin Oncol* 2006;24:2283-2289.
- Ribas A, Hanson DC, Noe DA, et al. Tremelimumab (CP-675,206), a cytotoxic T lymphocyte associated antigen 4 blocking monoclonal antibody in clinical development for patients with cancer. *Oncologist* 2007;12:873-883.
- Ribas A, Camacho LH, Lopez-Beres-

- tein G, et al. Antitumor activity in melanoma and anti-self responses in a phase I trial with the anti-cytotoxic T lymphocyte-associated antigen 4 monoclonal antibody CP-675,206. *J Clin Oncol* 2005;23:8968–8977.
24. Gomez-Navarro J, Antonia S, Sosman J, et al. Survival of patients (pts) with metastatic melanoma treated with the anti-CTLA4 monoclonal antibody (mAb) CP-675,206 in a phase I/II study. *J Clin Oncol* 2007;25(18S):8524.
25. Ribas A, Antonia S, Sosman J, et al. Results of a phase II clinical trial of 2 doses and schedules of CP-675,206, an anti-CTLA4 monoclonal antibody, in patients (pts) with advanced melanoma. *J Clin Oncol* 2007;25(18S):3000.
26. Elliott RL, Blobe GC. Role of transforming growth factor beta in human cancer. *J Clin Oncol* 2005;23:2078–2093.
27. Schlingensiepen R, Goldbrunner M, Szyrach MN, et al. Intracerebral and intrathecal infusion of the TGF-beta 2-specific antisense phosphorothioate oligonucleotide AP 12009 in rabbits and primates: toxicology and safety. *Oligonucleotides* 2005;15:94–104.
28. Schlingensiepen KH, Schlingensiepen R, Steinbrecher A, et al. Targeted tumor therapy with the TGF-beta2 antisense compound AP 12009. *Cytokine Growth Factor Rev* 2006;17:129–139.
29. Oettle H, Seufferlein T, Schmid R, et al. Preliminary results of a phase I/II study in pancreatic carcinoma, malignant melanoma, and colorectal carcinoma with the TGF- β 2 inhibitor AP 12009. *J Clin Oncol* 2007;25(18S):4607.
30. Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. *Proc Natl Acad Sci U S A* 2002;99:12293–12297.
31. Blank C, Kuball J, Voelkl S, et al. Blockade of PD-L1 (B7-H1) augments human tumor-specific T cell responses in vitro. *Int J Cancer* 2006;119:317–327.
32. Vonderheide RH. Prospect of targeting the CD40 pathway for cancer therapy. *Clin Cancer Res* 2007;13:1083–1088.
33. Bedian V, Donovan C, Gardner J, et al. In vitro characterization and pre-clinical pharmacokinetics of CP-870,893, a human anti-CD40 agonist antibody. *J Clin Oncol* 2006;24(18S):2539.
34. Gladue RP, Cole SH, Donovan C, et al. In vivo efficacy of the CD40 agonist antibody CP-870,893 against a broad range of tumor types: impact of tumor CD40 expression, dendritic cells, and chemotherapy. *J Clin Oncol* 2006;24(18S):2514.
35. Hunter TB, Alsarraj M, Gladue RP, Bedian V, Antonia SJ. An agonist antibody specific for CD40 induces dendritic cell maturation and promotes autologous anti-tumor T-cell responses in an in vitro mixed autologous tumor cell/lymph node cell model. *Scand J Immunol* 2007;65:479–486.
36. Vonderheide RH, Flaherty KT, Khalil M, et al. Clinical activity and immune modulation in cancer patients treated with CP-870,893, a novel CD40 agonist monoclonal antibody. *J Clin Oncol* 2007;25:876–883.
37. Dummer W, Bastian BC, Ernst N, Schanzle C, Schwaaf A, Brocker EB. Interleukin-10 production in malignant melanoma: preferential detection of IL-10-secreting tumor cells in metastatic lesions. *Int J Cancer* 1996;66:607–610.
38. Sato T, McCue P, Masuoka K, et al. Interleukin 10 production by human melanoma. *Clin Cancer Res* 1996;2:1383–1390.
39. Yue FY, Dummer R, Geertsens R, et al. Interleukin-10 is a growth factor for human melanoma cells and down-regulates HLA class-I, HLA class-II and ICAM-1 molecules. *Int J Cancer* 1997;71:630–637.
40. Garcia-Hernandez ML, Hernandez-Pando R, Gariglio P, Berumen J. Interleukin-10 promotes B16-melanoma growth by inhibition of macrophage functions and induction of tumor and vascular cell proliferation. *Immunology* 2002;105:231–243.
41. Gerlini G, Tun-Kyi A, Dudli C, Burg G, Pimpinelli N, Nestle FO. Metastatic melanoma secreted IL-10 down-regulates CD1 molecules on dendritic cells in metastatic tumor lesions. *Am J Pathol* 2004;165:1853–1863.
42. Brady J, Hayakawa Y, Smyth MJ, Nutt SL. IL-21 induces the functional maturation of murine NK cells. *J Immunol* 2004;172:2048–2058.
43. Zeng R, Spolski R, Finkelstein SE, et al. Synergy of IL-21 and IL-15 in regulating CD8⁺ T cell expansion and function. *J Exp Med* 2005;201:139–148.
44. Sondergaard H, Frederiksen KS, Thygesen P, et al. Interleukin 21 therapy increases the density of tumor infiltrating CD8⁺ T cells and inhibits the growth of syngeneic tumors. *Cancer Immunol Immunother* 2007;56:1417–1428.
45. He H, Wisner P, Yang G, et al. Combined IL-21 and low-dose IL-2 therapy induces anti-tumor immunity and long-term curative effects in a murine melanoma tumor model. *J Transl Med* 2006;4:24.
46. Ma HL, Whitters MJ, Konz RF, et al. IL-21 activates both innate and adaptive immunity to generate potent antitumor responses that require perforin but are independent of IFN-gamma. *J Immunol* 2003;171:608–615.
47. Davis ID, Skrumsager BK, Cebon J, et al. An open-label, two-arm, phase I trial of recombinant human interleukin-21 in patients with metastatic melanoma. *Clin Cancer Res* 2007;13:3630–3636.
48. Davis ID, Brady B, Millward M, et al. Anti-tumor activity of recombinant human interleukin-21 (rIL-21): preliminary data from a phase 2a study in patients with stage IV malignant melanoma (MM) without prior treatment. *J Clin Oncol* 2007;25(18S):3055.
49. Krieg AM, Vollmer J. Toll-like receptors 7, 8, and 9: linking innate immunity to autoimmunity. *Immunol Rev* 2007;220:251–269.
50. Pashenkov M, Goess G, Wagner C, et al. Phase II trial of a Toll-like receptor 9-activating oligonucleotide in patients with metastatic melanoma. *J Clin Oncol* 2006;24:5716–5724.
51. Wang HY, Wang RF. Regulatory T cells and cancer. *Curr Opin Immunol* 2007;19:217–223.
52. Kocak E, Lute K, Chang X, et al. Combination therapy with anti-CTL antigen-4 and anti-4-1BB antibodies enhances cancer immunity and reduces autoimmunity. *Cancer Res* 2006;66:7276–7284.
53. Dudley ME, Rosenberg SA. Adoptive-cell-transfer therapy for the treatment of patients with cancer. *Nat Rev Cancer* 2003;3:666–675.
54. Rosenberg SA, Yannelli JR, Yang JC, et al. Treatment of patients with metastatic melanoma with autologous tumor-infiltrating lymphocytes and interleukin 2. *J Natl Cancer Inst* 1994;86:1159–1166.
55. Cheever MA, Greenberg PD, Fefer A. Specificity of adoptive chemoimmunotherapy of established syngeneic tumors. *J Immunol* 1980;125:711–714.
56. North RJ. Cyclophosphamide-facilitated adoptive immunotherapy of an established tumor depends on elimination of tumor-induced suppressor T cells. *J Exp Med* 1982;155:1063–1074.
57. Eberlein TJ, Rosenstein M, Rosenberg SA. Regression of a disseminated syngeneic solid tumor by systemic transfer of lymphoid cells expanded in interleukin 2. *J Exp Med* 1982;156:385–397.
58. Rosenberg SA, Spiess P, Lafreniere R. A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. *Science* 1986;233:1318–1321.
59. Robbins PF, Dudley ME, Wunderlich J, et al. Cutting edge: persistence of transferred lymphocyte clonotypes correlates with cancer regression in patients receiving cell transfer therapy. *J Immunol* 2004;173:7125–7130.
60. Dudley ME, Wunderlich JR, Yang JC, et al. Adoptive cell transfer therapy following non-myceloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. *J Clin Oncol* 2005;23:2346–2357.

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