Published in final edited form as:

Am J Clin Dermatol. 2013 June ; 14(3): 179–194. doi:10.1007/s40257-013-0025-9.

Optimal Management of Metastatic Melanoma: Current Strategies and Future Directions

Marta Batus, Salman Waheed, Carl Ruby, Lindsay Petersen, Steven D. Bines, and Howard L. Kaufman

Rush University Melanoma Program and Departments of Medicine, General Surgery and Immunology and Microbiology, Rush University Medical Center, Chicago, IL, USA

Abstract

Melanoma is increasing in incidence and remains a major public health threat. Although the disease may be curable when identified early, advanced melanoma is characterized by widespread metastatic disease and a median survival of less than 10 months. In recent years, however, major advances in our understanding of the molecular nature of melanoma and the interaction of melanoma cells with the immune system have resulted in several new therapeutic strategies that are showing significant clinical benefit. Current therapeutic approaches include surgical resection of metastatic disease, chemotherapy, immunotherapy, and targeted therapy. Dacarbazine, interleukin-2, ipilimumab, and vemurafenib are now approved for the treatment of advanced melanoma. In addition, new combination chemotherapy regimens, monoclonal antibodies blocking the programmed death-1 (PD-1)/PD-ligand 1 pathway, and targeted therapy against CKIT, mitogen-activated protein/extracellular signal-regulated kinase (MEK), and other putative signaling pathways in melanoma are beginning to show promise in early-phase clinical trials. Further research on these modalities alone and in combination will likely be the focus of future clinical investigation and may impact the outcomes for patients with advanced melanoma.

1 Introduction

Skin cancer is the most common human malignancy. Globally, there are about 2–3 million cases of skin cancer each year, and while melanoma accounts for about 132,000 of these cases, it is linked to the most deaths.^[1] The incidence of melanoma has more than tripled in the Caucasian population over the past 20 years. Currently, it is the sixth most common cancer in the USA.^[2] In 2009, there were more than 8,500 deaths in the USA due to melanoma, with a slight male predominance.^[2] Melanoma is an aggressive tumor with advanced disease characterized by widespread metastatic lesions and the tumor has traditionally been resistant to most forms of treatment. Indeed, metastatic melanoma has a very poor prognosis with a median survival time of 8–9 months and an estimated 3-year survival rate of less than 15 %.^[3] These rates have not changed much in the last 25 years. The reason for this may be, in part, that effective therapies have been slow to emerge. Prior to 2011, the only agents approved for the treatment of metastatic melanoma were dacarbazine and interleukin-2 (IL-2).^[2] Recent advances in our understanding of the genetic profile of melanoma cells and the molecular factors that drive malignant transformation have resulted in the identification of numerous new therapeutic targets.^[1,2] In addition, major progress has been made in understanding the role of T lymphocytes in patients with melanoma, resulting in new forms of immunotherapy for the treatment of advanced

melanoma. This new understanding has led to several significant phase III clinical trials and the approval of the first BRAF inhibitor (vemurafenib) and T-cell checkpoint inhibitor (ipilimumab) for the treatment of stage IV melanoma. These new agents have provided the oncologist with new therapeutic options and efforts are underway to further evaluate the impact of dosing and sequencing of these agents to optimize the clinical benefit for patients with melanoma.^[4–5] This review summarizes the various modalities that are currently available for the treatment of advanced melanoma and touches briefly on some of the more promising agents in clinical development.

Melanoma can metastasize to any location in the body and detection of metastatic disease requires whole body imaging. While there are no data to support the routine imaging of high-risk patients, once metastatic disease is suspected complete imaging is indicated. This may consist of computed tomography (CT) scans of the chest, abdomen, and pelvis or whole body positron emission tomography (PET) scans. A magnetic resonance imaging (MRI) of the brain should also be done since CNS metastasis is also a major problem with melanoma. The American Joint Committee on Cancer (AJCC) TNM staging system for melanoma has suggested that the location of metastatic disease and serum lactate dehydrogenase (LDH) levels are highly predictive of prognosis for patients with advanced melanoma.^[7] In general, patients with distant skin, subcutaneous, or nodal metastases (M1a) have the best prognosis, with a worse prognosis for pulmonary only metastases (M1b), and the worst prognosis occurs for patients with extra-pulmonary visceral metastases or those with an elevated serum LDH (M1c). Once metastatic disease is identified there are several modalities that can be considered.

2 Surgical Therapy

Metastasectomy is the surgical excision of a metastatic tumor and this continues to be the standard of care for patients who present with a solitary melanoma metastasis. Clinical evaluation by a surgical oncologist is warranted in patients with isolated metastatic disease and this option should be reconsidered in patients following systemic therapy since it is not uncommon for some metastatic lesions to respond while others may be resistant to systemic therapy. There are no prospective, randomized clinical trials demonstrating a therapeutic benefit for metastasectomy in melanoma but there are many compelling retrospective studies that suggest selected patients may benefit from surgical treatment.

A review of the Surveillance Epidemiology and End Results (SEER) database for metastasectomy outcomes in melanoma suggested an improved survival for metastatic melanoma patients undergoing metastasectomy regardless of location. This effect was most pronounced for M1a patients.^[8] In this review, the median survival rate of patients with skin, subcutaneous, and lymph node metastases after surgical resection was reported to be 35 months compared with 6 months for patients without surgery.^[9] A similar benefit has been reported for regional lymphadenectomy in patients without an identified primary tumor.^[10] While these data may be biased by patient selection, they do suggest that selected patients with M1a disease may benefit from surgical resection.

The lung is the most common site of metastases of melanoma after distant skin, subcutaneous, and nodal metastases. Median survival after diagnosis of pulmonary metastatic disease is 13 months, but retrospective studies have found that surgical resection can result in median survival rates of up to 40 months and a 5-year survival rate of 35 % has been reported.^[11–12] In a single institution review of a prospective melanoma registry, 1,720 patients who had undergone pulmonary metastasectomy were identified.^[13] In this series, the median survival for the population after diagnosis of pulmonary metastases was 7.3 months. In patients who underwent metastasectomy, there was a 12-month survival benefit (19 vs. 7

months, $p < 0.01$). This benefit persisted even in the face of extrathoracic metastasis where surgical patients had a survival of 18 months compared with 8 months for those without surgery ($p < 0.01$). The investigators identified several factors on multivariate analysis that were associated with improved survival, including nodular histology, prolonged disease-free interval, fewer numbers of lesions, presence of extrapulmonary disease, and performance of pulmonary metastasectomy. Other retrospective studies describe similar advantages of surgery in patients with pulmonary-only metastasis, which has led some to argue surgery should be the standard of care for people with pulmonary metastatic melanoma.^[11–16,17]

Melanoma is the most common metastatic tumor to the gastrointestinal tract, and once identified, the survival prognosis is 4–6 months.^[18] As with pulmonary metastases, surgical resection is recommended, and 5-year survival rates are reported at 27 % after resection.^[19–21] Resection of hepatic metastases has been associated with increases in median survival from 6 to 22 months, although recurrence rate is as high as 75 %.^[22,23] There is a survival benefit for surgical resection of adrenal metastases as compared with patients managed without surgery, and laparoscopic adrenalectomy for metastatic melanoma is described in the literature.^[24,25] A review of pancreatic resections for metastatic melanoma showed that this approach may only offer a palliative benefit, with no improvement in long-term survival.^[26]

CNS metastases deserve special mention since these occur commonly and are associated with a bad prognosis. Whole brain and stereotactic radiation are useful in these patients but surgical resection may play a role in selected patients. In a retrospective review of brain metastases, a survival benefit was reported for surgical resection of solitary metastatic lesions, as well as improved palliation when surgical resection was performed, whether alone or combined with radiation.^[27] There are several other retrospective series suggesting a clinical benefit for craniotomy when only a single metastatic tumor is present in the brain.^[28–30] More recently, 41 patients who had undergone craniotomy for CNS melanoma metastasis were followed. In this study, 15 patients underwent subsequent whole brain radiation and 26 did not have radiation with no difference in survival between the groups. There was, however, a significant improvement in duration of survival for patients who achieved an objective systemic response to subsequent immunotherapy ($p < 0.05$). These data suggest that a multi-disciplinary approach to the management of melanoma CNS metastasis is warranted.^[31]

A number of factors have been associated with therapeutic benefit with metastasectomy. While there is clearly a benefit for resection of solitary lesions, the ability to completely resect all known sites of disease is a critical determinant of long-term survival.^[32–48] Surgical management also offers the opportunity for palliation in some cases and may be repeated, if necessary, whereas radiation and exposure to cytotoxic chemotherapy may be difficult after initial therapy.^[49–50] A patient who underwent five different metastatic resection surgeries has been described, and remains disease free for 67 months.^[51] The potential for combining surgical resection with other adjuvant therapies remains a topic of intense interest.^[52–56] These anecdotal and retrospective cases must be interpreted with caution as they are subject to significant selection bias. To date, there have been no prospective, randomized clinical trials evaluating metastasectomy in melanoma but surgical resection can be considered in appropriate patients.

3 Cytotoxic Chemotherapy

3.1 Dacarbazine

Dacarbazine is an alkylating agent and was approved for the treatment of metastatic melanoma in 1974. This is still the only cytotoxic chemotherapy agent approved by the US

FDA for the treatment of metastatic melanoma. Several studies have evaluated the efficacy of dacarbazine and objective response rates usually range from 6 % to 20 %.^[57] A careful review of the literature has further suggested that less than 5 % of patients achieve a complete response with dacarbazine and there is only a 2–6 % survival reported at 5 years.^[57] Dacarbazine has remained the standard chemotherapy of choice for metastatic melanoma largely because other single and combination chemotherapy regimens have failed to demonstrate any overall survival benefit in randomized clinical trials. New agents and combinations, however, are being evaluated and merit attention.

3.2 Temozolomide

Temozolomide (TMZ) is an oral prodrug of the active metabolite of dacarbazine (5-(3-methyltriazen-1-yl) imidazole-4-carboximide; or MTIC), is FDA approved for glioma, and has been used in advanced melanoma.^[57] TMZ also has an advantage over dacarbazine in being able to cross the blood-brain barrier, which has made it a popular choice for patients with CNS disease. A randomized clinical trial compared TMZ with dacarbazine, and demonstrated a modest improvement in median progression-free survival for patients in the TMZ group (1.9 vs. 1.5 months; $p = 0.012$).^[58] In this trial, there was no difference in overall survival (7.7 vs. 6.4 months; $p = 0.20$) or objective response rates (21 % TMZ group vs. 18 % dacarbazine group).

3.3 Combination Chemotherapy Regimens

A common chemotherapy regimen used in metastatic melanoma combines cisplatin, vinblastine, and dacarbazine (CVD). This regimen has been associated with objective response rates of 20–35 %.^[59] The regimen has been compared with dacarbazine alone, and no significant improvements in overall survival were observed.^[60] TMZ has been substituted for dacarbazine in this regimen and is currently under clinical investigation. Another combination regimen that has been under study is the combination of carboplatin and paclitaxel (CP), a regimen that is used in the treatment of non-small-cell lung cancer and advanced ovarian cancer.^[61] In a recent phase II clinical trial, 61 patients with metastatic melanoma were treated with CP and 20 patients were treatment naïve while 41 patients had received at least one prior therapy. An objective partial response was reported in 4.9 % of patients with an additional 23 % achieving stable disease. The median overall survival was 31 weeks for the patient population with those who had controlled disease (partial responders and stable disease patients) having 49 weeks median survival compared with 18 weeks for patients with progressive disease after treatment. The regimen was associated with myelosuppression, peripheral neuropathy, and fatigue. This regimen has not yet been directly compared with dacarbazine.

3.4 Biochemotherapy

Another popular approach has been to combine chemotherapy with immunotherapy in a regimen generally referred to as ‘biochemotherapy’ (BCT). The most widely studied BCT regimen has utilized dacarbazine-based regimens, such as CVD, with IL-2 and interferon- α -2b.^[62] A multi-institutional, cooperative group, phase III clinical trial compared BCT with chemotherapy (CVD) alone.^[63] The BCT arm demonstrated a higher response rate (19.5 % vs. 13.8 %; $p = 0.140$) and an improvement in median progression-free survival (4.8 vs. 2.9 months; $p = 0.015$). The investigators did not, however, demonstrate an advantage in overall survival (9.0 months in the BCT arm vs. 8.7 months in the CVD-alone arm; hazard ratio = 0.95, $p = 0.639$). Additionally, the BCT regimen was associated with severe toxicity (the incidence of grade 3 or worse toxicity was 95 % for BCT vs. 73 % for CVD; $p = 0.001$).^[63] Meta-analysis of several trials comparing various BCT regimens with chemotherapy showed significant increase in response rates for BCT regimens.^[64] In another trial that substituted

TMZ for dacarbazine in the BCT regimens, a decrease in the incidence of initial CNS progression was observed, but again, there was no improvement in overall survival.^[65] These data suggest that BCT should only be considered in the setting of a clinical study.

A more recent chemotherapy agent being investigated in metastatic melanoma is albumin-bound paclitaxel, or nab-paclitaxel.^[66] This agent has been approved for the treatment of patients with metastatic breast cancer.^[67] A phase II trial of albumin-bound paclitaxel as a single agent in previously treated and chemotherapy-naïve metastatic melanoma patients demonstrated partial response rates of 2.7 % and 21.6 %, respectively. In addition, median progression-free survival of 3.5 months and 4.5 months, and median overall survival of 12.1 months and 9.6 months, respectively, were seen in second- and first-line treatment settings.^[68] These figures compare favorably to dacarbazine-based regimens. Another phase II trial evaluated albumin-bound paclitaxel and carboplatin in previously treated and chemotherapy-naïve patients with unresectable stage IV metastatic melanoma. The results showed response rates of 8.8 % and 25.6 %, median progression-free survival of 4.2 months and 4.3 months, and median overall survival of 10.9 months and 11.1 months in previously treated and chemotherapy-naïve patients, respectively.^[69] In this trial, all objective responses were partial responses in the previously treated group, and all but one was a partial response in the chemotherapy-naïve group.^[69] These trials suggest promising clinical activity and nab-paclitaxel was associated with less toxicity than paclitaxel. A randomized phase III trial has been completed and results are expected soon.

4 Tumor Immunotherapy

Tumor immunotherapy is a modality that utilizes the immune system to recognize and eradicate cancer. Melanoma has been the prototypical tumor for immunotherapy based on the early identification of tumor-associated antigens within melanoma cells, recognition that T-cell infiltrates were related to good outcomes in primary melanoma lesions, the success of high-dose IL-2 in the treatment of metastatic melanoma, and recent survival benefits reported for ipilimumab, a T-cell checkpoint inhibitor. While it has long been recognized that the immune system could mediate tumor recognition and rejection, it is more recently been established that immunotherapy can be effective in mediating durable complete responses in selected patients with advanced melanoma.^[70] In Sects. 4.1–4.2, we review the two approved immunotherapy agents for metastatic melanoma and briefly describe some of the promising new agents in development. .

4.1 Interleukin-2

IL-2 is a cytokine that mediates the homeostatic function of T and natural killer (NK) cells. While originally described as a T-cell growth factor based on the need for IL-2 to expand effector T cells, emerging data have supported a role for IL-2 in expanding regulatory T cells (Tregs), which have innate suppressive functions in vitro and in vivo. The clinical application of IL-2 in melanoma patients began when IL-2 was used to support the survival of adoptively transferred T cells in the 1980s. Subsequent work in animal models and humans demonstrated that IL-2 when administered at high doses had independent anti-tumor activity.^[71] High-dose IL-2 was FDA approved for metastatic melanoma in 1998 based on an analysis of 270 metastatic melanoma patients treated with a high-dose (600,000 international units per kg) bolus (over 15 min every 8 h to 14 doses) of IL-2 with an overall response rate of 16 % and a complete response rate of 6 %.^[72] Long-term survival analysis of these patients demonstrated durable response with a median duration of complete response of over 59 months.^[73] Nearly 40 % of the responding patients that were alive during the report continued to be disease free for more than 70 months and without melanoma progression for more than 150 months following initiation of therapy. In addition, surgical resection of residual disease was performed in some patients who had a relapse and

these patients also attained prolonged relapse-free survival.^[74] The identification of patients likely to benefit from IL-2 has been the subject of considerable work and several putative biomarkers have been described, including serum vascular endothelial growth factor (VEGF) and fibronectin levels, although these require prospective validation. IL-2 requires treatment at specialized centers where the toxicity can be properly managed. Adverse effects include capillary leak syndrome, which is characterized by hypotension, tachycardia, peripheral edema, reversible multi-system organ failure, and cardiac arrhythmias.^[74] Additional toxicity consists of fever, pruritus, electrolyte abnormalities, cytopenias, and coagulopathy. Early reports of IL-2-related mortality stemmed from uncontrolled Gram-positive infections and antibiotic prophylaxis became routine after 1990.

Several strategies have been utilized to increase the therapeutic potential of IL-2 and these have largely centered on combinations with other modalities. While combinations with cytotoxic chemotherapy have generally been disappointing, a promising approach combining IL-2 with an HLA-0201-restricted gp100 peptide vaccine has been reported. The gp100:209–217(210M) peptide binds with a higher affinity to HLA-A2 than the native peptide, and induces stronger T-cell stimulation.^[75] An initial single-institution phase II trial with 210M combined with high-dose IL-2 demonstrated objective tumor responses in 13 of 31 (42 %) patients.^[76] A multi-institutional study evaluated three phase III trials of 210M and high-dose IL-2 administered in various schedules with the aim of achieving a target response rate of 30 %.^[75] None of the trials reached this target after a 60-month median follow-up; the overall response rate was 16.5 %. More recently, a prospective, randomized phase III trial was completed and a significant improvement in objective response was seen in patients treated with 210M and IL-2 compared with patients who received IL-2 alone (16 % vs. 6 %, $p = 0.03$).^[77] This trial also reported a significant improvement in progression-free survival (2.2 months vs. 1.6 months; $p = 0.008$) and a trend toward improved median overall survival (17.8 months vs. 11.1 months; $p = 0.06$) in the combination group compared with the HD-IL2-alone group.

4.2 Ipilimumab

The cytotoxic T-lymphocyte antigen 4 (CTLA-4) is an inhibitory checkpoint receptor that blocks T-cell activation and helps regulate the balance between immune activation and tolerance.^[78–80] T-cell activation is regulated by a series of signals from antigen-presenting cells that provide intra-cellular information to the T cell related to the type and strength of antigenic stimulation. The first signal is delivered through antigens, which for T cells is provided as processed peptides bound to major histocompatibility complex (MHC) molecules; antigenic peptides in the context of MHC are recognized by the T-cell receptor (TCR). For full activation, T cells also require a second, or costimulatory, signal. The best characterized costimulatory signal is provided by B7.1 and B7.2 (also known as CD80 and CD86) on the surface of antigen-presenting cells. CD80 and CD86 bind to CD28 on the surface of the T cell, resulting in signaling that allows the T cell to enter the cell cycle, differentiate, and produce cytokines.^[81] In the absence or insufficiency of the costimulation signal, T-cell responses are blunted and T cells can become tolerant, or unresponsive, to the presented antigen. Under normal physiologic conditions, CTLA-4 is mobilized to the surface of antigen-activated T cells after costimulation, and can effectively bind to B7 costimulatory molecules with much higher affinity than CD28.^[82] This interaction, in effect, reverses T-cell activation and provides an autocrine regulatory mechanism for preventing uncontrolled T-cell activation and autoimmunity. In the setting of an established tumor, however, this early inactivation of T-cell function may hinder tumor clearance. In fact, in an animal model of melanoma, the combination of a tumor cell vaccine with an antagonist CTLA-4 monoclonal antibody was shown to release T-cell responses from inhibition and treatment was able to mediate tumor regression of B16, a poorly immunogenic melanoma tumor.^[83]

This and other promising preclinical results led to clinical trials using a fully humanized anti-CTLA-4 monoclonal antibody (ipilimumab).

In a phase II clinical trial of 217 patients with unresectable stage III or IV melanoma, patients were randomized in a blinded manner to a fixed dose of ipilimumab at 0.3, 3.0, or 10.0 mg/kg. In this study, the best overall response rate was 11.1 % in the 10 mg/kg cohort with 4.2 % response at 3 mg/kg and no responses at the 0.3 mg/kg dose; responses were found to be durable (18–35 months).^[84–86] Ipilimumab has been associated with an interesting adverse effect profile that consists of autoimmune events, such as dermatitis, colitis, drug-related hepatitis, endocrinopathies, and rarely neuritis. These adverse effects appeared to be more common at the higher doses of ipilimumab.^[84] A prospective, double-blind, randomized, phase III trial was conducted in 676 HLA-A2 patients with advanced melanoma who were randomized to one of three treatment arms consisting of ipilimumab alone (at 3 mg/kg), ipilimumab (at 3 mg/kg) combined with the 210M gp100 peptide vaccine, or vaccine alone. This trial confirmed the therapeutic benefit of ipilimumab as patients who received the drug in either treatment arm had a significantly superior overall survival compared with vaccine-alone patients (10 months vs. 6 months; $p = 0.0026$).^[87] When comparing ipilimumab alone with ipilimumab plus gp100, the response rate was 10.9 % vs. 5.7 % ($p = 0.04$). Importantly, this trial also observed no significant difference in progression-free survival, which highlighted the delayed kinetics of response that is often observed with ipilimumab. Based on the results of this trial, ipilimumab was approved by the FDA for the treatment of advanced melanoma in 2011. A subsequent randomized clinical trial was reported that compared ipilimumab at 10 mg/kg and dacarbazine (850 mg/m² of body-surface area) with dacarbazine (850 mg/m²) and placebo.^[8] In this trial, 502 patients with previously untreated metastatic melanoma were enrolled. The overall survival was significantly prolonged in the ipilimumab-treated patients (11.2 vs. 9.1 months) with better 1-year survival in the combination arm compared with controls (47.3 % vs. 36.3 %). The survival effect persisted with prolonged 3-year survival for combination-treated patients compared with the control arm (20.8 % vs. 12.2 %; hazard ratio for death = 0.72; $p < 0.001$). The incidence of grade 3 or greater adverse events were reported in 56.3 % of patients treated in the combination arm compared with 27.5 % in the dacarbazine and placebo arm ($p < 0.001$). In this study, there were no drug-related deaths or gastrointestinal perforations reported.

These clinical trials focused attention on two important aspects of treatment with ipilimumab and potentially other T-cell checkpoint inhibitors. The first is the development of a diverse array of autoimmune adverse effects that appear to affect almost all tissues with the skin and gastrointestinal tract being the most common. These adverse effects can be effectively managed with corticosteroids, and rarely, more intense immunosuppressive medications. The second aspect is the delayed onset of both clinical responses and toxicity, leading to the need for caution in interpreting initial response and on-going vigilance for toxicity even after treatment has stopped. The delayed kinetics is presumably related to the longer time required for T-cell activation, migration to tumor sites, eradication of tumor cells, and clearance of necrotic debris. A new set of clinical guidelines known as the immune-related response criteria have been proposed as a more accurate method for monitoring patients treated with ipilimumab and other immune modulators.^[89–90] These guidelines will require further validation but should be kept in mind when evaluating melanoma patients being treated with these agents.

4.3 Programmed Death-1 (PD-1)/PD-Ligand 1 Blockade

Similar to CTLA-4, the programmed death-1 (PD-1) receptor is a T-cell co-inhibitory molecule that normally binds to the PD-1 and 2 ligands (PD-L1, PD-L2) on antigen-

presenting cells and suppresses T-cell activation. The PD-1/PD-L1 system is of particular interest because PD-1 is highly expressed on both activated T cells and 'exhausted' T cells following exposure to high antigen loads.^[91–92] Engagement of PD-1 by its ligands, PD-L1 and PD-L2, results in altered T-cell activation and function mediated by downregulation of TCR signaling and up-regulation of inhibitory tyrosine phosphatases.^[93–95] These ligands are typically expressed by antigen-presenting cells, but have also been found to be aberrantly expressed on a number of human tumors, including metastatic melanoma.^[96–97] Tumor PDL-1 expression was shown to correlate with the presence of infiltrating lymphocytes and a poor clinical prognosis.^[98] In preclinical animal models, antibodies that block PD-1 resuscitated melanoma-specific T-cell responses and augmented tumor regression.^[99–101] A fully humanized antibody to PD-1 has been tested in patients with refractory solid tumors, including melanoma.^[102] The antibody appears to be well tolerated without significant toxicity, except for one patient who developed inflammatory colitis. In another clinical trial that included 109 melanoma patients, 26 experienced objective responses that were durable in some cases for up to 12 months.^[103]

In a recent phase I clinical trial, an anti-PD-L1 monoclonal antibody in a dose escalation design ranging from 0.3 mg/kg to 10 mg/kg was administered to patients with selected metastatic tumors. The study enrolled 207 patients, including 55 with metastatic melanoma and treated patients until disease progression or toxicity developed. The median duration of treatment was 12 weeks and objective responses were reported in 9 of 52 evaluable melanoma patients as well as in two renal cell carcinoma patients, five non-small-cell lung carcinoma patients, and one ovarian cancer patient. The responses appeared to be durable with 50 % lasting at least 1 year after onset. The most common drug-related adverse effects were fatigue, infusion reactions, diarrhea, arthralgias, rash, nausea, pruritus, and headaches but only 9 % of patients experienced grade 3 or greater toxicity.^[104] Future studies are likely to include combinations of anti-PD-1 and anti-PD-L1 with ipilimumab and this is supported by pre-clinical mouse melanoma studies that demonstrated synergy between PD-1 and CTLA-4 blockade.^[105]

4.4 Adoptive Cell Transfer

Adoptive cell transfer is a form of passive immunotherapy in which patients are infused with a large number of melanoma-specific T cells. Recent studies, particularly when cells are administered in the setting of host lymphodepletion, have reported objective response rates as high as 70 %.^[106] The process of generating the large number of tumor-antigen-specific T cells is very labor and time intensive. Briefly, T cells are selected for their ability to produce interferon- γ in the presence of tumor or specific antigen(s) and carefully expanded in culture for up to 2 weeks using IL-2 to produce large numbers of cells (up to 50×10^9) to be re-infused into the patient.^[107] The success of this approach appears dependent on (1) the source of tumor-specific T cells, which can come from tumor-infiltrating lymphocytes (TILs) or transgenically modified, peripheral blood T cells that express a melanoma-specific TCR, (2) ex vivo IL-2 expansion, and (3) conditioning patients with a regimen of non-myeloablative chemotherapy and/or irradiation before infusion and high-dose IL-2 after infusion.^[108]

The lymphodepleting conditioning regimens have included non-myeloablative chemotherapy with fludarabine, cyclophosphamide, and total body irradiation (2–12 Gy). The mechanism by which host lymphocyte depletion supports the anti-tumor activity of adoptive cell transfer (ACT) is incompletely understood but may include suppression of Tregs, removal of cytokine sinks, and eradication of host tumor immunosuppressive factors. The source of the T cells also strongly influences the success of ACT. TILs provide the ideal source for the tumor-specific T cells and a number of studies that utilized this source have generated impressive response rates of up to 70 %.^[106, 109, 110] However, a drawback to this

source is the high 'dropout' rate (up to 40 %) due to the inability to generate enough functional T cells in culture. To combat the high 'dropout' rate seen in the TIL studies, peripheral blood T cells can be genetically engineered to express high- or low-affinity tumor-antigen-specific TCRs.^[111–112] The results from studies utilizing these approaches were not as impressive as the TIL studies, with 19–30 % of the patients experiencing objective responses.^[113–114] Adverse effects are often related to the conditioning regimens (e.g., fludarabine, cyclophosphamide, and irradiation) and those seen with high-dose IL-2 administration. In some cases, autoimmune events such as the destruction of normal melanocytes in eyes and skin occurs and immunosuppression is sometimes required to control these.^[109] The ACT approach will require further prospective randomized trials with survival endpoints as well as feasibility analyses at more centers before this can be incorporated into the therapeutic armamentarium for melanoma treatment.

4.5 Regulatory T-Cell Inhibition

Tregs are defined by high expression of the IL-2 receptor (IL-2R) α chain (CD25) and the Forkhead box P3 (FoxP3) transcription factor; these cells suppress activated effector T cells and can inhibit anti-tumor immunity in animal models.^[115–117] The impact of Tregs in patients with melanoma has been suspected to be based on reports of increased Treg numbers in both the peripheral circulation and at the tumor microenvironment.^[118] This increase in Treg frequency was found to correlate with poor clinical outcomes and may also affect the ratio of effector CD8⁺ T cells (cytolytic) to Tregs. For example, a ratio that favors CD8⁺ T cells has been shown to strongly correlate with improved survival ($p < 0.0001$).^[119] Thus, a potentially promising approach to release anti-tumor immunity from Treg suppression is to deplete the host of Tregs. Several strategies have been developed for this purpose, although none is completely specific for Tregs. In addition to non-myeloablative conditioning, the most well conducted studies have taken advantage of the propensity for Tregs to express high levels of CD25 on the cell surface.^[120–122]

Ontak, a fusion of the IL-2 protein and diphtheria toxin, selectively eliminates IL-2R-expressing Tregs from the peripheral blood of renal cell carcinoma patients. The decrease in Treg frequency was transient and was associated with augmented effector T-cell proliferation and cytotoxicity following active immunization.^[120] Furthermore, results from a recent clinical trial of Ontak in metastatic melanoma patients demonstrated an abrogation of the suppressive activity of Tregs in vivo and enhanced de novo appearance of tumor-specific CD8⁺ T cells. Of the 60 patients treated, 17 % had objective partial responses with impressive 1-, 2-, and 3-year survival (40.0 %, 17.9 %, and 9.2%).^[123] Despite these data, further calibration of the timing and dosage of Ontak is needed, as other studies failed to obtain similar results.^[124] This discrepancy may be due to the unwanted depletion of anti-tumor effector cells (e.g., CD4⁺ helper and CD8⁺ cytotoxic T cells), which also express CD25. Another agent, anti-IL-2R monoclonal antibody (daclizumab), originally developed to prevent organ transplant rejection, may also attenuate Treg immune suppression in melanoma patients and is currently being explored.^[125–126]

4.6 Oncolytic Virus Therapy

One strategy to generate a systemic anti-melanoma immune response is to induce the destruction of cancer cells by the use of an engineered oncolytic herpes simplex, type 1 virus. This virus encoding human granulocyte-macrophage colony-stimulating factor (talimogene laherparepvec) is injected directly into an established metastatic melanoma lesion and while it is taken up by both normal and malignant cells, it replicates only in the melanoma cells. The oncolytic nature of this virus should result in the release of tumor antigens that can be taken up by antigen-presenting cells to activate melanoma-specific T-cell responses.^[127] In a phase II clinical trial in which patients with refractory stage IV or

unresectable stage III melanoma were treated with talimogene laherparepvec, an objective clinical response of 28 % was observed.^[128] This included regression of injected and non-injected visceral disease. Further evaluation of peripheral blood and tumor biopsy specimens suggested that regressing tumors were associated with the appearance of melanoma-associated antigen (MART)-1-specific effector CD8+ T cells and a decrease in Tregs.^[129] These early-phase clinical trials also confirmed the safety of this agent and only minor grade I adverse events were reported, such as fever and local injection-site pain. These data demonstrate the potential therapeutic potential of local oncolytic virus treatment and a follow-up multi-institutional, prospective, randomized phase III clinical trial is currently in progress. Other vectors, including Cocksackievirus A21, adenovirus, Newcastle disease virus, and others are also being explored.

4.7 Toll-Like Receptor Agonists

The toll-like receptors (TLRs) are a collection of molecules that belong to the IL-1R superfamily and recognize repeated patterns that are common among host pathogens, often referred to as pathogen-associated molecular patterns (PAMPs). Activation of the innate immune system (e.g., macrophages and dendritic cells) through TLRs promotes the activation of naïve B and T cells, and fosters the development of adaptive immunity against an array of pathogenic organisms.^[130] Ligation of TLRs induces the production of local cytokines, such as interferon- α and IL-12, which enhance local immune responses.^[130] Although originally described in the induction of pathogen-specific immunity, there is emerging evidence that TLRs may also mediate anti-tumor immunity. Imiquimod is a topical TLR 7/8 agonist that has been approved by the FDA for the treatment of viral warts and basal cell carcinoma. Early reports have also suggested that imiquimod may induce regression of superficial melanocytic lesions.^[131–132] The tumors that regressed following TLR agonist treatment have been associated with a significant immune response, including an increase in CD8+ T-cell infiltration at the site of application. Imiquimod has also been used in combination with tumor vaccines to elicit more potent systemic anti-tumor immune responses.^[133] The endpoint of this study was induction of antigen-specific T-cell responses, which were seen in 44 % of the treated patients, suggesting imiquimod could boost the immunity of tumor vaccines.^[133] Although tumor regression was local, the data suggest that this may be a potent adjuvant for vaccines, and perhaps other forms of immunotherapy.

5 Targeted Therapy

A major advance in the treatment of cutaneous melanoma has been the identification of activating mutations in the genes of a number of key cell signaling molecules that drive melanoma cell proliferation and the malignant phenotype. These molecular mutations can be identified and the development of small molecular inhibitors that target these mutations has been validated as a clinically useful strategy for the treatment of advanced melanoma. Overall, mutations in the genes of these key signaling molecules can be identified in approximately 70 % of patients with cutaneous melanoma. The most common is *BRAF*, which can be mutated in 50–60 % of cutaneous melanomas, and has been successfully used as a target in melanoma.^[134] *NRAS* mutations can be identified in almost 20 % of cutaneous melanomas. Although *NRAS* has eluded drug development to date, there is interest in several *NRAS* downstream molecular targets.^[135] Mutations in *c-KIT* have also been identified in approximately 1–6 % of cutaneous melanomas but the frequency may be higher in mucosal and acral lesions. Studies are underway to better understand the potential of *c-KIT* as a target for drug therapy in melanoma.^[136]

5.1 BRAF Inhibitors

The studies of BRAF inhibitors have provided important insights into the role of targeted therapy for melanoma treatment. In August 2011, the first targeted drug for melanoma, the selective oral BRAF inhibitor vemurafenib, was approved by the FDA for the treatment of advanced melanoma in patients harboring a mutation in *BRAF*.^[137] BRAF is a member of the mitogen-activated protein kinase (MAPK) pathway and plays a role in transferring cell surface growth signals to the nucleus of the cell. BRAF is one of three ras-activating factors (RAF) that are stimulated by RAS and subsequently activate mitogen-activated protein/extracellular signal-regulated kinase (MEK) in this signaling pathway. Although CRAF is the isoform most commonly activated by RAS, it is the *BRAF* isoform that is most commonly mutated in malignant cells. A rather unique feature of *BRAF* mutations is that most (>90 %) occur in one location at amino acid 600, in which the normal valine is substituted with other amino acids, most frequently a glutamic acid resulting in the well defined *BRAF V600E* mutation. Overall, *BRAF* mutations have been found in 8 % of all cancers but seem to occur with a higher frequency in tumor cells of neural crest origin with the peak being approximately 50–60 % of melanomas. The mutations result in constitutive activation of MEK regardless of RAS status in the cell. *BRAF* appears to be a critical oncogene for melanoma as the introduction of a *BRAF* mutation in wild-type cells is sufficient to induce malignant transformation.^[138] Furthermore, *BRAF* mutations occur early in melanoma pathogenesis as mutations have been reported in severely dysplastic nevi.^[139]

A major advance in the field has been the development of small molecule BRAF inhibitors. These agents generally fall into two classes, those that inhibit a large spectrum of protein kinases and those that are more selective for RAF kinases. The more selective agents have achieved much better results in clinical melanoma trials and include the oral drugs, vemurafenib and dabrafenib. These inhibitors selectively block BRAF signaling in cells with *BRAF* mutations and may actually promote signaling in cells that harbor wild-type *BRAF*. Vemurafenib has now been widely tested in phase I, II, and III clinical trials for metastatic melanoma patients with a known *BRAF* mutation. These studies have documented that almost 90 % of patients demonstrate at least some degree of tumor regression following exposure to vemurafenib.^[137,140–141] The largest clinical trial was a prospectively randomized phase III study in 675 previously untreated patients with metastatic melanoma harboring a *BRAF V600E* mutation. In this study patients were randomized to treatment with vemurafenib administered at 960 mg orally twice a day or dacarbazine given at 1,000 mg/m² of body surface area intravenously every 3 weeks. This trial reported a statistically significant improvement in overall survival (hazard ratio = 0.37, $p < 0.001$) and progression-free survival (5.3 vs. 1.6 months; hazard ratio = 0.26, $p < 0.001$) favoring vemurafenib treatment.^[137] The 6-month survival was 84 % in the vemurafenib group and 64 % in the dacarbazine group. The objective response rate according to Response Evaluation Criteria In Solid Tumors (RECIST) criteria was 48 % in the vemurafenib-treated patients compared with 5 % in the dacarbazine-treated patients. The authors concluded that there was a 63 % relative reduction in the risk of death for patients treated with vemurafenib. Based on these study results, the FDA approved vemurafenib for the treatment of metastatic melanoma harboring a *BRAF* mutation in August of 2011.

The toxicity profile of vemurafenib included skin reactions consisting of photosensitivity, rash, pruritus, hyperkeratosis, keratocanthomas, and squamous cell carcinomas. Additional adverse effects were fatigue, arthralgias, alopecia, headache, nausea, vomiting, and diarrhea.^[137] In the phase III clinical trial, 38 % of patients required dose modification because of adverse effects. Subsequent data have suggested that treatment with vemurafenib may also result in prolonged QT interval.^[142] Thus, patients treated with vemurafenib should have a baseline skin examination and EKG and be instructed to use sunscreen when

going outdoors. The patients must be closely monitored for skin reactions, especially the development of squamous cell carcinoma. These tumors appear to be localized and responsive to local excision if they do arise.

In the phase III trial, the median time to response with vemurafenib was 1.45 months and this confirms the rapid onset of therapeutic benefit that can be seen with selective BRAF inhibitors. A potential limiting factor, however, is that across all vemurafenib trials, the median duration of response was about 5.5–7.5 months with a majority of patients eventually experiencing drug resistance and progression of disease. The mechanisms of this resistance remain incompletely defined but may include incomplete inhibition of the MAPK pathway, increased expression of *NRAS* in *BRAF* blocked cells, downstream activating mutations in MEK or other pathway factors, and activation of alternative signaling pathways, such as the PI3 kinase pathway.^[135–137] A better understanding of these resistance mechanisms and an intense focus on combination therapy are promising strategies for dealing with drug resistance with BRAF inhibitors.

Clinical trials of dabrafenib, another oral selective BRAF inhibitor, are on-going. In fact, early data from a phase I trial with patients treated with dabrafenib showed that nine of ten patients with metastatic melanoma with untreated brain metastases had reductions in size of brain lesions.^[143] The overall reduction in size in brain metastases ranged from 20 % to 100 %.^[143] More recently, an open-label phase III randomized trial of dabrafenib was conducted in patients with treatment-naïve, *BRAF*-mutated, and unresectable stage III or IV melanoma.^[144] In this trial, 733 patients were screened and 250 patients were randomized 3:1 to receive dabrafenib (150 mg by mouth twice a day) or dacarbazine (1,000 mg per m² by intravenous administration every 3 weeks). The most common adverse effects with dabrafenib were skin-related effects, fever, fatigue, arthralgia, and headache, while the most frequent toxicity seen with dacarbazine was nausea, vomiting, neutropenia, fatigue, and asthenia. Patients treated with dabrafenib have an improved progression-free survival of 5.1 months compared with 2.7 months for dacarbazine.

5.2 CKIT Inhibitors

Similar to *BRAF*, activating mutations have been identified in *CKIT*, which encodes a cell surface tyrosine kinase that delivers growth signals to cells. Mutations in *CKIT* have been well documented in other cancers, including chronic myelogenous leukemia (CML) and gastrointestinal stromal tumors (GIST) where treatment with imatinib, an oral CKIT inhibitor, has been associated with a significant survival advantage.^[145] In melanoma, mutations in *CKIT* have been described in up to 6 % of cutaneous melanomas, although the presence of *CKIT* mutations is much higher in certain subtypes of melanoma, such as mucosal melanoma (39 %), acral lentiginous melanoma (36 %), and cutaneous melanomas arising in areas of chronic sun-damaged skin (28 %), providing perhaps a partial explanation for the unusual clinical behavior of these melanoma subtypes.^[146] The recognition that these types of melanoma are more likely to harbor *CKIT* mutations has helped plan clinical trials of CKIT inhibitors in patients with melanoma, although mutation analysis is routinely used as eligibility criteria for participation in clinical trials of CKIT inhibitors. This may be particularly important because, in contrast to *BRAF* mutations which appear to be highly restricted to a single locus, *CKIT* mutations have been reported across several exons with some more likely to be associated with emergence of drug resistance.^[147] A higher frequency of *CKIT* mutations are found within exon 11 at a site that encodes the juxtamembraneous region of the receptor. *CKIT* is emerging as an important target in melanoma since many of the melanoma variants harboring *CKIT* mutations have traditionally been resistant to other therapeutic modalities available for melanoma treatment.

Imatinib is an oral CKIT inhibitor and has demonstrated dramatic clinical responses in patients with melanoma harboring *CKIT* mutations.^[148] In a recent open-label, single-arm, phase II clinical trial, 295 patients with metastatic mucosal, acral, or cutaneous melanoma arising in chronic sun-damaged skin were screened for the presence of a mutation in *CKIT*.^[149] Of the 295 patients, 51 patients with mutations were identified and 28 patients were treated with imatinib administered at a dose of 400 mg orally twice a day. The trial reported two complete objective responses, two durable responses, and two transient partial responses among 25 patients evaluable for response. The overall objective response rate was 16 % with a 12-week median time to disease progression. In this report, the authors did find an association between clinical response and mutation pattern. Further clinical studies with other CKIT inhibitors, including dasatinib and nilotinib, are underway in melanoma.^[137]

5.3 MEK Inhibitors

Another factor in the MAPK pathway is MEK. MEK is downstream from CKIT, NRAS and BRAF/CRAF but has also been reported to harbor activating mutations.^[150] Selumetinib is an orally available selective MEK inhibitor and has shown anti-tumor activity against *BRAF V600E*-mutated melanoma cell lines and therapeutic activity in pre-clinical melanoma models.^[151–152] This agent was evaluated in an open-label, randomized, phase II clinical trial in which 200 patients with unresectable stages III or IV melanoma were evenly randomized to selumetinib administered at 100 mg orally twice a day in 28-day cycles or temozolomide given at 200 mg/m² of body surface area orally each day for 5 days in each 28-day cycle.^[153] The authors reported a toxicity profile that included dermatitis acneiform, diarrhea, nausea, and peripheral edema for the selumetinib-treated patients. There was no difference in objective response rate or progression-free survival between the treatment groups. This trial, however, did not require mutation status testing prior to study enrollment. The authors did report that five of the six partial responders were subsequently found to harbor *BRAF V600E* mutations. Thus, future studies focusing on melanoma patients with defined *BRAF* or *MEK* mutations are expected.

5.4 Vascular Endothelial Growth Factor Inhibitors

Melanoma, like many tumors, produces high levels of VEGF. The presence of high levels of VEGF has been correlated with poor prognosis, immune suppression, and growth of tumor neovasculature and tumor progression. Thus, VEGF may be an important target for therapeutic intervention in melanoma. Several clinical trials have attempted to test this hypothesis with equivocal results. In a single-arm, multi-institutional, phase II clinical trial, 62 patients with previously untreated metastatic melanoma were treated with oral temozolomide at a dose of 150 mg/m² on days 1–7 and the anti-VEGF monoclonal antibody, bevacizumab, at a dose of 10 mg/kg by intravenous administration on day 1 every 2 weeks until disease progression or unacceptable toxicity.^[154] The objective response rate was 16 % (one complete response and nine partial responses) with an additional 22 patients with stable disease for an overall disease control rate of 52 %. The median progression-free survival was 4.2 months and overall survival was 9.6 months. The authors reported an improvement in overall survival for patients with *BRAF V600E*-mutated melanoma (12.0 vs. 9.2 months, $p = 0.014$).

In another single-arm, phase II clinical trial, bevacizumab (15 mg/kg) was administered intravenously on day 1 of a 2-week cycle in combination with interferon- α -2B given at a dose of 5 MU/m² subcutaneously three times a week during cycle 1 and at a dose of 10 MU/m² subcutaneously three times a week during subsequent cycles. The patients were restaged every six cycles and a total of 25 patients were enrolled.^[155]

The authors reported that adverse effects included interferon-related fatigue and myalgias requiring dose reduction in 11 patients, bevacizumab-related proteinuria in six patients, and three grade 4 adverse events: one pulmonary embolism, one myocardial infarction, and one stroke. Overall, six patients had an objective partial response and five patients had stable disease. The median progression-free and overall survival rates were 4.8 and 17 months, respectively. The regimen was considered to have clinical activity. A placebo-controlled, prospective, randomized clinical trial was conducted to compare the combination of bevacizumab (15 mg/kg), carboplatin (area under the plasma concentration curve, 5), and paclitaxel (175 mg/m²) administered every 3 weeks with chemotherapy alone.^[156] A total of 214 patients were randomized 2:1 to the three drug combination treatment or to chemotherapy and placebo. At a median follow-up of 13 months, the bevacizumab/chemotherapy group had a median progression-free survival of 5.6 months compared with 4.2 months in the chemotherapy-alone group (hazard ratio = 0.78, $p = 0.14$). The objective response rate was 25.5 % for the bevacizumab group compared with 16.4 % for the control group ($p = 0.16$). The authors concluded that the study did not meet its initial endpoint of improved progression-free survival. Collectively, these studies suggest the potential of VEGF as a target but failed to confirm a significant improvement in clinically meaningful endpoints for bevacizumab.

5.5 Other Targets

A variety of additional molecular targets has been identified for potential drug development in melanoma.

5.5.1 AKT/PI3K/mTor—In addition to the MAPK pathway, the P13K/AKT/mammalian target of rapamycin (mTOR) pathway has also been shown to harbor activating mutations that cooperate with *BRAF* mutations to promote tumor pathogenesis.^[157] In addition, the tumor suppressor gene, *PTEN*, which normally inhibits this pathway is frequently lost in melanoma cells through both missense mutations and allelic deletion. Although selective drugs targeting AKT, PI3K, and mTOR are in development, and have shown promise in other tumors, convincing data of single-agent activity in melanoma are lacking at present.

5.5.2 CDK4/p16—Melanoma cells have been known for some time to harbor alterations in several cell cycle factors, including the cyclin-dependent kinase 4 (CDK4/p16).^[158] The presence of point mutations is rare and it has been more common to find complete deletion of the p16 locus or cyclin D amplifications. While these genetic aberrations in melanoma cells suggest this may be an attractive therapeutic target, no preliminary data or specific drugs have yet been tested despite the development of CDK inhibitors.

5.5.3 ErbB4—Mutations in the ErbB family of tyrosine kinase receptors have been implicated in a variety of cancers, although only ErbB4 has been identified with mutations in melanoma.^[159] This raises the potential to utilize non-selective inhibitors of ErbB in melanoma and will likely spur additional research to develop more selective inhibitors of ErbB4 for melanoma therapy.

5.5.4 GNAQ—GNAQ is a G protein of the G1 family and has been shown to harbor activating mutations in an α subunit in ocular melanomas.^[160] In contrast to cutaneous and mucosal melanomas, uveal melanomas do not appear to have mutations in *BRAF*, *CKIT*, or *NRAS*. The activating mutation in *GNAQ* does result in stimulation of the MAPK signaling pathway and cell lines harboring *GNAQ* mutations are sensitive to MEK inhibitors, and studies with selumetinib seem likely.^[160]

5.5.5 MITF—The master melanoma-lineage transcription factor, MITF, has been suggested as a potential therapeutic target and has been found to be amplified in up to 20 % of melanoma cells.^[161] MITF regulates the transcriptional control of several critical genes, including the cyclin-dependent kinase 2 (CDK2), suggesting a role as an oncogene in melanoma cells. Further interest in MITF has been based on recent findings suggesting that BRAF may partially control MITF expression, possibly by mediating MITF phosphorylation by the MAPK pathway and subsequent ubiquitin-mediated proteolysis.^[162]

5.5.6 NRAS—The role of mutated *NRAS* in melanoma has been widely recognized and can be found in approximately 20 % of melanoma cells. The importance of NRAS is also highlighted by the clinical activity of the selective BRAF inhibitors and the increased expression of NRAS as a mechanism of resistance in vemurafenib-treated melanoma cells.^[163] To date, NRAS has eluded selective therapeutic drug development.

5.5.7 p53—Although p53 is usually intact in melanoma cells, the functional status of p53 can be altered through genetic and epigenetic modifications of p53 messengers. For example, MDM2 has been shown to be amplified in melanoma cell lines resulting in lack of p53 function.^[164] Thus, there is interest in using recently identified MDM2 antagonists for treatment of melanoma. Further studies are needed to better understand the utility of functional p53 as a target in melanoma therapy.

5.6 Combination Therapy

While the development of targeted therapy has already revolutionized the clinical management of patients with melanoma, it is clear that single-agent inhibitors are unlikely to result in significant advances in overall survival for most patients with advanced melanoma. The success of vemurafenib, however, has validated the study of targeted therapy and has generated considerable interest in understanding the mechanisms of resistance in vemurafenib-treated patients. The identification of additional molecular targets within the MAPK pathway and others leads to the potential for combining inhibitors of two or more targets within the same or across different cell signaling pathways. In addition, it may be possible to consider combining targeted therapy with other therapeutic modalities, such as cytotoxic chemotherapy and immunotherapy. The recent finding that vemurafenib induces an early local T-cell infiltration into melanoma sites further supports the potential for combining *BRAF* inhibition with immunotherapy designed to stimulate tumor-infiltrating lymphocytes.^[165] Future clinical trials evaluating a variety of rationally designed, combination treatment regimens will be necessary to better define optimal therapeutic strategies for patients with advanced melanoma.^[166]

6 Future Directions

The advances made in the past few years for the treatment of advanced melanoma have been dramatic with the approval of two new drugs in the past year, ipilimumab and vemurafenib.^[88,138] An improved understanding of the basic molecular and cellular changes in melanoma and the interactions with the host immune system have yielded important insights into the basic biology of melanoma while providing new therapeutic targets for drug development. Clearly, there will be an intense focus on new molecular targets based on the success of vemurafenib and new immunotherapy strategies based on the ipilimumab data. Research will undoubtedly include combination studies within and across the various modalities available for melanoma treatment. The studies conducted have highlighted important issues in the management of melanoma, including the potential for autoimmune toxicity, delayed kinetics of therapeutic response, and the likelihood of

emerging drug resistance with molecularly targeted therapies. These challenges will be the subject of further investigation in the coming years.

Metastatic melanoma remains a challenging clinical problem but the availability of several new drugs has improved the therapeutic options for patients. In addition to dacarbazine and IL-2, the recent approval of ipilimumab and vemurafenib are changing the clinical landscape of melanoma treatment. Studies need to be conducted to better understand the optimal sequencing of these agents and questions about dosing, particularly for ipilimumab, need to be resolved. The role of surgical intervention may also need to be reconsidered given the potential effectiveness of these new agents, which must be balanced against the emergence of drug resistance. The next few years promise to be exciting ones with so many new agents on the horizon.

Acknowledgments

No sources of funding were received to prepare this article. The authors have no conflicts of interest that are directly relevant to the content of this article.

References

1. Gray-Schopfer V, Wellbrock C, Marias R. Melanoma biology and new targeted therapy. *Nature*. 2007; 445:851–857. [PubMed: 17314971]
2. Ko JM, Fisher DE. A new era: melanoma genetics and therapeutics. *J Pathol*. 2011; 223:241–250. [PubMed: 21125678]
3. Eggermont AM. Advances in systemic treatment of melanoma. *Ann Oncol*. 2010; 21(Suppl. 7):viii339–viii344.
4. Garbe C, Eigentler TK, Keilholz U, et al. Systematic review of medical treatments in melanoma: current status and future prospects. *Oncologist*. 2011; 16:5–24. [PubMed: 21212434]
5. Nathanson KL. Using genetics and genomics strategies to personalize therapy for cancer: focus on melanoma. *Biochem Pharmacol*. 2010; 80:755–761. [PubMed: 20412787]
6. Atallah E, Flaherty L. Treatment of metastatic malignant melanoma. *Curr Treat Options Oncol*. 2005; 6(3):185–93. [PubMed: 15869730]
7. Balch CM, Gershenwald JE, Soong SJ, et al. Final version of 2009 AJCC melanoma staging and classification. *J Clin Oncol*. 2009; 27:6199–6206. [PubMed: 19917835]
8. Wasif N, Bagaria SP, Ray P, et al. Does metastasectomy improve survival in patients with Stage IV melanoma? A cancer registry analysis of outcomes. *J Surg Oncol*. 2011; 104(2):111–115. [PubMed: 21381040]
9. Essner R, Lee JH, Wanek LA, et al. Contemporary surgical treatment of advanced-stage melanoma. *Arch Surg*. 2004; 139(9):961–966. [PubMed: 15381613]
10. Lee CC, Faries MB, Wanek LA, et al. Improved survival after lymphadenectomy for nodal metastasis from an unknown primary melanoma. *J Clin Oncol*. 2008; 26(4):535–541. [PubMed: 18235114]
11. Neuman HB, Patel A, Hanlon C, et al. Stage-IV melanoma and pulmonary metastases: factors predictive of survival. *Ann Surg Oncol*. 2007; 14(10):2847–2853. [PubMed: 17680317]
12. Schuhan C, Muley T, Dienemann H, et al. Survival after pulmonary metastasectomy in patients with malignant melanoma. *Thorac Cardiovasc Surg*. 2011; 59(3):158–162. [PubMed: 21480136]
13. Petersen RP, Hanish SI, Haney JC, et al. Improved survival with pulmonary metastasectomy: an analysis of 1720 patients with pulmonary metastatic melanoma. *J Thorac Cardiovasc Surg*. 2007; 133(1):104–110. [PubMed: 17198792]
14. Conill C, Gimferrer JM, Marruecos J, et al. Clinical outcome after surgical resection of lung metastases from melanoma. *Clin Transl Oncol*. 2007; 9(1):48–52. [PubMed: 17272230]
15. Andrews S, Robinson L, Cantor A, et al. Survival after surgical resection of isolated pulmonary metastases from malignant melanoma. *Cancer Control*. 2006; 13(3):218–223. [PubMed: 16885918]

16. Lewis CW Jr, Harpole D. Pulmonary metastasectomy for metastatic malignant melanoma. *Semin Thorac Cardiovasc Surg.* 2002; 14(1):45–48. [PubMed: 11977016]
17. Yang SC. Pulmonary metastasectomy for melanoma: beyond the standard of care. *Ann Surg Oncol.* 2007; 14(10):2696–2697. [PubMed: 17674107]
18. Liang KV, Sanderson SO, Nowakowski GS, et al. Metastatic malignant melanoma of the gastrointestinal tract. *Mayo Clin Proc.* 2006; 81(4):511–516. [PubMed: 16610571]
19. Chua TC, Saxena A, Morris DL. Surgical metastasectomy in AJCC stage IV M1c melanoma patients with gastrointestinal and liver metastases. *Ann Acad Med Singapore.* 2010; 39(8):634–639. [PubMed: 20838706]
20. Sanki A, Scolyer RA, Thompson JF. Surgery for melanoma metastases of the gastrointestinal tract: indications and results. *Eur J Surg Oncol.* 2009; 35(3):313–319. [PubMed: 18590949]
21. Baars A, van Riel JM, Cuesta MA, et al. Metastasectomy and active specific immunotherapy for a large single melanoma metastasis. *Hepatogastroenterology.* 2002; 49(45):691–693. [PubMed: 12063971]
22. Woon WW, Haghighi KS, Zuckerman RS, et al. Liver resection and cryotherapy for metastatic melanoma. *Int Surg.* 2008; 93(5):274–277. [PubMed: 19943429]
23. Pawlik TM, Zorzi D, Abdalla EK, et al. Hepatic resection for metastatic melanoma: distinct patterns of recurrence and prognosis for ocular versus cutaneous disease. *Ann Surg Oncol.* 2006; 13(5):712–720. [PubMed: 16538410]
24. Mittendorf EA, Lim SJ, Schacherer CW, et al. Melanoma adrenal metastasis: natural history and surgical management. *Am J Surg.* 2008; 195(3):363–368. [PubMed: 18206850]
25. Sturgeon C, Leong SP, Duh QY. Laparoscopic surgery for melanoma metastases to the adrenal gland. *Expert Rev Anticancer Ther.* 2004; 4(5):837–841. [PubMed: 15485317]
26. Sanki A, Scolyer RA, Thompson JF. Surgery for melanoma metastases of the gastrointestinal tract: indications and results. *Eur J Surg Oncol.* 2009; 35(3):313–319. [PubMed: 18590949]
27. Zacest AC, Bassar M, Stevens G, et al. Surgical management of cerebral metastases from melanoma: outcome in 147 patients treated at a single institution over two decades. *J Neurosurg.* 2002; 96(3):552–558. [PubMed: 11883841]
28. Carrubba CJ, Vitaz TW. Factors affecting the outcome after treatment for metastatic melanoma to the brain. *Surg Neurol.* 2009; 72(6):707–711. [PubMed: 19604550]
29. Majer M, Samlowski WE. Management of metastatic melanoma patients with brain metastases. *Curr Oncol Rep.* 2007; 9(5):4111–4116.
30. Douglas JG, Margolin K. The treatment of brain metastases from malignant melanoma. *Semin Oncol.* 2002; 29(5):518–524. [PubMed: 12407517]
31. Lonser RR, Song DK, Klapper J, et al. Surgical management of melanoma brain metastases in patients treated with immunotherapy. *J Neurosurg.* 2011; 115(1):30–36. [PubMed: 21476810]
32. Ollila DW, Gleisner AL, Hsueh EC. Rationale for complete metastasectomy in patients with stage IV metastatic melanoma. *J Surg Oncol.* 2011; 104(4):420–424. [PubMed: 21858837]
33. Morton DL. Melanoma: are we there yet? *J Surg Oncol.* 2011; 104(4):337.
34. Faries MB, Ariyan S. Current surgical treatment in melanoma. *Curr Probl Cancer.* 2011; 35(4):173–184. [PubMed: 21911181]
35. Sosman JA, Moon J, Tuthill RJ, et al. A phase 2 trial of complete resection for stage IV melanoma: results of Southwest Oncology Group Clinical Trial S9430. *Cancer.* 2011; 117(20):4740–4746. [PubMed: 21455999]
36. Caudle AS, Ross MI. Metastasectomy for stage IV melanoma: for whom and how much? *Surg Oncol Clin N Am.* 2011; 20(1):133–144. [PubMed: 21111963]
37. Hussussian CJ. Surgical treatment of advanced melanoma. *Clin Plast Surg.* 2010; 37(1):161–168. [PubMed: 19914466]
38. Wargo JA, Tanabe K. Surgical management of melanoma. *Hematol Oncol Clin North Am.* 2009; 23(3):565–581. [PubMed: 19464603]
39. Smylie M, Claveau J, Alanen K, et al. Management of malignant melanoma: best practices. *J Cutan Med Surg.* 2009; 13(2):55–73. [PubMed: 19459245]

40. Mosca PJ, Teicher E, Nair SP, et al. Can surgeons improve survival in stage IV melanoma? *J Surg Oncol.* 2008; 97(5):462–468. [PubMed: 18270974]
41. Young SE, Martinez SR, Essner R. The role of surgery in treatment of stage IV melanoma. *J Surg Oncol.* 2006; 94(4):344–351. [PubMed: 16917867]
42. Faries MB, Morton DL. The promise of metastasectomy in melanoma. *Ann Surg Oncol.* 2006; 13(5):607–609. [PubMed: 16538408]
43. Ollila DW, Caudle AS. Surgical management of distant metastases. *Surg Oncol Clin N Am.* 2006; 15(2):385–398. [PubMed: 16632222]
44. Wong SL, Coit DG. Role of surgery in patients with stage IV melanoma. *Curr Opin Oncol.* 2004; 16(2):155–160. [PubMed: 15075909]
45. Essner R. Surgical treatment of malignant melanoma *Surg Clin North Am.* 2003; 83(1):109–156.
46. Allen PJ, Coit DG. The surgical management of metastatic melanoma. *Ann Surg Oncol.* 2002; 9(8):762–770. [PubMed: 12374659]
47. Allen PJ, Coit DG. The role of surgery for patients with metastatic melanoma. *Curr Opin Oncol.* 2002; 14(2):221–226. [PubMed: 11880715]
48. Komorowski AL, Wysocki WM, White RL Jr. Surgical management of solitary metastatic melanoma. *Acta Chir Belg.* 2009; 109(2):155–158. [PubMed: 19499673]
49. Martinez SR, Young SE. A rational surgical approach to the treatment of distant melanoma metastases. *Cancer Treat Rev.* 2008; 34(7):614–620. [PubMed: 18556133]
50. Tauceri F, Mura G, Roseano M, et al. Surgery and adjuvant therapies in the treatment of stage IV melanoma: our experience in 84 patients. *Langenbecks Arch Surg.* 2009; 394(6):1079–1084. [PubMed: 18317795]
51. Tomov T, Siegel R, Bembenek A. Long-term survival in stage IV melanoma after repetitive surgical therapy. *Onkologie.* 2008; 31(5):259–261. [PubMed: 18497515]
52. Spanknebel K, Kaufman HL. Surgical treatment of stage IV melanoma. *Clin Dermatol.* 2004; 22(3):240–250. [PubMed: 15262311]
53. Tagawa ST, Cheung E, Banta W, et al. Survival analysis after resection of metastatic disease followed by peptide vaccines in patients with stage IV melanoma. *Cancer.* 2006; 106(6):1353–1357. [PubMed: 16475151]
54. Lotem M, Peretz T, Drize O, et al. Autologous cell vaccine as a post operative adjuvant treatment for high-risk melanoma patients (AJCC stages III and IV): the new American Joint Committee on Cancer. *Br J Cancer.* 2002; 86(10):1534–1539. [PubMed: 12085200]
55. Guba M, Steinbauer M, Ruhland V, et al. Elevated MIA serum levels are predictors of poor prognosis after surgical resection of metastatic malignant melanoma. *Oncol Rep.* 2002; 9(5):981–984. [PubMed: 12168059]
56. Lens M. Current clinical overview of cutaneous melanoma. *Br J Nurs.* 2008; 17(5):300–305. [PubMed: 18414292]
57. Kim C, Lee CW, Kovacic L, et al. Long-term survival in patients with metastatic melanoma treated with DTIC or temozolomide. *Oncologist.* 2010; 15:765–771. [PubMed: 20538743]
58. Middleton MR, Grob JJ, Aronson N, et al. Randomized phase III study of temozolomide versus dacarbazine in the treatment of patients with advanced metastatic melanoma. *J Clin Oncol.* 2000; 18:158–166. [PubMed: 10623706]
59. Bajetta E, Del Vecchio M, Nova P, et al. Multicenter phase III randomized trial of polychemotherapy (CVD regimen) versus the same chemotherapy (CT) plus subcutaneous interleukin-2 and interferon- α 2b in metastatic melanoma. *Ann Oncol.* 2006; 17:571–577. [PubMed: 16469753]
60. O'Day SJ, Kim CJ, Reintgen DS. Metastatic melanoma: chemotherapy to biochemotherapy. *Cancer Control.* 2002; 9(1):31–38. [PubMed: 11907464]
61. Pflugfelder A, Eigentler TK, Keim U, et al. Effectiveness of carboplatin and paclitaxel as first- and second-line treatment of 61 patients with metastatic melanoma. *PLoS One.* 2011; 6(2):e16882. [PubMed: 21359173]
62. Chapman PB, Panageas KS, Williams L, et al. Clinical results using biochemotherapy as a standard of care in advanced melanoma. *Melanoma Res.* 2002; 12:381–387. [PubMed: 12170188]

63. Atkins MB, Hsu J, Lee S, et al. Phase III trial comparing concurrent biochemotherapy with cisplatin, vinblastine, dacarbazine, interleukin-2, and interferon alpha-2b with cisplatin, vinblastine, and dacarbazine alone in patients with metastatic malignant melanoma (E3695): a trial coordinated by the Eastern Cooperative Oncology Group. *J Clin Oncol*. 2008; 26:5748–5754. [PubMed: 19001327]
64. Ives NJ, Stowe RL, Lorigan P, et al. Chemotherapy compared with biochemotherapy for the treatment of metastatic melanoma: a meta-analysis of 18 trials involving 2621 patients. *J Clin Oncol*. 2007; 25:5426–5434. [PubMed: 18048825]
65. Atkins MB, Gollob JA, Sosman JA, et al. A phase II pilot trial of concurrent biochemotherapy with cisplatin, vinblastin, temozolomide, interleukin 2, and IFN- α 2B in patients with metastatic melanoma. *Clin Cancer Res*. 2002; 8:3075–3081. [PubMed: 12374674]
66. Stinchcombe TE, Socinski MA, Walko CM, et al. Phase 1 and pharmacokinetic trial of carboplatin and albumin-bound paclitaxel, ABI-007 (Abraxane[®]) on three treatment schedules in patients with solid tumors. *Cancer Chemother Pharmacol*. 2007; 6:759–766. [PubMed: 17285317]
67. Gradishar WJ, Tjulandin S, Davidson N, et al. Superior efficacy of albumin-bound paclitaxel, ABI-007, compared with polyethylated castor oil-based paclitaxel in women with metastatic breast cancer: results of a phase III trial. *J Clin Oncol*. 2005; 23:7794–7803. [PubMed: 16172456]
68. Hersh EM, O'Day SJ, Ribas A, et al. A phase 2 clinical trial of nab-paclitaxel in previously treated and chemotherapy-naïve patients with metastatic melanoma. *Cancer*. 2010; 116:155–163. [PubMed: 19877111]
69. Kottschade LA, Suman VJ, Amatruda T III, et al. A phase II trial of nab-paclitaxel (ABI-007) and carboplatin in patients with unresectable stage IV melanoma. *Cancer*. 2011; 117:1704–1710. [PubMed: 21472717]
70. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science*. 2011; 331(6024):1565–1570. [PubMed: 21436444]
71. Krieg C, Létourneau S, Pantaleo G, et al. Improved IL-2 immunotherapy by selective stimulation of IL-2 receptors on lymphocytes and endothelial cells. *Proc Natl Acad Sci U S A*. 2010; 107(26):11906–11911. [PubMed: 20547866]
72. Atkins MB, Lotze MT, Dutcher JP, et al. High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. *J Clin Oncol*. 1999; 17(7):2105–2116. [PubMed: 10561265]
73. Atkins MB, Kunkel L, Sznol M, et al. High-dose recombinant interleukin-2 therapy in patients with metastatic melanoma: long-term survival update. *Cancer J Sci Am*. 2000; 6(Suppl. 1):S11–S14. [PubMed: 10685652]
74. Bhatia S, Tykodi SS, Thompson JA. Treatment of metastatic melanoma: an overview. *Oncology (Williston Park)*. 2009; 23(6):488–496. [PubMed: 19544689]
75. Sosman JA, Carillo C, Urba WJ, et al. Three phase II cytokine working group trials of gp100 (210M) peptide plus high-dose interleukin-2 in patients with HLA-A2-positive advanced melanoma. *J Clin Oncol*. 2008; 26(14):2292–2298. [PubMed: 18467720]
76. Rosenberg SA, Yang JC, Schwartzentruber DJ, et al. Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. *Nat Med*. 1998; 4(3):321–327. [PubMed: 9500606]
77. Schwartzentruber DJ, Lawson DH, Richards JM, et al. gp100 peptide vaccine and interleukin-2 in patients with advanced melanoma. *N Engl J Med*. 2011; 364(22):2119–2127. [PubMed: 21631324]
78. Waterhouse P, Penninger JM, Timms E, et al. Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. *Science*. 1995; 270(5238):985–988. [PubMed: 7481803]
79. Tivol EA, Borriello F, Schweitzer AN, et al. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity*. 1995; 3(5):541–547. [PubMed: 7584144]
80. Chambers CA, Sullivan TJ, Allison JP. Lymphoproliferation in CTLA-4-deficient mice is mediated by costimulation-dependent activation of CD4⁺ T cells. *Immunity*. 1997; 7(6):885–895. [PubMed: 9430233]

81. Greenwald RJ, Freeman GJ, Sharpe AH. The B7 family revisited. *Annu Rev Immunol.* 2005; 23:515–548. [PubMed: 15771580]
82. Salama AK, Hodi FS. Cytotoxic T-lymphocyte-associated antigen-4. *Clin Cancer Res.* 2011; 17(14):4622–4628. [PubMed: 21467163]
83. 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(3):355–366. [PubMed: 10430624]
84. Wolchok JD, Neyns B, Linette G, et al. Ipilimumab monotherapy in patients with pretreated advanced melanoma: a randomised, double-blind, multicentre, phase 2, dose-ranging study. *Lancet Oncol.* 2010; 11(2):155–164. [PubMed: 20004617]
85. O'Day SJ, Maio M, Chiarion-Sileni V, et al. Efficacy and safety of ipilimumab monotherapy in patients with pretreated advanced melanoma: a multicenter single-arm phase II study. *Ann Oncol.* 2010; 21(8):1712–1717. [PubMed: 20147741]
86. Weber J, Thompson JA, Hamid O, et al. A randomized, double-blind, placebo-controlled, phase II study comparing the tolerability and efficacy of ipilimumab administered with or without prophylactic budesonide in patients with unresectable stage III or IV melanoma. *Clin Cancer Res.* 2009; 15(17):5591–5598. [PubMed: 19671877]
87. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med.* 2010; 363(8):711–723. [PubMed: 20525992]
88. Robinson MR, Chan CC, Yang JC, et al. Cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma: a new cause of uveitis. *J Immunother.* 2004; 27(6):478–479. [PubMed: 15534492]
89. Blansfield JA, Beck KE, Tran K, et al. Cytotoxic T-lymphocyte-associated antigen-4 blockade can induce autoimmune hypophysitis in patients with metastatic melanoma and renal cancer. *J Immunother.* 2005; 28(6):593–598. [PubMed: 16224277]
90. 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(14):8372–8377. [PubMed: 12826605]
91. Freeman GJ, Long AJ, Iwai Y, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med.* 2000; 192(7):1027–1034. [PubMed: 11015443]
92. Barber DL, Wherry EJ, Masopust D, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature.* 2006; 439(7077):682–687. [PubMed: 16382236]
93. Hirano F, Kameko K, Tamura H, et al. Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity. *Cancer Res.* 2005; 65(3):1089–1096. [PubMed: 15705911]
94. Sheppard KA, Fitz LJ, Lee JM, et al. PD-1 inhibits T-cell receptor induced phosphorylation of the ZAP70/CD3zeta signalosome and downstream signaling to PKCtheta. *FEBS Lett.* 2004; 574(1–3): 37–41. [PubMed: 15358536]
95. Okazaki T, Maeda A, Nishimura H, et al. PD-1 immunoreceptor inhibits B cell receptor-mediated signaling by recruiting src homology 2-domain-containing tyrosine phosphatase 2 to phosphotyrosine. *Proc Natl Acad Sci U S A.* 2001; 98(24):13866–13871. [PubMed: 11698646]
96. Yamazaki T, Akiba H, Iwai H, et al. Expression of programmed death 1 ligands by murine T cells and APC. *J Immunol.* 2002; 169(10):5538–5545. [PubMed: 12421930]
97. Iwai Y, Ishida M, Tanaka Y, et al. 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(19):12293–12297. [PubMed: 12218188]
98. Hino R, Kabashima K, Kato Y, et al. Tumor cell expression of programmed cell death-1 ligand 1 is a prognostic factor for malignant melanoma. *Cancer.* 2010; 116(7):1757–1766. [PubMed: 20143437]

99. He YF, Zhang GM, Wang XH, et al. Blocking programmed death-1 ligand-PD-1 interactions by local gene therapy results in enhancement of antitumor effect of secondary lymphoid tissue chemokine. *J Immunol.* 2004; 173(8):4919–4928. [PubMed: 15470033]
100. Strome SE, Dong H, Tamura H, et al. B7-H1 blockade augments adoptive T-cell immunotherapy for squamous cell carcinoma. *Cancer Res.* 2003; 63(19):6501–6505. [PubMed: 14559843]
101. Okazaki T, Honjo T. PD-1 and PD-1 ligands: from discovery to clinical application. *Int Immunol.* 2007; 19(7):813–824. [PubMed: 17606980]
102. Brahmer JR, Drake CJ, Wollner I, et al. 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.* 2010; 28(19):3167–3175. [PubMed: 20516446]
103. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med.* 2012; 366(26):2443–2454. [PubMed: 22658127]
104. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med.* 2012; 366(26):2455–2465. [PubMed: 22658128]
105. Curran MA, Montalvo W, Yagita H, Allison JP. 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 U S A.* 2010; 107(9):4275–4280. [PubMed: 20160101]
106. Dudley ME, Yang JC, Sherry R, et al. Adoptive cell therapy for patients with metastatic melanoma: evaluation of intensive myeloablative chemoradiation preparative regimens. *J Clin Oncol.* 2008; 26(32):5233–5239. [PubMed: 18809613]
107. Besser MJ, Shapira-Frommer R, Treves AJ, et al. Minimally cultured or selected autologous tumor-infiltrating lymphocytes after a lympho-depleting chemotherapy regimen in metastatic melanoma patients. *J Immunother.* 2009; 32(4):415–423. [PubMed: 19342963]
108. Muranski P, Boni A, Wrzesinski C, et al. Increased intensity lymphodepletion and adoptive immunotherapy: how far can we go? *Nat Clin Pract Oncol.* 2006; 3(12):668–681. [PubMed: 17139318]
109. Dudley ME, Wunderlich JR, Yang JC, et al. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. *J Clin Oncol.* 2005; 23(10):2346–2357. [PubMed: 15800326]
110. Dudley ME, Wunderlich JR, Robbins PF, et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. *Science.* 2002; 298(5594):850–854. [PubMed: 12242449]
111. Murphy A, Westwood JA, Teng MW, et al. Gene modification strategies to induce tumor immunity. *Immunity.* 2005; 22(4):403–414. [PubMed: 15845446]
112. Sadelain M, Riviere I, Brentjens R. Targeting tumours with genetically enhanced T lymphocytes. *Nat Rev Cancer.* 2003; 3(1):35–45. [PubMed: 12509765]
113. Johnson LA, Morga RA, Dudley ME, et al. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. *Blood.* 2009; 114(3):535–546. [PubMed: 19451549]
114. Morgan RA, Dudley ME, Wunderlich JR, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science.* 2006; 314(5796):126–129. [PubMed: 16946036]
115. Han Y, Guo Q, Zhang M, et al. CD69+ CD4+ CD25- T cells, a new subset of regulatory T cells, suppress T cell proliferation through membrane-bound TGF-beta 1. *J Immunol.* 2009; 182(1): 111–120. [PubMed: 19109141]
116. Curotto de Lafaille MA, Lafaille JJ. Natural and adaptive foxp3+ regulatory T cells: more of the same or a division of labor? *Immunity.* 2009; 30(5):626–635. [PubMed: 19464985]
117. Fontenot JD, Rasmussen JP, Williams LM, et al. Regulatory T cell lineage specification by the forkhead transcription factor foxp3. *Immunity.* 2005; 22(3):329–341. [PubMed: 15780990]
118. Jacobs JF, Nierkens S, Figdor CG, et al. Regulatory T cells in melanoma: the final hurdle towards effective immunotherapy? *Lancet Oncol.* 2012; 13(1):e32–e42. [PubMed: 22225723]
119. Sato E, Olson SH, Ahn J, et al. Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. *Proc Natl Acad Sci U S A.* 2005; 102(51):18538–18543. [PubMed: 16344461]

120. Dannull J, Su Z, Rizzieri D, et al. Enhancement of vaccine-mediated antitumor immunity in cancer patients after depletion of regulatory T cells. *J Clin Invest*. 2005; 115(12):3623–3633. [PubMed: 16308572]
121. Rasku MA, Clem AL, Telang S, et al. Transient T cell depletion causes regression of melanoma metastases. *J Transl Med*. 2008; 6:12. [PubMed: 18334033]
122. Litzinger MT, Fernando R, Curiel TJ, et al. IL-2 immunotoxin denileukin difitox reduces regulatory T cells and enhances vaccine-mediated T-cell immunity. *Blood*. 2007; 110(9):3192–3201. [PubMed: 17616639]
123. Telang S, Rasku MA, Clem AL, et al. Phase II trial of the regulatory T cell-depleting agent, denileukin difitox, in patients with unresectable stage IV melanoma. *BMC Cancer*. 2011; 11(1): 515. [PubMed: 22165955]
124. Attia P, Maker AV, Haworth LR, et al. Inability of a fusion protein of IL-2 and diphtheria toxin (denileukin difitox, DAB389IL-2, ONTAK) to eliminate regulatory T lymphocytes in patients with melanoma. *J Immunother*. 2005; 28(6):582–592. [PubMed: 16224276]
125. Jacobs JF, Punt CJ, Lesterhuis WJ, et al. Dendritic cell vaccination in combination with anti-CD25 monoclonal antibody treatment: a phase I/II study in metastatic melanoma patients. *Clin Cancer Res*. 2010; 16(20):5067–5078. [PubMed: 20736326]
126. Jones E, Dahm-Vicker M, Simon AK, et al. Depletion of CD25+ regulatory cells results in suppression of melanoma growth and induction of autoreactivity in mice. *Cancer Immun*. 2002; 2:1. [PubMed: 12747746]
127. Liu BL, Robinson M, Han ZQ, et al. ICP34.5 deleted herpes simplex virus with enhanced oncolytic, immune stimulating, and anti-tumour properties. *Gene Ther*. 2003; 10(4):292–303. [PubMed: 12595888]
128. Senzer NN, Kaufman HL, Amatruda T, et al. Phase II clinical trial of a granulocyte-macrophage colony-stimulating factor-encoding, second-generation oncolytic herpesvirus in patients with unresectable metastatic melanoma. *J Clin Oncol*. 2009; 27(34):5763–5771. [PubMed: 19884534]
129. Kaufman HL, Kim DW, DeRaffele G, et al. Local and distant immunity induced by intralesional vaccination with an oncolytic herpes virus encoding GM-CSF in patients with stage IIIc and IV melanoma. *Ann Surg Oncol*. 2010; 17(3):718–730. [PubMed: 19915919]
130. Barton GM, Kagan JC. A cell biological view of toll-like receptor function: regulation through compartmentalization. *Nat Rev Immunol*. 2009; 9(8):535–542. [PubMed: 19556980]
131. Krieg AM. Toll-like receptor 9 (TLR9) agonists in the treatment of cancer. *Oncogene*. 2008; 27(2):161–167. [PubMed: 18176597]
132. Schulze HJ, Cribier B, Requena L, et al. Imiquimod 5% cream for the treatment of superficial basal cell carcinoma: results from a randomized vehicle-controlled phase III study in Europe. *Br J Dermatol*. 2005; 152(5):939–947. [PubMed: 15888150]
133. Adams S, O'Neill DW, Nonaka D, et al. Immunization of malignant melanoma patients with full-length NY-ESO-1 protein using TLR7 agonist imiquimod as vaccine adjuvant. *J Immunol*. 2008; 181(1):776–784. [PubMed: 18566444]
134. Flaherty TK. Targeting metastatic melanoma. *Annu Rev Med*. 2012; 63:21.1–21.13.
135. Flaherty KT, Fisher DE. New strategies in metastatic melanoma: oncogene-defined taxonomy leads to therapeutic advances. *Clin Cancer Res*. 2011; 17(15):4922–4928. [PubMed: 21670085]
136. Flaherty TK, Hodi FS, Bastian BC. Mutation-driven drug development in melanoma. *Curr Opin Oncol*. 2010; 22(3):178–183. [PubMed: 20401974]
137. Chapman PB, Hauschild A, Robert C, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med*. 2011; 364(26):2507–2516. [PubMed: 21639808]
138. Tsao H, Goel V, Wu H, et al. Genetic interaction between NRAS and BRAF mutations and PTEN/MMAC1 inactivation in melanoma. *J Invest Dermatol*. 2004; 122:337–341. [PubMed: 15009714]
139. Uribe P, Wistuba II, Gonzalea S. BRAF mutation: a frequent event in benign, atypical and malignant melanocytic lesions of the skin. *Am J Dermatopathol*. 2003; 25(5):365–370. [PubMed: 14501284]
140. Flaherty KT, Puzanov I, Kim KB, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med*. 2010; 363:809–819. [PubMed: 20818844]

141. Ribas AK, Kim KB, Schuchter L, et al. BRIM-2: an open-label, multicenter phase II study of vemurafenib (PLX4032, RG7204) in previously treated patients with BRAFV600E mutation-positive metastatic melanoma [abstract]. *J Clin Oncol*. 2011; 29(Suppl):8509.
142. Zelboraf [package insert]. San Francisco (CA): Genetech USA; Aug. 2011
143. Falchook GS, Long GV, Kurzrock R, et al. Dabrafenib in patients with melanoma, untreated brain metastases, and other solid tumours: a phase 1 dose-escalation trial. *Lancet*. 2012; 379(9829): 1858–1859. [PubMed: 22608322]
144. Hauschild A, Grob JJ, Demidov LV, et al. Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. *Lancet*. 2012; 380(9839):358–365. [PubMed: 22735384]
145. Hochhaus A. Imatinib mesylate (Glivec, Gleevec) in the treatment of chronic myelogenous leukemia (CML) and gastrointestinal stromal tumors (GIST). *Ann Hematol*. 2004; 83(Suppl. 1):S65–S66. [PubMed: 15124676]
146. Beading C, Jacobson-Dunlop E, Hodi FS, et al. KIT gene mutations and copy number in melanoma subtypes. *Clin Cancer Res*. 2008; 14(21):6821–6828. [PubMed: 18980976]
147. Heinrich MC, Corless CL, Demetri GD, et al. Kinase mutations and imatinib responses in patients with metastatic gastrointestinal tumor. *J Clin Oncol*. 2003; 21:4342–4349. [PubMed: 14645423]
148. Hodi FS, Friedlander P, Corless CL, et al. Major response to imatinib mesylate in KIT-mutated melanoma. *J Clin Oncol*. 2008; 26(12):2046–2051. [PubMed: 18421059]
149. Carvajal RD, Antonescu CR, Wolchok JD, et al. KIT as a therapeutic target in metastatic melanoma. *JAMA*. 2011; 305(22):2327–2334. [PubMed: 21642685]
150. Smalley KS, Flaherty KT. Integrating BRAF/MEK inhibitors into combination therapy for melanoma. *Br J Cancer*. 2009; 100(3):431–435. [PubMed: 19156138]
151. Yeh TC, Marsh V, Bennet BA, et al. Biological characterization of AARY-142886 (AZD6244), a potent, highly selective mitogen-activated protein kinase kinase 1/2 inhibitor. *Clin Cancer Res*. 2007; 13:1576–1583. [PubMed: 17332304]
152. Friday BB, Yu C, Dy GK, et al. BRAF V600E disrupts AZD6244-induced abrogation of negative feedback pathways between extracellular signal-regulated kinase and Raf proteins. *Cancer Res*. 2008; 68:6145–6153. [PubMed: 18676837]
153. Kirkwood JM, Bastholt L, Robert C, et al. Phase II, open-label, randomized trial of the MEK 1/2 inhibitor selumetinib as monotherapy versus temozolomide in patients with advanced melanoma. *Clin Cancer Res*. 2011; 18(2):555–567. [PubMed: 22048237]
154. von Moos R, Seifert B, Simcock M, et al. First-line temozolomide combined with bevacizumab in metastatic melanoma: a multicentre phase II trial (SAKK 50/07). *Ann Oncol*. 2011; 23(2):531–536. [PubMed: 21527587]
155. Grignol V, Olencki T, Relekar K, et al. A phase II trial of bevacizumab and high-dose interferon alpha 2B in metastatic melanoma. *J Immunother*. 2011; 34(6):509–515. [PubMed: 21654521]
156. Kim KB, Sosman JA, Fruehauf JP, et al. BEAM: a randomized phase II study evaluating the activity of bevacizumab in combination with carboplatin plus paclitaxel in patients with previously untreated advanced melanoma. *J Clin Oncol*. 2012; 30(1):34–41. [PubMed: 22124101]
157. Dankort D, Curley DP, Cartlidge RA, et al. Braf(V600E) cooperates with Pten loss to induce metastatic melanoma. *Nature Genet*. 2008; 41:544–552. [PubMed: 19282848]
158. Malley KS, Contractor R, Nguyen TK, et al. Identification of a novel subgroup of melanomas with MKIT/cyclin-dependent kinase-4 overexpression. *Cancer Res*. 2008; 68(14):5743–5752. [PubMed: 18632627]
159. Prickett TD, Agrawal NS, Wei X, et al. Analysis of the tyrosine kinome in melanoma reveals recurrent mutations in ERBB4. *Nature Genet*. 2009; 41(10):1127–1132. [PubMed: 19718025]
160. Van Raamsdonk CD, Berzrokov V, Green G, et al. Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi. *Nature*. 2009; 457:599–602. [PubMed: 19078957]
161. Garraway LA, Widlund HR, Rubin MA, et al. Integrative genomic analyses identify MITF as a lineage survival oncogene amplified in malignant melanoma. *Nature*. 2005; 436:117–122. [PubMed: 16001072]

162. Wellbroick C, Rana S, Peterson H, et al. Oncogenic BRAD regulates melanoma proliferation through the lineage specific factor MITF. PLoS ONE. 2008; 3:e2734. [PubMed: 18628967]
163. Nazarian R, Shi H, Wang Q, et al. Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. Nature. 2010; 468:973–977. [PubMed: 21107323]
164. Muthusamy V, Hobbs C, Noguera C, et al. Amplification of CDK4 and MDM2 in malignant melanoma. Genes Chrom Cancer. 2006; 45:447–454. [PubMed: 16419059]
165. Wilmott JS, Long GV, Howle JR, et al. Selective BRAF inhibitors and induce marked T cell infiltration into human metastatic melanoma. Clin Cancer Res. 2012; 18(5):1386–1394. [PubMed: 22156613]
166. Blank CU, Hooijkaas AI, Haanen JB, et al. Combination of targeted therapy and immunotherapy in melanoma. Cancer Immunol Immunother. 2011; 60:1359–1371. [PubMed: 21847631]