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Immunotherapy for Metastatic Melanoma

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ABSTRACT

Melanoma has traditionally been considered an immunogenic tumor. A number of approaches have been studied for enhancement of antitumor immunity. The first cytokine approved for the treatment of metastatic melanoma, interleukin-2, has resulted in prolonged responses in a small subset of patients, providing hope that immunotherapy might be useful for this disease. Ipilimumab, a monoclonal antibody to CTLA-4, was recently approved and a number of other promising investigational approaches are currently being pursued. This manuscript discusses more recent advances in the treatment of melanoma employing a variety of immune-enhancing approaches. J. Cell. Biochem. 113: 725–734, 2012.

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Melanoma is the most aggressive form of skin cancer. Recent decades have seen a rise in both incidence and mortality from metastatic disease [Jilaveanu et al., 2009]. In 2011, an estimated 70,230 new cases of melanoma will be diagnosed (approximately 40,010 males and 30,220 females) and 8,790 deaths are expected (approximately 5,750 males and 3,040 females) [Siegel et al., 2011]. This is a sharp rise when compared with a decade ago; in 2001, there were 51,400 cases and 7,800 deaths [Greenlee et al., 2001]. With median survivals of roughly 7 months until recently and a miniscule 5-year survival rate of less than 10%, metastatic melanoma remains one of the most difficult cancers to treat effectively. With the advent of new innovations in treatment of advanced melanoma, treatment options are likely to be substantially less limited and, more importantly, overall survival (OS) is expected to improve significantly.

Immunotherapy has been used for a number of decades to treat cancer. Melanoma, in particular, as a disease that has the ability to invoke a spontaneous immune response, has been a prime target for immunotherapeutic approaches. Though immunotherapy-based approaches have had varying degrees of success, they are demonstrating increasing promise in the treatment of metastatic melanoma.

Since the initial success with interleukin-2 (IL-2) and interferon-α, and the subsequent clinical approval of IL-2, the focus has included alternative cytokines, adoptive immunotherapy, immunomodulators, dendritic cell therapies, peptide vaccines, and combinational immunotherapies. Though the clinical responses to these immunotherapies until recently were somewhat disappointing, advances have been made in the past decade that provide renewed optimism that immune modulation will continue to improve. Perhaps the biggest advances have been made in the disruption of immune checkpoints and adoptive cell therapy. Further work is needed to identify predictive biomarkers to enhance the therapeutic ratio for melanoma patients. Though these approaches are still evolving, the intention of this article is to discuss the recent advances and future directions.

CYTOKINES

Cytokines are small protein molecules secreted by cells of the immune system. Cytokines are signaling molecules that function as regulators and immune-modulating agents. The extent of the effect that a particular cytokine might have on immune activity depends greatly on a number of factors including abundance of the cytokine or the complementary receptor in the host system, ability and potency of signaling to downstream targets, and redundancy of function, to name a few.

The first immunotherapy approved by the Federal Drug Administration (FDA) for the treatment of unresectable melanoma was the cytokine IL-2. Treatment with high-dose IL-2 can result in prolonged responses in a minority of patients [Jilaveanu et al., 2009]. The overall response rate to IL-2 is in the order of 10%, and approximately half of those are very durable [Jilaveanu et al., 2009].

Conflicts of interest: None.

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Lower doses of IL-2 have been used alone and in combination with interferon and chemotherapy (biochemotherapy). The response rate to biochemotherapy is higher, but the OS was not superior to that of chemotherapy alone [Jilaveanu et al., 2009]. Attempts have been made to identify factors that might predict response or resistance to IL-2 [Sabatino et al., 2009]. However, no biomarkers have been validated on independent patient cohorts.

With the initial activity of IL-2 in the treatment of advanced melanoma, other interleukins, such as interleukin-21 (IL-21), have been studied in early phase clinical trials. IL-21 regulates B cells, and also induces activation of CD8+ T cells and/or NK cells, promoting a potent antitumor effect in pre-clinical models and in patients [Frederiksen et al., 2008]. Phase I and II studies utilizing IL-21 for treatment of melanoma have been completed [Davis et al., 2009; Rasmussen et al., 2010; Hashmi and Van Veldhuizen, 2010; Petrella et al., 2010]. Antitumor activity was reported at a dose of 30 μg/kg, which was generally well tolerated. In one phase II clinical study, 40 patients with stage IV melanoma were given three different IL-21 dose regimens. Of the 37 evaluable patients, 9 had a partial response (PR) (ORR = 24.3%; median duration = 5 months), 16 had stable disease (SD), and 12 had progressive disease (PD). The likelihood of response was not dependent on dose, IL-21 receptor expression, or B-Raf mutational status. The adverse effects reported with IL-21 were mild, minor fatigue and muscle pain (flu-like symptoms), transient lymphopenia and a rash, similar to those induced by other cytokines. Based on studies in mouse tumor models, intratumoral delivery of IL-21 could potentially inhibit tumor growth more potently [Costanzo et al., 2010]. Recent pre-clinical studies have shown increased efficacy when combining IL-21 with other cytokines or fusing it to GMCSF [Williams et al., 2010; Zhao et al., 2010]. The latter has been shown to induce distinct, but complimentary, elements of the immune response not seen with either of the two cytokines alone [Williams and Galipeau, 2011]. These unique synergistic effects can be exploited in the future for treatment of melanoma.

Other cytokines in clinical development include IL-7, IL-15, and IL-18, all of which activate cytotoxic T cells. Immunotherapy targeted to induce differentiation of CD8(+) T cells at the tumor site toward effector and memory stages is believed to be a key step for the efficacy of antitumor response. In this regard, cytokines such as IL-7 and IL-15 alone or in combination may be exploited to promote antigen-independent maturation of antitumor CD8(+) T cells [Le et al., 2009]. IL-7 and IL-15 belong to the IL-2 gamma-chain receptor cytokine family. IL-7 and IL-15 have been shown to induce faster and more prolonged T-cell proliferation and less apoptosis of activated T cells than IL-2 [Caserta et al., 2010; Li et al., 2010]. A phase I trial of IL-7 in combination with peptide vaccine therapy comprising gp100 and MART-1 antigens in patients with metastatic melanoma has been completed, while a phase I/II trial using IL-15 in patients with resected stage IIIc/IV melanoma is currently accruing participants (www.clinicaltrial.gov). To avoid systemic toxicities, attempts are underway to develop methods of targeted delivery of cytokines to the tumor site. These antibodies allow targeting surface markers on melanoma cells for the specific delivery of chemically or genetically linked immune-stimulating cytokines. In a phase I/II clinical trial, the activity of EMD 273063 (hu14.18-IL2) (EMD Lexigen Research Center), a humanized anti-GD2 monoclonal antibody fused to IL-2, was tested in patients with unresectable melanoma [Ribas et al., 2009]. EMD 273063 showed some biologic activity with increased immune-related cytokines and intratumoral changes in some patients. Post-treatment tumors showed decreased staining for GD2, the target of EMD 273063, in four out of seven cases studied, and in most cases a trend was seen toward increased intratumoral CD3+ T cells and CD8+ T cells.

Another class of cytokines used for melanoma, the interferons, activate immune cells, such as macrophages and natural killer (NK) cells, and also up-regulate antigen presentation to T lymphocytes, resulting in an increase in recognition of antigens or tumor cells. Interferon-alpha (INFa) has both antiproliferative and immunomodulatory effects. For this reason, INFa administered by a variety of schedules was studied in several trials, but were not shown to significantly improve survival for metastatic melanoma patients, when given as a single agent or in combination with chemotherapy, and these regimens were highly toxic [Jilaveanu et al., 2009]. Subsequent trials were performed at lower doses aimed at reducing toxicity, but still resulting in no significant difference in OS. We note that in the adjuvant setting, in patients with resected stage II or III disease, while use of interferon has not consistently resulted in significant improvement in OS, studies have consistently demonstrated significant improvement in relapse-free survival [Mocellin et al., 2010]. Granulocyte-Macrophage-Colony-Stimulating Factor (GMCSF) is another cytokine that has been studied for melanoma treatment. GMCSF recruits monocytes that have the potential to be cytotoxic to tumor cells, activate macrophages, and activate dendritic cells responsible for immune response by T cells [Grabstein et al., 1986; Szabolcs et al., 1995]. GMCSF has been given to patients with both unresected and resected disease. In one phase II trial, 31 patients with unresectable metastatic disease were treated with oral temozolomide followed by subcutaneous GMCSF, interferon-alfa, and recombinant IL-2. CR was documented for four patients (13%) with an additional four patients (13%) demonstrating PR [Weber et al., 2005]. In a recent phase III study in the adjuvant setting, GMCSF was administered to patients with resected Stage III/IV disease. Although the study recruited a large patient sample (815 patients), there was no statistically significant difference in OS, but there was a trend toward improved survival in the treatment arm (72.1 months) compared to the placebo arm (59.8 months) [Lawson et al., 2010]. The 250 mcg, 14-day regimen (every 28 days for up to 1 year) was well tolerated, with primarily grade I/II toxicities. Further analysis of this study suggested that patients with Stage IV disease may see the most benefit. However, further study in randomized trials is necessary to corroborate this subset analysis [Lawson et al., 2010].

VACCINES

Another avenue that has been pursued for many years is the utilization of vaccines. Cancer vaccines have been studied since William Coley's fortuitous findings that bacterial toxins administered to cancer patients resulted in some patient benefit [Kirkwood et al., 2008]. More recent findings in the field have led to the first

FDA-approved cancer vaccine, Sipuleucel-T (from Dendreon Corporation) for prostate cancer. This is an autologous dendritic cell (DC) immunotherapy, which involves ex-vivo engineering of DCs to recognize prostate acid phosphatase. The primary function of DCs is to process antigenic material and in turn present that antigen on its surface to other cells, functioning as messengers between innate and adaptive immunities of the host. The surface presentation typically activates cells such as CD8+ cytotoxic T cells to respond to that antigen. The primary antitumor effector function is believed to lie with the activation of cytotoxic CD8+ T cells and of NK cells. However, since tumors are considered to poorly present antigens, and also due to high numbers of DC-inhibiting regulatory T Cells (Treg cells) associated with melanoma, this cytotoxic T cell/NK cell response has to be further augmented in order to be effective for treating melanoma [Ilkovitch and Lopez, 2008]. The objective of modulating DCs by vaccine is to elicit an immune response, activating cytotoxic T lymphocytes (CTLs), which will react with and eventually reduce, or hopefully even eradicate, the tumor. DCs can process peptides from various tumor antigens, present the antigens, and activate immune responses [Alexandrescu et al., 2010]. Several experimental regimens have used peptide antigens, autologous, and/or allogenic tumor lysates.

Various clinical trials incorporating DC vaccines have been conducted, as reviewed by Engell-Noerregaard et al. [2009]. Though repeated T-cell activation with antigen-loaded DCs does occur, most of these studies have demonstrated that expansion of specific immune responses to tumor-antigen is often ephemeral, seldom yielding durable responses [Banchereau et al., 2001; Rosenberg et al., 2005; Alexandrescu et al., 2010]. Some successes have been noted with peptide- or tumor lysate-pulsed DCs, rendering them capable of eliciting CTL response. However, autologous DCs of cancer patients lack responsiveness without ex vivo manipulation to trigger their activation [Sivendran et al., 2010]. A recently completed phase I/II clinical trial utilizing DC-based therapy specifically for the treatment of MM resulted in treatment-associated SD for a reported 24% of patients, with SD being correlated to prolonged survival. However, only 13% of patients demonstrated continued survival after 4 months, and a mere 6% had prolonged SD for more than 6 months [Trepiakas et al., 2010]. Advancing the clinical efficacy of these regimens will require both more efficient stimulation of DCs and more effective immunogenic adjuvants.

Manipulation of Toll-like receptors (TLRs) is one approach to enhance DC-based therapy. TLRs bind to agonists that are capable of inducing inflammatory response. Typically these antigenic agonists are delivered via the antigen presenting cells (APCs) as part of the innate immune response. The most common and potent of these APCs are DCs. As DCs present antigenic agonists to the TLRs, pro-inflammatory responses are elicited. The use of TLR agonists as adjuvants to mature DCs is an enticing approach to trigger and enhance antitumor responses. This pro-inflammatory response in combination with response from more specific, targeted therapies has been studied in advanced melanoma [Brichard and Lejeune, 2007; Bogunovic et al., 2011]. Successfully augmented immunologic responses were found in melanoma patients treated with either peptide- or protein-based vaccines coupled with adjuvant TLR agonists Imiquimod (TLR7) and Resiquimod (TLR7/8) [Shackleton et al., 2004; Adams et al., 2008; Bogunovic et al., 2011]. Radboud University Nijmegen Medical Centre is currently recruiting patients for a study of TLR ligand matured DC vaccination in melanoma patients (www.clinicaltrials.gov).

Recent in vitro and mouse in vivo work with TLR agonists demonstrates that certain TLR agonists or combinations thereof can be synergistic in maturing DCs while others are actually immunosuppressive, inhibiting inflammatory cytokine production and or T-cell priming [Bogunovic et al., 2011]. Efforts are also ongoing to enhance DC antigen presentation, such as the clinical trial currently being conducted by Celldex, Inc., using CDX-1401 in conjunction with resiquimod in metastatic melanoma expressing NY-ESO-1 (www.clinicaltrials.gov).

ANTIBODIES TARGETING IMMUNE CO-STIMULATORY OR CO-INHIBITORY MOLECULES

Therapeutic antibodies have been used in a number of cancer settings. Antibodies can circulate freely in the blood and the lymphatic system, where they can bind to targets either on the cell surface or in the blood. Monoclonal antibodies to inhibit or activate immune co-inhibitory or co-stimulatory molecules, respectively, have been used in recent trials, as summarized below.

The TNFR super-family comprises a growing list of receptors for membrane-bound and soluble cytokines all of which are expressed predominantly on cells of hematopoietic lineage. Examples of TNFR super-family members include: 4-1BB (CD137), OX-40R (CD134), CD40, the CD30 Ag, CD27, FAS (CD95), and DR3. A common function of the TNFR super-family members is the regulation of activation/proliferation or induction of apoptosis of lymphocytes, by delivering co-stimulatory signals to T cells. Co-stimulation is a requirement for T-cell activation and a lack of co-stimulation results in peripheral T-cell tolerance, which results in immune unresponsiveness or T-cell death through apoptosis. Animal models have shown that sustaining positive co-stimulatory signals will boost T-cell responses against tumor antigens and will facilitate the generation of cytotoxic T-cell (CTL) responses, which will ultimately mediate or induce tumor cell death [Croft, 2009].

AGONISTIC ANTIBODIES TO 4-1BB (CD137), OX-40 (CD134) AND CD40

Anti-4-1BB (CD137). 4-1BB (CD137) is an inducible costimulatory receptor expressed on various activated immune cells such as T cells, NK cells, DCs, eosinophils, and mast cells. Upon ligand binding, CD137 delivers anti-apoptotic signals, prevents T-cell death and can enhance antigen-specific T-cell activity [Sivendran et al., 2010].

Agonistic antibodies targeting 4-1BB (CD137) have been developed and tested in phase I/II clinical studies. A phase I dose-escalation study of BMS-663513 (anti-CD137 (4-1BB), Bristol Myers Squibb Co.) was conducted in patients with advanced solid-tumor malignancies including melanoma [Sznol et al., 2008]. BMS-663513 was administered every 3 weeks intravenously. The drug was well tolerated across a wide dose range (0.3–15 mg/kg) and

demonstrated limited clinical activity as a single agent in some patients, including patients with melanoma. Fatigue, transaminitis, and neutropenia were the primary reported side effects. Concomitant biomarker studies demonstrated increased expression of IFN-inducible genes in peripheral blood, circulating activated CD8 and CD4 T cells and serum neopterin levels. In a subset of patients, post-treatment biopsies showed increased expression of CD8a and IFNγ. Another randomized, multi-dose, phase II study of BMS-663513 administered at doses of 0.1, 1, or 5 mg/kg every 3 weeks or 1 mg/kg every 6 weeks, as second-line monotherapy in patients with stage III or IV melanoma, has been completed (www.clinicaltrials.gov).

Anti-OX-40R (CD134). OX-40 (CD134) is a co-stimulatory receptor, transiently expressed on activated CD4 and CD8 T cells. CD134 regulates T-cell function and survival. Upon ligand binding, CD134 enhances cytokine production and augments proliferation of both CD4 and CD8 T cells.

In a phase I trial, a mouse antihuman OX40 agonistic antibody was tested in patients with advanced tumors at escalating doses [Kovacsovics-Bankowski et al., 2009]. The overall toxicity was low and partial tumor regression was seen in 5 out of 20 patients. Correlative analyses showed induction of CD4+ T helper cells, CD8+ T cells, and NK cells in a dose-dependent manner.

Anti-CD40. CD40 is a transmembrane receptor expressed on various immune cells, as well as endothelium, platelets, and tumor cells. Activation of CD40 by ligand binding promotes B-cell and T-cell activation and stimulates apoptosis and growth of tumor cells [Sivendran et al., 2010]. Agonistic anti-CD40 antibodies trigger antitumor immunity. In a phase I clinical trial, a human IgG2 agonistic anti-CD40 antibody, CP870,893 (Pfizer, New York), was tested in 29 patients with advanced solid tumors, including melanoma. Patients received doses from 0.01 to 0.3 mg/kg and the dose-limiting toxicity was reported at 0.3 mg/kg (venous thromboembolism and headache). Four patients with melanoma (14% of all patients and 27% of melanoma patients) had objective PRs [Vonderheide et al., 2007].

ANTAGONISTIC ANTIBODIES TO IMMUNE CO-INHIBITORY MOLECULES: ANTI-CTLA4 AND ANTI-PD1 INHIBITION OF CYTOTOXIC T-LYMPHOCYTE ANTIGEN 4 (CTLA-4)

The first antibody-based immune therapy for melanoma, ipilimumab, was approved by the FDA in March 2011. Ipilimumab is a monoclonal antibody that inhibits CTLA-4, resulting in induction or augmentation of antitumor immunity. CTLA-4 is a member of the immunoglobulin super-family and acts as a negative regulator of the immune response. Up-regulation of CTLA-4 on the surface of CTLs, which are important mediators of specific antitumor responses, induces cell cycle arrest of CTLs and inhibits proliferation of these cells, contributing to immune evasion [Korman et al., 2006]. An inhibitory antibody against CTLA-4 eradicates inhibitory downstream signals and enhances T-cell activation, eliciting their cytotoxic antitumor response. Ipilimumab (MDX-010/BMS-734016, Medarex Inc./Bristol Myers Squibb Co., Princeton, NJ) was studied in a large number of clinical trials, as summarized by Thumar and Kluger [2010]. The pivotal trial that led to its approval for treatment of late-stage melanoma patients with unresectable stage III or IV melanoma randomized 676 patients to one of three arms [Thumar and Kluger, 2010]. Patients received either ipilimumab alone (137 patients), ipilimumab in combination with a gp100 peptide vaccine (403 patients), or gp100 alone (136 patients). Ipilimumab was administered in both arms at a dose of 3 mg/kg every 3 weeks for four treatments. In this relatively poor prognosis group of patients, there was significant improvement in median OS in the two arms that received ipilimumab compared to patients that received gp100 peptide alone, 10 months versus 6.4 months, respectively [Hodi et al., 2010]. Despite the reported OS benefit in patients treated with ipilimumab, response rates in this trial and other clinical trials with ipilimumab for melanoma range from 5 to 15% only, although many of these responses are durable [Thumar and Kluger, 2010]. Additional phase III trials have been completed, most notably a randomized trial comparing ipilimumab plus dacarbazine to dacarbazine alone in metastatic melanoma. Patients were randomized to receive ipilimumab (10 mg/kg) and DTIC (850 mg/m^2) or placebo and DTIC (850 mg/m^2) at weeks 1, 4, 7, 10 followed by DTIC alone every 3 weeks through week 22. Ipilimumab and DTIC significantly improved OS when compared to DTIC alone (median survival of 11.2 months vs. 9.1 months), with higher survival rates in the ipilimumab–dacarbazine group at 1 year (47.3% vs. 36.3%), at 2 years (28.5% vs. 17.9%), and at 3 years (20.8% vs. 12.2%) [Robert et al., 2011].

Combination studies including ipilimumab are underway. A combination trial of ipilimumab and anti-PD1 (described below) is ongoing (www.clinicaltrials.gov). A combination trial with ipilimumab and the inhibitor of mutated BRAF (present in approximately 50% of melanomas), vemurafenib, will start shortly. Ipilimumab has been given in combination with bevacizumab (a monoclonal antibody to VEGF) in a phase I trial for metastatic melanoma. Patients received 10 mg/kg ipilimumab plus 7.5 mg/kg or 15 mg/kg bevacizumab. Toxicity was not negligible, but the response rate appeared to be superior to that of ipilimumab alone. Among the 21 evaluable patients, eight PRs and six incidences of SD were reported [Hodi et al., 2011].

Tremelimumab (CP-675,206) is a fully human IgG2 monoclonal anti-CTLA-4 antibody which has been investigated in phase I, II, and III clinical studies. In the phase III trial for patients with metastatic melanoma, tremelimumab (15 mg/kg every 3 months for up to four doses) was compared to standard single agent chemotherapy [Ribas et al., 2008]. Single agent tremelimumab was well tolerated, but failed to demonstrate an OS benefit when compared to standard chemotherapy, temozolomide or dacarbazine (median OS was 11.8 months in the tremelimumab arm and 10.7 in the chemotherapy arm). Although targeting the same molecule, tremelimumab and ipilimumab might in fact have totally different mechanisms of inhibition that could explain the difference in their clinical efficacy. Tremelimumab is an IgG2 antibody, while ipilimumab is an IgG1 isotype, and even though in general IgG subclasses show more than 95% homology in the amino acid sequence, they can uniquely modulate immune response with differences in complement activation, binding to the Fc receptor and triggering of immune effector cells. It is also possible that the timing and dosing of the two anti-CTLA-4 regimens may be critical, and every 3-week ipilimumab induction might be more advantageous for effective immune stimulation compared to the every 90-day tremelimumab induction.

To improve the therapeutic ratio of CTLA-4 inhibitors, efforts are underway to discover predictive biomarkers and study optimal use of combinations of ipilimumab with other drugs [Sondak et al., 2011].

Ipilimumab treatment is associated with unique challenges in terms of managing toxicities (immune-related adverse events, irAEs) and interpreting imaging studies. Toxicities associated with ipilimumab are mainly immune-related adverse events (irAEs), and are somewhat related to dosage and scheduling. Grade 3–4 irAEs were reported in 20–40% of patients, with the majority of toxicities being skin, endocrine, and gastrointestinal. Though this percentage is sizeable, most irAEs are reversible with corticosteroids. Dermatological adverse effects include pruritis, rash, and vitiligo. Gastrointestinal adverse effects include colitis with diarrhea, bleeding, and bowel perforation. Endocrine adverse effects include hypophysitis, adrenalitis, and thyroiditis. Ocular irAEs are less common, but can affect vision. Toxicities can be life-threatening, with a mortality rate of less than 2% from treatment-related bowel perforation and autoimmune colitis.

The kinetics of response to ipilimumab therapy are different from those observed with conventional chemotherapeuties. Treatment with ipilimumab can be associated with delayed responses, initial tumor growth followed by shrinkage and mixed responses, making standard radiographic criteria difficult to apply. Tumor inflammation resulting from infiltration by CTLs could be the likely cause of initial tumor growth followed by belated shrinkage [Thumar and Kluger, 2010]. New radiographic criteria are being investigated [Wolchok et al., 2009].

INHIBITION OF PROGRAMMED CELL DEATH-1 (PD-1)

Another antagonistic antibody-based treatment is an antiprogrammed cell death-1 (PD-1) antibody. PD-1 is expressed on activated T and B cells, monocytes, NK-T cells, and DC and has two ligands, PD-L1 (B7-H1) and PD-L2 (B7-H2). PD-L1 is expressed on T and B cells, macrophages, and DCs, while the latter is more restricted to DCs [Weber, 2010]. High levels of PD-L1 were found on tumors including melanoma, where it is believed to mediate immune evasion by promoting T-cell apoptosis [Dong et al., 2002]. A positive correlation was observed between PD-L1 expression on melanoma cells and OS [Kronig et al., 2011]. PD-1 is aberrantly expressed on circulating melanoma antigen-specific T cells and tumor-infiltrating lymphocytes (TILs). It is believed that melanoma cells may initiate and sustain durable PD-1 signaling, and in turn, T-cell exhaustion and T-lymphocyte dysfunction. Therefore, since tumors and their microenvironment express PD-1 and PD-L1, PD-1 blockade might reverse its aberrant expression and signaling, restore function of immune effector cells, and induce an antitumor immune response.

Preliminary studies in PD-1-deficient mice injected with B16 melanoma cells resulted in heightened T-cell response and cytokine production, and inhibition of hematogenous spread of melanoma cells [Iwai et al., 2002]. Based on these preclinical findings, early phase clinical trials were initiated with anti-PD-1 (MDX-1106; Bristol Myers Squibb) as a single agent [Brahmer et al., 2010; Sznol et al., 2010]. In one study, MDX-1106 was administered at doses of 1, 3, and 10 mg/kg IV. MTD was not reached and drug-related adverse effects were mild and included fatigue, nausea, diarrhea, xerostomia, and pruritus. 37.5% of the evaluable patients had an objective tumor response and included patients treated at 1, 3, or 10 mg/kg [Sznol et al., 2010]. Perhaps the most remarkable results of this initial trial were the duration of response—median progression free survival had not been yet at the time of data analysis, and some of the initial responses observed over 3 years ago are ongoing [Sznol et al., 2010]. The toxicity profile appears to be superior to that of ipilimumab, although the two drugs have not been compared in a randomized fashion. Also, of interest, the phase I trial included patients with renal cell carcinoma and non-small cell lung cancer, with similar response rates in these two diseases.

In vitro studies have shown that combining PD-1 and CTLA-4 blockade is more effective than either one alone in shifting the melanoma tumor immune microenvironment from suppressive to inflammatory and restoring function of immune effector cells [Curran et al., 2010a]. A phase I clinical trial combining MDX-1106 with ipilimumab is ongoing, as is a trial that includes use of peptide vaccines in combination with MDX-1106, in patients with stage III or IV melanoma (www.clinicaltrials.gov).

OTHER APPROACHES TO MANIPULATING IMMUNE CO-STIMULATION OR INHIBITION

Immune escape by can occur by dysregulation of other immune modulating molecules such as CD200, LAG3, TIM3, TGF-b, and IDO, each of which contributes to the induction of anergy and immune escape in melanoma [Sakuishi et al., 2010]. Antibodies specifically developed to target such molecules or deplete T regulatory cells might represent good future strategies to restore the lymphocyte-mediated tumor inhibition and anti-tumor immunity. Such strategies can be studied alone or in combination with other immunotherapies to complement the relieving of the T cell anergy/exhaustion/tolerance pathways.

ADOPTIVE IMMUNOTHERAPY

Adoptive immunotherapy is another highly promising approach that is currently under intense investigation. Adoptive immunotherapy utilizes autologous tumor-reactive T cells that can be transferred into the host after elimination of suppresser T-cells to gain antitumor responses. Since melanoma tumors have the capacity to elicit production of antitumor lymphocytes, it is possible to harvest lymphocytes from the melanoma tumors, grow them ex vivo, and select for cells with specific tumor antigens for transfer back into the host. The transfer of TILs, for example, has proven highly effective at mediating tumor responses and treatment with TILs have resulted in durable objective responses in patients with advanced melanoma [Rosenberg et al., 2011].

Early studies targeting human tumors with autologous TILs showed the ability to mediate tumor regression. However, these early studies produced responses with little to no durability and response rates were moderate at best [Rosenberg et al., 1988; Park et al., 2011]. It was not until pre-conditioning regimens were added to TIL-based treatment to deplete immune suppresser cells that improved response rates and duration of response were observed. These lymphocyte depletion regimens include pretreatment with

chemotherapy and whole body irradiation [Rosenberg and Dudley, 2009]. With these regimens up to 70% of patients had measurable tumor regression, with 32% of patients demonstrating complete response, and the majority of responders being durable for over 3 years [Park et al., 2011].

A number of phase I/II trials with variations of TIL therapy have been completed. Examples include a phase II trial in which TILs were expanded via anti-CD3 and IL-2 and then administered to patients following transient lymphodepletion, followed by high-dose IL-2 therapy. Thirteen out of 25 (52%) evaluable patients had either a partial or complete response. A high percentage of CD8+ T cells and a lower percentage of CD4+ T cells in the infused TIL was significantly associated with a higher probability of response. Interestingly, in some patients clinical response was seen after a prolonged period of SD [Radvanyi et al., 2010].

Toxicities associated with these regimens are considerable and are primarily related to the lymphodepletion with high dose chemotherapy or radiotherapy. Moreover, high doses of IL-2 are administered after TIL infusion, resulting in additional toxicity, and there is a small treatment mortality rate. In addition to the considerable toxicity, TIL therapy has a number of other limitations. There is a requirement to surgically isolate a sizeable tumor, which is not feasible for all patients. Not all tumors have enough melanoma-specific T cells for culture, and it is not always possible to grow enough TIL ex-vivo, despite improved methods of stimulation. It takes a few weeks for TILs to grow, making the use of unselected, short-term cultured (young) TILs a more rapid and attractive treatment option for patients with aggressive disease. Recent results from studies utilizing young TILs in treatment of metastatic melanoma demonstrate that young TILs can mediate tumor regression in 42% of metastatic melanoma patients with manageable toxicity [Shapira-Frommer et al., 2011]. The advantage of using young TIL is shortening of the process and elimination of the waiting period during which many patients succumb to their disease. Another limitation of adoptive immunotherapy is that it can only be administered in select institutions that have the appropriate facilities and resources. Nonetheless, adoptive immunotherapy is associated with a very high durable response rate in patients who are robust enough to undergo therapy, possibly the highest of all currently available therapies for this disease, and is an excellent choice for patients who meet eligibility criteria.

Attempts are underway to enhance the efficacy of TIL therapy. One of the primary focuses has been the discovery of new tumor antigens that possess greater specificity in order to more efficiently direct adoptive immune response with TILs. One strategy is to generate CD8+ and CD4+ antigen-specific T-cell clones by stimulating peripheral T cells with peptides derived from tumor antigens. In one study, autologous CD4+ T-cell clones with specificity for the melanoma-associated antigen NY-ESO-1 were isolated and expanded in vitro and then infused into a patient with metastatic melanoma inducing a durable clinical remission [Hunder et al., 2008]. TIL-based therapy is also focusing on genetic engineering of normal T cells to become tumor-reactive. One way to accomplish this is by engineering highly active T-cell receptors (TCRs) specific for tumor antigens, to elicit antitumor activity [Johnson et al., 2006]. The advantage of this pursuit is the elimination of the surgical isolation of tumors, since normal T cells could be engineered to express TCRs and elicit tumor responses. Another modified TIL therapy currently being developed is the modified expression of chimeric antigen receptors (CARs) in T cells. CARS are cellular receptors engineered to be a fusion of a tumor–antigen-binding domain (derived from a single chain antibody) and intracellular signaling domains. This fusion protein allows for the intracellular signaling domains to activate T cells upon binding to antigens expressed by tumor cells. Though CARs have been shown to be feasible and safe antitumor reagents, several obstacles still limit their use. One such limitation is the necessity for the antigen to be expressed on the cell surface to trigger a response, a limitation not seen with TCRs which are capable of recognizing intracellular and extracellular processed peptides [Park et al., 2011]. Likewise, the rapid half-life of CAR-modified T cells infused into the host produce only limited antitumor responses [Cartellieri et al., 2010]. Several recent clinical trials have reported effectiveness of gene-modified T cells targeting tumor antigens such as GD2, CD20, MART-1, gp100, CD19, CEA, and NY-ESO-1 in melanoma and other cancers. In these trials, TCR- or CAR-engineered T cells have shown clinical benefit in melanoma, colorectal cancer, synovial cell cancer, neuroblastoma, and lymphoma [Park et al., 2011]. In one clinical study 36 patients with metastatic melanoma were treated, 20 patients with TCR recognizing the melanoma antigen MART-1 (human derived) and 16 patients with TCR recognizing the restricted melanoma antigen gp100 (mouse derived). Objective response rates of 30 and 19% were seen in patients who received the human or mouse TCR, respectively [Johnson et al., 2009]. In another study of TCR gene therapy to target the melanoma-associated antigen NY-ESO-1, patients with NY-ESO-1 positive tumors were treated with autologous TCR-transduced T cells plus IL-2. Measurable response rates were observed in four of six patients with synovial cell sarcoma (66%) and five of 11 patients with melanoma (45%). Two patients with metastatic melanoma had a complete response that persisted over a year and one patient with synovial cell sarcoma had a PR which lasted 18 months [Robbins et al., 2011]. Clinical trials utilizing CAR-modified T cells in metastatic renal cell carcinoma, ovarian cancer, and chronic lymphocytic leukemia (CLL) showed that this approach was only moderately effective, although persistent CAR-modified T cells in the host was associated with decreased tumor burden [Kershaw et al., 2006; Lamers et al., 2006; Brentjens et al., 2011].

With the advent of new immunotherapeutic agents, adoptive T-cell therapy might be further improved by combining TILs with these agents. One possibility is combining TILs with anti-PD-1 antibodies. Pre-clinical studies have used microRNA to silence PD-1expression in TILs; PD-1 expression was downregulated by 50–70% in T-cell lines and peripheral blood lymphocytes [Park et al., 2010]. The downregulation of PD-1 in TILS may lead to improved antitumor activity in vitro and in vivo. Blocking inhibitory immune signals on reactive lymphocytes via pre-treatment with a CTLA-4 inhibitor to enhance baseline TIL might represent another strategy. Preclinical studies show that 4-1BB co-stimulation by an agonistic anti 4-1BB antibody can improve TIL survival and boost antitumor activity [Hernandez et al., 2009].

Likewise, TIL treatments may be enhanced by modifying host lymphodepletion, selectively depleting CD4+ cells or T regulatory

cells. Also, though trials have been performed with IL-2 treatment after TIL infusion, administering alternative cytokines such as IL-7, IL-15, IL-21, IL-12, might be useful to support cell growth. Adoptive transfer of activated T cells, grown in IL-7 + IL-15 can be tried [Le et al., 2009]. Moreover, IL-7 might be superior to IL-2 for expansion of tumor-specific CD4(+) T cells [Caserta et al., 2010]. Additionally, one preclinical study showed that IL-15 is superior to IL-2 in supporting long-term survival and expansion of MART-1+ CD8+ TILs after stimulation [Li et al., 2010].

COMBINATION STRATEGIES—APPROACHES FOR CLINICAL DEVELOPMENT

Although some of the novel immunotherapy approaches discussed here have shown benefit, response rates to the two FDA approved agents (ipilimumab and IL-2) are low and toxicity is not negligible. Tumor molecular diversity along with the uniqueness of the host immunological background is responsible for the high degree of variability in response to treatment. In an effort to improve clinical benefit and overcome tumor evasive- and immune suppression, current strategies are focusing on targeting multiple molecules or pathways to treat this disease. Clinical studies are combining conventional antimelanoma treatments (chemotherapy) or molecular targeted therapies with immunotherapies. These approaches might demonstrate synergy in antitumor activity. One such example is the combination of dacarbazine and ipilimumab compared to dacarbazine alone, described above, which has been tested in a phase III trial and demonstrated superior OS in the combination arm [Robert et al., 2011]. We do not know, however, if ipilimumab plus dacarbazine is superior to ipilimumab alone; this trial has yet to be conducted. Small molecule inhibitors targeted to "addictive oncogenes" in the melanocyte might also synergize with immune therapies. For example, approximately 50% of metastatic melanomas harbor activating mutations in B-RAF. PLX4032 (RG7204/RO5185426/Vemurafenib, Genentech), an inhibitor of mutated B-RAF, was recently approved by the FDA for treatment of metastatic melanoma. Given the total lack of target overlap between vemurafenib and ipilimumab, combining these therapies is unlikely to add toxicity, but highly likely to increase efficacy, and a trial combining these therapies is being planned. Other MAPK pathway inhibitors for melanoma, such as GSK2118436 (Dabrafenib, GlaxoSmithKline), a selective inhibitor of mutant B-RAF, and GSK1120212 (JTP-74057/Trametinib, GlaxoSmithKline), a MEK inhibitor, can also be studied in combination with ipilimumab and anti-PD-1 antibodies [Flaherty et al., 2010; Infante et al., 2010; Kefford et al., 2010; Chapman et al., 2011]. Combining different immunotherapeutic regimens might also increase effectiveness and clinical benefit. One possibility would be combining antibodies blocking co-inhibitory receptors (such as CTLA-4) with antibodies activating co-stimulatory receptors (4-1BB). This combination therapy approach showed some level of synergy in tumor rejection in preclinical models [Curran et al., 2010b]. Combining cytokines with monoclonal antibodies or combining monoclonal antibodies that have already shown promise in clinical settings, such as anti-PD-1 and anti-CTLA-4, are also being studied. A phase II clinical trial comparing ipilimumab 10 mg/kg plus the DC stimulator Sargramostim (GM-CSF) to ipilimumab alone in advance melanoma has just completed accrual (www.clinicaltrials.gov). This trial was based on pre-clinical data showing synergism between these agents in mouse models [van Elsas et al., 1999].

Another possible combination strategy is high-dose IL-2 with other agents. A combination trial of high-dose IL-2 with Sargramostim (GM-CSF) administered for 7 days prior to initiation of IL-2 and continued for 4 weeks showed response rates similar to those seen with IL-2 alone. However, toxicity was comparable to that of high-dose IL-2 treatment alone, and this treatment regimen triggered increases in DC, IL-2R, and Treg in most of the patients tested, even though this did not result in an improvement in the response rates [Lutzky et al., 2010].

Combining vaccines with IL-2 is also a reasonable strategy as peptides, in spite of having limited activity by themselves, could potentially prime the immune system and augment the IL-2 effects. IL-2 can serve as an immune adjuvant to enhance the specific T-cell response and antitumor activity induced by the vaccine. Route and timing of administration of IL-2 and/or the vaccine might be critical. A phase multicenter phase III clinical study for patients who are HLA-A201 positive, showed that peptide (gp100:209-217[210M]) vaccination increases the response rates when compared to high dose IL-2 alone (16% vs. 6%, $P = 0.03$), and prolongs the progression-free survival (2.2 months vs. 1.6 months, $P = 0.008$). The median OS was also longer in the vaccine-IL-2 group than in the IL-2-only group, but the study was not powered to demonstrate an OS benefit (17.8 months vs. 11.1 months, $P = 0.06$) [Schwartzentruber et al., 2011].

CHALLENGES AND FUTURE DIRECTIONS

Although in the last decade we have seen dramatic advances in immunotherapy for the treatment of melanoma, several challenges still lie ahead. Predictive biomarker studies for immunotherapies are lacking, and improved efficacy by using combination therapies, perhaps at lower doses, might improve the therapeutic ratios for immune-based regimens. Improved understanding of the interaction between the tumor and the host immune system will result in advances in clinical care. The host system contains a multitude of variables, some of which may be effected by previous treatment regimens, resulting in increased complexity in selecting immunotherapies for melanoma patients. For example, although the half-life of ipilimumab is limited, CTLA-4 blockade after ipilimumab therapy can be ongoing for years, leading to difficulties in interpreting responses to subsequent therapies. An additional variable to take into consideration is genetic variability of the tumor within a given patient and emergence of subclones that might have different drug sensitivity/resistance patterns and elicit variable immune responses.

One major roadblock in the development of effective immunotherapy is the expense and complexity of immune monitoring. Since animal models are typically used for pre-clinical testing of drugs and drug combinations, when studying immune therapies, these studies have to be conducted in immune-competent animals. We currently have a limited number of spontaneous melanoma murine models that can be used for these purposes [Dankort et al., 2009]. While these models are helpful, they remain limited due to differences between mice and humans in terms of drug pharmacokinetic properties, genetics, etc. An additional challenge for the

development of immunotherapy is the increasing treatment options currently available. A number of different therapeutic options for treatment exist, albeit many of them substandard with limited efficacy, yet this does limit clinical trial accrual and referrals to academic centers. Although these challenges to development of improved immunotherapies need to be addressed, progress in the past decade has been dramatic, and treatment regimens are likely to be refined as biotechnology evolves and understanding of the immune responses improves. Thus, future clinical treatment for melanoma will clearly include immunotherapies as part of targeted patient- and disease-specific care.

REFERENCES

Adams S, O'Neill DW, Nonaka D, Hardin E, Chiriboga L, Siu K, Cruz CM, Angiulli A, Angiulli F, Ritter E, Holman RM, Shapiro RL, Berman RS, Berner N, Shao Y, Manches O, Pan L, Venhaus RR, Hoffman EW, Jungbluth A, Gnjatic S, Old L, Pavlick AC, Bhardwaj N. 2008. Immunization of malignant melanoma patients with full-length NY-ESO-1 protein using TLR7 agonist imiquimod as vaccine adjuvant. J Immunol 181(1):776–784.

Alexandrescu DT, Ichim TE, Riordan NH, Marincola FM, Di Nardo A, Kabigting FD. Dasanu CA. 2010. Immunotherapy for melanoma: Current status and perspectives. J Immunother 33(6):570–590.

Banchereau J, Palucka AK, Dhodapkar M, Burkeholder S, Taquet N, Rolland A, Taquet S, Coquery S, Wittkowski KM, Bhardwaj N, Pineiro L, Steinman R, Fay J. 2001. Immune and clinical responses in patients with metastatic melanoma to CD34(+) progenitor-derived dendritic cell vaccine. Cancer Res 61(17):6451–6458.

Bogunovic D, Manches O, Godefroy E, Yewdall A, Gallois A, Salazar AM, Marie I, Levy DE, Bhardwaj N. 2011. TLR4 engagement during TLR3-induced pro-inflammatory signaling in dendritic cells promotes IL-10-mediated suppression of anti-tumor immunity. Cancer Res. 71:5467–5476.

Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, Sharfman WH, Stankevich E, Pons A, Salay TM, McMiller TL, Gilson MM, Wang C, Selby M, Taube JM, Anders R, Chen L, Korman AJ, Pardoll DM, Lowy I, Topalian SL. 2010. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: Safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol 28(19):3167–3175.

Brentjens RJ, Riviere I, Park J, Davilla M, Wang X, Yeh R, Lamanna N, Frattini MG, Sadelain M. 2011. Lymphodepletion and tumor burden govern clinical responses in patients with B-cell malignancies treated with autologous, CD19-targeted T cells. J Clin Oncol 29:18s (abstr 2534).

Brichard VG, Lejeune D. 2007. GSK's antigen-specific cancer immunotherapy programme: Pilot results leading to Phase III clinical development. Vaccine 25(Suppl 2):B61–B71.

Cartellieri M, Bachmann M, Feldmann A, Bippes C, Stamova S, Wehner R, Temme A, Schmitz M. 2010. Chimeric antigen receptor-engineered T cells for immunotherapy of cancer. J Biomed Biotechnol 2010:956304.

Caserta S, Alessi P, Basso V, Mondino A. 2010. IL-7 is superior to IL-2 for ex vivo expansion of tumour-specific CD4(+) T cells. Eur J Immunol 40(2):470–479.

Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, Dummer R, Garbe C, Testori A, Maio M, Hogg D, Lorigan P, Lebbe C, Jouary T, Schadendorf D, Ribas A, O'Day SJ, Sosman JA, Kirkwood JM, Eggermont AM, Dreno B, Nolop K, Li J, Nelson B, Hou J, Lee RJ, Flaherty KT, McArthur GA. 2011. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med 364(26):2507–2516.

Costanzo A, Chimenti MS, Botti E, Caruso R, Sarra M, Monteleone G. 2010. IL-21 in the pathogenesis and treatment of skin diseases. J Dermatol Sci 60(2):61–66.

Croft M. 2009. The role of TNF superfamily members in T-cell function and diseases. Nat Rev Immunol 9(4):271–285.

Curran MA, Montalvo W, Yagita H, Allison JP. 2010a. PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. Proc Natl Acad Sci USA 107(9):4275–4280.

Curran MA, Kim M, Al-Shamkhaniw A, Allison JP. 2010b. 4-1BB activation induces the master-regulator Eomes and A broad-spectrum TH1 phenotype which synergizes with CTLA-4 blockade to reject B16 Melanoma. J Immunother Abstracts for the 25th Annual Scientific Meeting of the International Society for Biological Therapy of Cancer 33:894.

Dankort D, Curley DP, Cartlidge RA, Nelson B, Karnezis AN, Damsky WE, Jr., You MJ, DePinho RA, McMahon M, Bosenberg M 2009. Braf(V600E) cooperates with Pten loss to induce metastatic melanoma. Nat Genet 41(5): 544–552.

Davis ID, Brady B, Kefford RF, Millward M, Cebon J, Skrumsager BK, Mouritzen U, Hansen LT, Skak K, Lundsgaard D, Frederiksen KS, Kristjansen PE, McArthur G. 2009. Clinical and biological efficacy of recombinant human interleukin-21 in patients with stage IV malignant melanoma without prior treatment: A phase IIa trial. Clin Cancer Res 15(6):2123–2129.

Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, Roche PC, Lu J, Zhu G, Tamada K, Lennon VA, Celis E, Chen L. 2002. Tumor-associated B7-H1 promotes T-cell apoptosis: A potential mechanism of immune evasion. Nat Med 8(8):793–800.

Engell-Noerregaard L, Hansen TH, Andersen MH, Thor Straten P, Svane IM. 2009. Review of clinical studies on dendritic cell-based vaccination of patients with malignant melanoma: Assessment of correlation between clinical response and vaccine parameters. Cancer Immunol Immunother 58(1):1–14.

Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA, O'Dwyer PJ, Lee RJ, Grippo JF, Nolop K, Chapman PB. 2010. Inhibition of mutated, activated BRAF in metastatic melanoma. N Engl J Med 363(9):809–819.

Frederiksen KS, Lundsgaard D, Freeman JA, Hughes SD, Holm TL, Skrumsager BK, Petri A, Hansen LT, McArthur GA, Davis ID, Skak K. 2008. IL-21 induces in vivo immune activation of NK cells and CD8(+) T cells in patients with metastatic melanoma and renal cell carcinoma. Cancer Immunol Immunother 57(10):1439–1449.

Grabstein KH, Urdal DL, Tushinski RJ, Mochizuki DY, Price VL, Cantrell MA, Gillis S, Conlon PJ. 1986. Induction of macrophage tumoricidal activity by granulocyte-macrophage colony-stimulating factor. Science 232(4749): 506–508.

Greenlee RT, Hill-Harmon MB, Murray T, Thun M. 2001. Cancer statistics, 2001. CA Cancer J Clin 51(1):15–36.

Hashmi MH, Van Veldhuizen PJ. 2010. Interleukin-21: Updated review of Phase I and II clinical trials in metastatic renal cell carcinoma, metastatic melanoma and relapsed/refractory indolent non-Hodgkin's lymphoma. Expert Opin Biol Ther 10(5):807–817.

Hernandez JA, Li Y, Wang Y, Hwu P, Radvanyi L. 2009. Improving Tumor Infiltrating Lymphocyte Survival, Function for Melanoma Adoptive T-cell Therapy Through 4-1BB Costimulation. J Immunother International Society for Biological Therapy of Cancer 24th Annual Meeting Abstracts 32:944.

Hodi FS, O'Day SJ, McDermott DF, WeberRW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, Akerley W, van den Eertwegh AJ, Lutzky J, Lorigan P, Vaubel JM, Linette GP, Hogg D, Ottensmeier CH, Lebbe C, Peschel C, Quirt I, Clark JI, Wolchok JD, Weber JS, Tian J, Yellin MJ, Nichol GM, Hoos A, Urba WJ. 2010. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 363(8): 711–723.

Hodi FS, Friedlander PA, Atkins MB, McDermott DF, Lawrence DP, Ibrahim N, Wu X, Zhou J, Giobbie-Hurder A, Murphy G, Hollman T, Velazquez E, Russell S, Dipiro P, Yap JT, Van Den Abbeele AD. 2011. A phase I trial of

ipilimumab plus bevacizumab in patients with unresectable stage III or stage IV melanoma. J Clin Oncol 29:15S 2011 (abstr 8511).

Hunder NN, Wallen H, Cao J, Hendricks DW, Reilly JZ, Rodmyre R, Jungbluth A, Gnjatic S, Thompson JA, Yee C. 2008. Treatment of metastatic melanoma with autologous CD4+ T cells against NY-ESO-1. N Engl J Med 358(25): 2698–2703.

Ilkovitch D, Lopez DM. 2008. Immune modulation by melanoma-derived factors. Exp Dermatol 17(12):977–985.

Infante JR, Fecher LA, Nallapareddy S, Gordon MS, Flaherty KT, Cox DS, DeMarini DJ, Burris HA, Messersmith WA. 2010. Safety and efficacy results from the first-in-human study of the oral MEK 1/2 inhibitor GSK1120212. J Clin Oncol 28:15s (abstr 2503).

Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N. 2002. 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 USA 99(19):12293–12297.

Jilaveanu LB, Aziz SA, Kluger HM. 2009. Chemotherapy and biologic therapies for melanoma: Do they work? Clin Dermatol 27(6):614–625.

Johnson LA, Heemskerk B, Powell DJ, Jr., Cohen CJ, Morgan RA, Dudley ME, Robbins PF, Rosenberg SA. 2006. Gene transfer of tumor-reactive TCR confers both high avidity and tumor reactivity to nonreactive peripheral blood mononuclear cells and tumor-infiltrating lymphocytes. J Immunol 177(9):6548–6559.

Johnson LA, Morgan RA, Dudley ME, Cassard L, Yang JC, Hughes MS, Kammula US, Royal RE, Sherry RM, Wunderlich JR, Lee CC, Restifo NP, Schwarz SL, Cogdill AP, Bishop RJ, Kim H, Brewer CC, Rudy SF, VanWaes C, Davis JL, Mathur A, Ripley RT, Nathan DA, Laurencot CM, Rosenberg SA. 2009. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. Blood 114(3):535–546.

Kefford R, Arkenau H, Brown MP, Millward M, Infante JR, Long GV, Ouellet D, Curtis M, Lebowitz PF, Falchook GS. 2010. Phase I/II study of GSK2118436, a selective inhibitor of oncogenic mutant BRAF kinase, in patients with metastatic melanoma and other solid tumors. J Clin Oncol 28:15s (abstr 8503).

Kershaw MH, Westwood JA, Wang G, Eshhar Z, Mavroukakis SA, White DE, Wunderlich JR, Canevari S, Rogers-Freezer L, Chen CC, Yang JC, Rosenberg SA, Hwu P. 2006. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. Clin Cancer Res 12(20 Pt 1):6106–6115.

Kirkwood JM, Tarhini AA, Panelli MC, Moschos SJ, Zarour HM, Butterfield LH, Gogas HJ. 2008. Next generation of immunotherapy for melanoma. J Clin Oncol 26(20):3445–3455.

Korman AJ, Peggs KS, Allison JP. 2006. Checkpoint blockade in cancer immunotherapy. Adv Immunol 90:297–339.

Kovacsovics-Bankowski M, Walker E, Floyd K, Urba W, Curti B, Weinberg AD, Robert W. 2009. Increased CD4 and CD8 memory T-cell proliferation after anti-OX40 administration to cancer patients: Immunologic assessment of a phase I clinical trial. J Immunother International Society for Biological Therapy of Cancer 24th Annual Meeting Abstracts 32:952.

Kronig H, Falchner KJ, Odendahl M, Brackertz B, Conrad H, Muck D, Hein R, Blank C, Peschel C, Haller B, Schulz S, Bernhard H. 2011. PD-1 expression on MelanA-reactive T cells increases during progression to metastatic disease. Int J Cancer [Epub ahead of print].

Lawson DH, Lee SJ, Tarhini AA, Margolin KA, Ernstoff MS, Kirkwood JM. 2010. E4697: Phase III cooperative group study of yeast-derived granulocyte macrophage colony-stimulating factor (GM-CSF) versus placebo as adjuvant treatment of patients with completely resected stage III-IV melanoma. J Clin Oncol 28:15s (abstr 8504).

Lamers CH, Sleijfer S, Vulto AG, Kruit WH, Kliffen M, Debets R, Gratama JW, Stoter G, Oosterwijk E. 2006. Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: First clinical experience. J Clin Oncol 24(13):e20–e22.

Le HK, Graham L, Miller CH, Kmieciak M, Manjili MH, Bear HD. 2009. Incubation of antigen-sensitized T lymphocytes activated with bryostatin 1+ionomycin in IL-7+IL-15 increases yield of cells capable of inducing regression of melanoma metastases compared to culture in IL-2. Cancer Immunol Immunother 58(10):1565–1576.

Li Y, Hernandez J, Wu R, Hwu P, Radvanyi L. 2010. IL-15 is superior to IL-2 in supporting Antigen-specific expansion and maintenance of T-cell memory in melanoma. J Immunother International Society for Biological Therapy of Cancer 24th Annual Meeting Abstracts 33:988.

Lutzky J, Lawson DH, Enriquez-Nunez Y, Gabrilovich D. 2010. Phase II trial of high-dose interleukin-2 (IL-2) with priming and concomitant sargramostim (GM-CSF) in patients with advanced melanoma. J Clin Oncol 28:15s (abstr 8560).

Mocellin S, Pasquali S, Rossi CR, Nitti D. 2010. Interferon alpha adjuvant therapy in patients with high-risk melanoma: A systematic review and meta-analysis. J Natl Cancer Inst 102(7):493–501.

Park TS, Inozume T, Ahmadzadeh M, Hanada K, Yang J, Rosenberg S, Morgan R. 2010. Artifical microRNA targeting programmed death receptor-1 to enhance adoptive cell transfer therapy for cancer. J Immunother Abstracts for the 25th Annual Scientific Meeting of the International Society for Biological Therapy of Cancer 33:863.

Park TS, Rosenberg SA, Morgan RA. 2011. Treating cancer with genetically engineered T cells. Trends Biotechnol [Epub ahead of print].

Petrella TM, Tozer R, Belanger K, Savage KJ, Wong R, Kamel-Reid S, Tron V. 2010. Interleukin-21 (IL-21) activity in patients (pts) with metastatic melanoma (MM). J Clin Oncol 28:15s (abstr 8507).

Radvanyi LG, Bernatchez C, Zhang M, Miller P, Glass M, Papadopoulos N, Hwu P. 2010. adoptive T cell therapy for metastatic melanoma: The MD Anderson experience. J Immunother Abstracts for the 25th Annual Scientific Meeting of the International Society for Biological Therapy of Cancer 33:863.

Rasmussen MA, Colding-Jorgensen M, Hansen LT, Bro R. 2010. Multivariate evaluation of pharmacological responses in early clinical trials - a study of rIL-21 in the treatment of patients with metastatic melanoma. Br J Clin Pharmacol 69(4):379–390.

Ribas A, Hauschild A, Kefford R, Punt CJ, Haanen JB, Marmol M, Garbe C, Gomez-Navarro J, Pavlov D, Marshall M. 2008. Phase III, open-label, randomized, comparative study of tremelimumab (CP-675,206) and chemotherapy (temozolomide [TMZ] or dacarbazine [DTIC]) in patients with advanced melanoma. J Clin Oncol 26:15s (abstr. LBA9011).

Ribas A, Kirkwood JM, Atkins MB, Whiteside TL, Gooding W, Kovar A, Gillies SD, Kashala O, Morse MA. 2009. Phase I/II open-label study of the biologic effects of the interleukin-2 immunocytokine EMD 273063 (hu14.18-IL2) in patients with metastatic malignant melanoma. J Transl Med 7:68.

Robert C, Thomas L, Bondarenko I, O'Day S, M DJ, Garbe C, Lebbe C, Baurain JF, Testori A, Grob JJ, Davidson N, Richards J, Maio M, Hauschild A, Miller WH, Jr., Gascon P, Lotem M, Harmankaya K, Ibrahim R, Francis S, Chen TT, Humphrey R, Hoos A, Wolchok JD. 2011. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med 364(26):2517–2526.

Robbins PF, Morgan RA, Feldman SA, Yang JC, Sherry RM, Dudley ME, Wunderlich JR, Nahvi AV, Helman LJ, Mackall CL, Kammula US, Hughes MS, Restifo NP, Raffeld M, Lee CC, Levy CL, Li YF, El-Gamil M, Schwarz SL, Laurencot C, Rosenberg SA. 2011. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. J Clin Oncol 29(7):917–924.

Rosenberg SA, Packard BS, Aebersold PM, Solomon D, Topalian SL, Toy ST, Simon P, Lotze MT, Yang JC, Seipp CA, et al. 1988. Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. A preliminary report. N Engl J Med 319(25):1676–1680.

Rosenberg SA, Sherry RM, Morton KE, Scharfman WJ, Yang JC, Topalian SL, Royal RE, Kammula U, Restifo NP, Hughes MS, Schwartzentruber D, Berman

DM, Schwarz SL, Ngo LT, Mavroukakis SA, White DE, Steinberg SM. 2005. Tumor progression can occur despite the induction of very high levels of self/tumor antigen-specific CD8+ T cells in patients with melanoma. J Immunol 175(9):6169–6176.

Rosenberg SA, Dudley ME. 2009. Adoptive cell therapy for the treatment of patients with metastatic melanoma. Curr Opin Immunol 21(2):233–240.

Rosenberg SA, Yang JC, Sherry RM, Kammula US, Hughes MS, Phan GQ, Citrin DE, Restifo NP, Robbins PF, Wunderlich JR, Morton KE, Laurencot CM, Steinberg SM, White DE, Dudley ME. 2011. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res 17(13):4550–4557.

Sabatino M, Kim-Schulze S, Panelli MC, Stroncek D, Wang E, Taback B, Kim DW, Deraffele G, Pos Z, Marincola FM, Kaufman HL. 2009. Serum vascular endothelial growth factor and fibronectin predict clinical response to high-dose interleukin-2 therapy. J Clin Oncol 27(16):2645–2652.

Sakuishi K, Apetoh L, Sullivan JM, Blazar BR, Kuchroo VK, Anderson AC. 2010. Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. J Exp Med 207(10):2187–2194.

Schwartzentruber DJ, Lawson DH, Richards JM, Conry RM, Miller DM, Treisman J, Gailani F, Riley L, Conlon K, Pockaj B, Kendra KL, White RL, Kuzel TM, Curti B, Leming PD, Whitman ED, Balkissoon J, Reintgen DS, Kaufman H, Marincola FM, Merino MJ, Rosenberg SA, Choyke P, Vena D, Hwu P. 2011. gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma. N Engl J Med 364(22):2119–2127.

Siegel R, Ward E, Brawley O, Jemal A. 2011. Cancer statistics, 2011: The impact of eliminating socioeconomic and racial disparities on premature cancer deaths. CA Cancer J Clin 61(4):212–236.

Shackleton M, Davis ID, Hopkins W, Jackson H, Dimopoulos N, Tai T, Chen Q, Parente P, Jefford M, Masterman KA, Caron D, Chen W, Maraskovsky E, Cebon J. 2004. The impact of imiquimod, a toll-like receptor-7 ligand (TLR7L), on the immunogenicity of melanoma peptide vaccination with adjuvant Flt3 ligand. Cancer Immun 4:9.

Shapira-Frommer R, Besser M, Kuchuk I, Nave R, Zippel D, Treves A, Nagler A, Apter S, Shimoni A, Yerushalmi R, Ben-Ami E, Ben-Nun A, Markel G, Itzhaki O, Catane R, Schachter J. 2011. Adoptive transfer of short-term cultured tumor-infiltrating lymphocytes (young TIL) in metastatic melanoma patients. J Clin Oncol 29:18s (abstr 8510).

Sivendran S, Glodny B, Pan M, Merad M, Saenger Y. 2010. Melanoma immunotherapy. Mt Sinai J Med 77(6):620–642.

Sondak VK, Smalley KS, Kudchadkar R, Grippon S, Kirkpatrick P. 2011. Ipilimumab. Nat Rev Drug Discov 10(6):411–412.

Szabolcs P, Moore MA, Young JW. 1995. Expansion of immunostimulatory dendritic cells among the myeloid progeny of human CD34+ bone marrow precursors cultured with c-kit ligand, granulocyte-macrophage colony-stimulating factor, and TNF-alpha. J Immunol 154(11):5851–5861.

Sznol M, Hodi FS, Margolin K, McDermott DF, Ernstoff MS, Kirkwood JM, Wojtaszek C, Feltquate D, Logan T. 2008. Phase I study of BMS-663513, a fully human anti-CD137 agonist monoclonal antibody, in patients (pts) with advanced cancer (CA). J Clin Oncol 26:15s (abstr 3007).

Sznol M, Powderly JD, Smith DC, Brahmer JR, Drake CJ, McDermott DF, Lawrence DP, Wolchok JD, Topalian SL, Lowy I. 2010. Safety and antitumor activity of biweekly MDX-1106 (Anti-PD-1, BMS-936558/ONO-4538) in patients with advanced refractory malignancies. J Clin Oncol 28:15s (abstr 2506).

Thumar JR, Kluger HM. 2010. Ipilimumab: A promising immunotherapy for melanoma. Oncology (Williston Park) 24(14):1280–1288.

Trepiakas R, Berntsen A, Hadrup SR, Bjorn J, Geertsen PF, Straten PT, Andersen MH, Pedersen AE, Soleimani A, Lorentzen T, Johansen JS, Svane JS. 2010. Vaccination with autologous dendritic cells pulsed with multiple tumor antigens for treatment of patients with malignant melanoma: Results from a phase I/II trial. Cytotherapy 12(6):721–734.

van Elsas A, Hurwitz AA, Allison JP. 1999. 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 190(3): 355–366.

Vonderheide RH, Flaherty KT, Khalil M, Stumacher MS, Bajor DL, Hutnick NA, Sullivan P, Mahany JJ, Gallagher M, Kramer A, Green SJ, O'Dwyer PJ, Running KL, Huhn RD, Antonia SJ. 2007. Clinical activity and immune modulation in cancer patients treated with CP-870,893, a novel CD40 agonist monoclonal antibody. J Clin Oncol 25(7):876–883.

Weber RW, O'Day S, Rose M, Deck R, Ames P, Good J, Meyer J, Allen R, Trautvetter S, Timmerman M, Cruickshank S, Cook M, Gonzalez R, Spitler LE. 2005. Low-dose outpatient chemobiotherapy with temozolomide, granulocyte-macrophage colony stimulating factor, interferon-alpha2b, and recombinant interleukin-2 for the treatment of metastatic melanoma. J Clin Oncol 23(35):8992–9000.

Weber J. 2010. Immune checkpoint proteins: A new therapeutic paradigm for cancer–preclinical background: CTLA-4 and PD-1 blockade. Semin Oncol 37(5):430–439.

Williams P, Galipeau J. 2011. GMCSF-interleukin fusion cytokines induce novel immune effectors that can serve as biopharmaceuticals for treatment of autoimmunity and cancer. J Intern Med 269(1):74–84.

Williams P, Rafei M, Bouchentouf M, Raven J, Yuan S, Cuerquis J, Forner KA, Birman E, Galipeau J. 2010. A fusion of GMCSF and IL-21 initiates hyper-signaling through the IL-21Ralpha chain with immune activating and tumoricidal effects in vivo. Mol Ther 18(7):1293–1301.

Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, Lebbe C, Maio M, Binder M, Bohnsack O, Nichol G, Humphrey R, Hodi FS. 2009. Guidelines for the evaluation of immune therapy activity in solid tumors: Immune-related response criteria. Clin Cancer Res 15(23):7412–7420.

Zhao F, Dou J, He XF, Wang J, Chu L, Hu W, Yu F, Wu Y, Gu N. 2010. Enhancing therapy of B16F10 melanoma efficacy through tumor vaccine expressing GPI-anchored IL-21 and secreting GM-CSF in mouse model. Vaccine 28(16):2846–2852.