

Targeted Therapy for Metastatic Melanoma

Ravi K. Amaravadi, MD, and Keith T. Flaherty, MD

Dr. Amaravadi is Instructor of Medicine and Dr. Flaherty is Assistant Professor of Medicine at the Developmental Therapeutics Program, Abramson Cancer Center at the University of Pennsylvania in Philadelphia.

Address correspondence to:
Keith T. Flaherty, MD, Medical Arts Building Suite 103, Penn Presbyterian Medical Center, 39th and Market Street, Philadelphia, PA 19104
E-mail: ktflaherty@aol.com

Abstract: Metastatic melanoma remains one of the most treatment-refractory malignancies. Despite decades of clinical trials testing chemotherapy and immunotherapy, a standard first-line treatment for metastatic melanoma has not yet been established. This review summarizes recent advances in our understanding of the role of targeted therapies including MAPK signaling inhibitors, VEGF signaling inhibitors, survival kinase inhibitors, and cyclin-dependent kinase inhibitors. An overview of melanoma biology and established targets, followed by a summary of completed and ongoing early-phase clinical trials highlights the failure of the first generation of targeted therapies to improve outcomes as single agents. In contrast, early hints of improved outcomes have been generated by clinical trials testing the combination of sorafenib and chemotherapy. The potential of targeted therapies in combination with chemotherapy or regimens consisting of multiple targeted therapies is explored, as increasing evidence suggests that combination therapeutics could finally impact the outcome of metastatic melanoma.

The majority of cutaneous melanoma lesions are diagnosed as stage I tumors, conferring a 90% 5-year survival rate when treated with surgery with or without adjuvant interferon.¹ However, once malignant melanoma cells obtain access to the blood or lymphatic system, and are beyond the scope of surgical resection, melanoma represents one of the most treatment-refractory malignancies.

For the purposes of the present discussion, targeted therapies are defined as drugs that specifically bind or perturb the function of a molecular target that has been implicated in the pathogenesis of cancer. These agents offer the theoretical advantage of decreased toxicity and increased antineoplastic efficacy compared to traditional cytotoxic chemotherapy. This review outlines the limited successes achieved with chemotherapy and immunotherapy, describes recent insights into the biology of melanoma, introduces some of the known targets in melanoma, and discusses the impact of recent results of clinical trials testing the first generation of targeted therapy for metastatic melanoma (Table 1).

Keywords

Metastatic melanoma, MAPK, VEGF, Bcl-2, growth factor signaling

Table 1. Targeted Therapies and Their Targets

Therapeutic Agent	Target	Therapeutic Mechanism	Clinical Trial Phase
MAPK activation: tumor cell proliferation, angiogenesis			
Sorafenib	RAF	KI	III
Raf-265	RAF	KI	I
PLX4032	Mutant BRAF	KI	I
R1155777	NRAS	Farnesyl transferase inhibitor	II
PD0325901	MEK	KI	I
VEGF/VEGF receptors/angiogenesis: tumor vascularization			
Sorafenib	VEGFR2	KI	III
Avastin	VEGF	mAb	II
Thalidomide	Angiogenesis/unknown	unknown	II
Volociximab	Integrin $\alpha 5\beta 1$	mAB	II
Growth Factor Receptor and downstream kinase activation: tumor cell growth, survival			
Imatinib	PDGFR	KI	II
CCI-779	mTOR	KI	II
RAD-001	mTOR	KI	II
Bcl-2 family members: tumor cell apoptosis resistance			
Oblimersen	Bcl-2	DNA antisense	III
Proteasome activation: tumor cell apoptosis resistance			
Bortezomib	Proteasome	Catalytic inhibitor	II
PI3K/Akt activation: tumor cell growth, survival, proliferation			
17-AAG	HSP90	Inhibitor	I
Perifosine	Akt	KI	II
p16 deletion and CDK4 activation: cell cycle progression			
PD-0332991	CDK4/6	KI	I

KI: Kinase inhibitor; mAB: monoclonal antibody

Clinical Characteristics and the Biology of Metastatic Melanoma

The clinical characteristics of melanoma that pose unique challenges to achieving advances in treatment include primary resistance to chemotherapy, a rapid tumor doubling time, and a particular tendency to metastasize to the brain. These characteristics can lead to rapid morbidity and limit the use of potentially active but toxic therapies to patients with excellent performance status. The underpinnings of these clinical characteristics may stem from the inherent resilience of melanocytes, the recurring genetic events that lead to progression from normal melanocyte to benign nevi, and the rare genetic defects that lead to the development of invasive melanoma. Melanocytes originate from the neural crest, differentiate and hone to

skin and other organs, and sparsely populate those end organs. Two peculiarities of melanoma are that it can arise de novo, independent of a preexisting melanocytic lesion, and it can arise in sites where the dominant etiologic factor—UV irradiation—is unlikely to have contributed to carcinogenesis. For the vast majority of metastatic melanoma, however, a cutaneous lesion on sun-exposed skin can be identified as the site of primary malignancy.

Cutaneous melanocytes exist in a hypoxic environment and rely heavily on growth factor signaling provided by neighboring keratinocytes and inflammatory cells for their survival.² The melanocyte's chief function is to secrete melanin, a pigment that serves as an antioxidant and free-radical scavenger and protects skin cells, including the melanocytes themselves, from carcinogenic mutations induced by UV light.³ Fair-skinned individuals have reduced melanin production compared to dark-skinned

individuals, and hence an increased susceptibility to developing skin cancers, supporting this protective role.

The formation of benign nevi appears to be a consequence of mutations in the *BRAF*⁴ and *NRAS*⁵ genes, found throughout the spectrum of benign-to-malignant melanocytic lesions. Activating mutations of these oncogenes in benign pigmented lesions appear to be an early, but insufficient, step in the development of invasive melanoma. Accumulation of subsequent mutations, especially in tumor suppressor genes, is likely necessary to convert benign pigmented lesions into malignant melanoma. Melanoma tumors first grow horizontally and later enter the vertical growth phase, which has been associated with an upregulation of genes associated with tumor angiogenesis, invasion, and metastasis.⁶ Along with their inherent fitness to migrate and survive cellular stress, these and other genetic events described below equip melanoma cells with multiple mechanisms to invade, metastasize, and resist therapy.

Limited Success of Chemotherapy and Immunotherapy for Metastatic Melanoma

We have entered the fourth decade of clinical trials involving cytotoxic chemotherapy drugs for the treatment of metastatic melanoma without identifying a clear standard-of-care, first-line treatment for this disease. Numerous small and large phase II and phase III trials testing platinum drugs, alkylating agents, nitrosoureas, vinca alkaloids, taxanes, topoisomerase inhibitors, and anthracyclines, both as single agents and in combination regimens, have yielded low response rates and progression-free and overall survival rates that are not clearly different from the natural history of metastatic disease.⁷⁻¹³ (For a recent review, see Gogas et al, 2007.¹⁴) Efforts to improve these numbers by adding immunotherapy,¹⁵⁻²⁰ or tamoxifen to chemotherapy²¹⁻²⁴ have resulted in increased toxicity without improving progression-free or overall survival in phase III trials. A meta-analysis of 20 randomized trials found no survival difference between dacarbazine (DTIC) alone and non-DTIC combination regimens with or without immunotherapy.²⁵ Despite these repeated negative results, combination regimens such as biochemotherapy and the Dartmouth regimen (DTIC, cisplatin, carmustine, and tamoxifen) are still widely prescribed outside of clinical trials for the treatment of metastatic melanoma.

Case reports of spontaneous tumor regression in patients with metastatic melanoma suggested that immunotherapy may have a higher impact on the outcome of metastatic melanoma than in other cancers. Despite initial successes in phase II clinical trials, phase III trials have demonstrated that immunotherapy based on interleukin (IL)-2 benefits only a small subset of patients with meta-

static melanoma.²⁶ High-dose IL-2 can result in durable partial and complete responses in some patients²⁷ and should be considered a treatment option for good-risk patients at a center experienced in managing IL-2 toxicity. Efforts to improve immunotherapy for melanoma have been extensively reviewed²⁶⁻²⁸ and will not be discussed here.

In 1975, DTIC became the first US Food and Drug Administration (FDA)-approved chemotherapeutic agent for the treatment of metastatic melanoma on the basis of phase II data. Numerous phase III trials have tested combination regimens against single-agent DTIC and have failed to demonstrate superiority in overall survival.²⁵ Despite low objective response rates of 5–10% in recent trials, DTIC remains the only FDA-approved chemotherapy for metastatic melanoma and is typically employed as the reference standard in phase III trials. The FDA approval of temozolomide (Temodar, Schering), the orally available analog of DTIC with the added advantage of central nervous system penetration, for malignant glioblastoma multiforme has been followed by testing of temozolomide-based regimens in melanoma. A phase III trial comparing temozolomide to DTIC found no difference in overall survival,²⁹ but a statistically significant increase in progression-free survival (1.9 vs 1.5 months) was observed. The ease of administration and favorable toxicity profile have resulted in widespread use of temozolomide off-label for the treatment of metastatic melanoma despite these poor outcomes. More recently, preclinical studies have identified recurring molecular abnormalities in melanoma cell lines and patient samples and ushered in the first generation of clinical trials testing targeted therapies for the clinical management of metastatic melanoma.

Targets in Metastatic Melanoma

A number of signaling pathways are commonly dysregulated in melanoma, and therefore have been proposed as targets for drug therapy (Figure 1). Clinical trials with single-agent targeted therapies, developed for other cancers, have been uniformly negative. One reason why these agents have thus far failed to alter the course of metastatic melanoma may be because they antagonize signaling mediators that, although present in melanoma, are not essential to the pathogenesis and spread of melanoma. This distinction becomes clearer when considering the experience with several of the agents that are discussed below.

It has been hypothesized that melanoma is resistant to cytotoxic chemotherapy due to the frequent overexpression of antiapoptotic proteins such as Bcl-2 and Bcl-XL. Despite initial reports that overexpression of Bcl-2 in melanoma cell lines may contribute to melanoma invasion

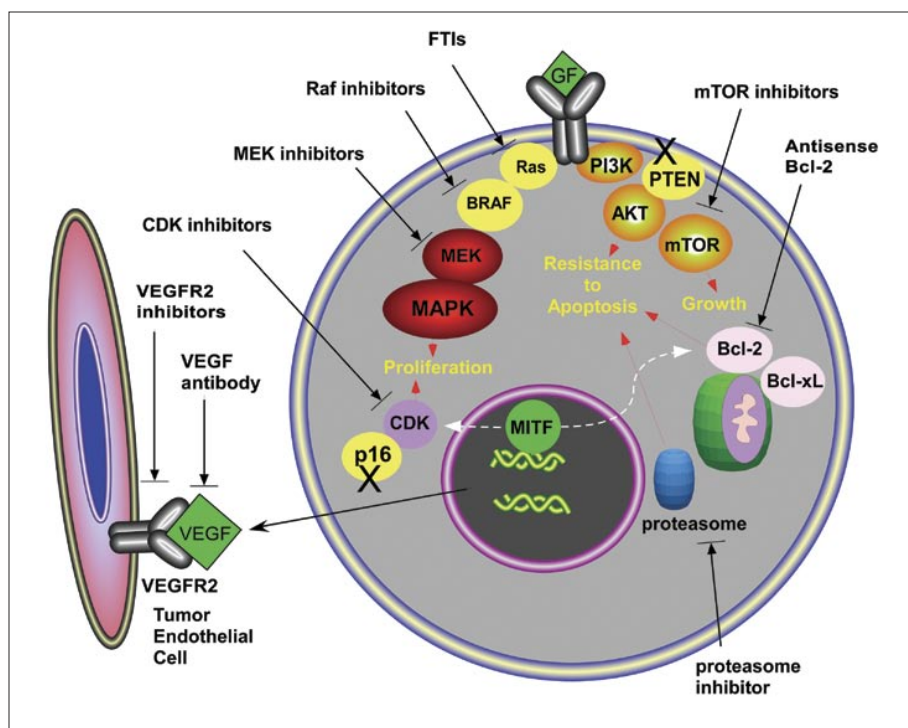


Figure 1. Targeted therapy currently being evaluated for melanoma. A melanoma cell and tumor endothelial cell are shown. Yellow indicates targets with known recurring somatic or germline mutations in patients with melanoma; X: inactivating mutations or deletions have been described.

GF=growth factor, FTIs=farnesyl transferase inhibitors.

and angiogenesis,^{29,30} the true prevalence of high Bcl-2 expression and its prognostic significance in melanoma patients remain uncertain.

Other dysregulated signaling pathways, without an identified genetic defect, can also drive melanoma pathogenesis and have been identified as targets because they are activated in most melanoma preclinical models. In particular, vascular endothelial growth factor (VEGF) and the VEGF receptors are important targets in melanoma.³¹⁻³³ Other growth factor receptors such as platelet-derived growth factor (PDGFR) and c-KIT are present on the majority of primary melanomas.³⁴ Targeting these receptors has been pursued with imatinib (Gleevec, Novartis). Finally, preclinical evidence suggests that proteasomal degradation is dysregulated in melanoma, leading to increased nuclear factor-kappa B (NF-κB) signaling, amongst other consequences.³⁵

A number of the targets described thus far are activated as a result of somatic genetic defects in specific oncogenes and tumor suppressor genes in melanoma. Directing drug development toward these genetically defined targets provides an opportunity to pair a patient with melanoma that harbors a particular genetic defect with a therapy that targets this defect. For example the *BRAF* V600E activating mutation can be found in 30–70%^{4,5,36} of metastatic melanomas and activating mutations in *NRAS* can be found in an additional 5–15%^{5,36} of metastatic melanomas. Because of the high prevalence rates of these activating mutations in metastatic melanoma, tar-

geting the kinases in the mitogen-activated protein kinase (MAPK) signaling pathway has become a focal point for new drugs in melanoma. Besides MAPK signaling, inactivating mutations or deletion of the tumor suppressor gene *PTEN* have been identified in 30% of melanomas,³⁶ and genetic amplification and increased expression of Akt³⁷ has been identified in 40–60% of melanomas. Together these defects result in constitutive activation of survival kinase signaling through Akt and its downstream effector mammalian target of rapamycin (mTOR). Inactivating mutations of the tumor suppressor genes *p16INK4A* and *p14ARF*, and activating mutations of the *CDK4* gene are high-penetrance genetic germline mutations that have been identified in 20–40% of familial melanomas³⁸ as well as multiple primary melanoma syndrome.³⁹ Somatic p16 mutations can be found in a high proportion of sporadic melanomas.^{40,41} Although these predisposition genes have multiple additional functions, the prevalence of these defects in both familial and sporadic melanoma suggests that disinhibition of retinoblastoma protein (Rb)-mediated cell cycle progression may be a necessary event in a majority of invasive melanomas. Another common genetic event in invasive melanoma is amplification of the *MITF* gene, leading to overexpression of the *MITF* transcription factor.⁴² *MITF* acts as an oncogene and has been shown to contribute to resistance to cytotoxic chemotherapy in preclinical models.⁴³

Outlined below are some of the targeted therapies that are currently being tested in clinical trials for patients

with metastatic melanoma. In addition to determining if targeted therapies can improve response rates and can affect progression-free survival, the focus of these early-phase clinical trials of targeted therapies is on the biologic effect of the targeted therapy in tumor samples and the impact of tumor genetics on clinical outcomes. The correlative endpoints of many of the phase I and phase II trials of targeted therapies are arguably as critical as clinical outcomes. Because of the availability of powerful tools to determine the drug-to-target effect and the gene-to-drug relationship in patient samples, even if the first generation of targeted therapies fail to improve survival outcomes in phase III trials, this approach could increase the likelihood of identifying which target is worthy of further drug development, and ultimately which patient could benefit from a particular agent. Unlike the success of single-agent targeted therapies in other treatment-refractory malignancies such as renal cell carcinoma,⁴⁴ there is little evidence to support the use of these targeted therapies as single agents in the treatment of metastatic melanoma. The development of combination regimens that simultaneously counter multiple abnormalities is the strategy most likely to lead to significant increases in survival.⁴⁵ While we await the development of additional targeted agents for melanoma, there is increasing evidence that combining some of the available targeted therapies with cytotoxic chemotherapy may lead to improved clinical outcomes and manageable toxicities.

Targeting Bcl-2

The rationale for targeting the antiapoptotic protein Bcl-2 stems from preclinical studies in which overexpression of this protein in melanoma cells led to increased secretion of angiogenic factors and resistance to chemotherapy.³¹ Oblimersen (Genasense, Genta), a first-in-class DNA antisense molecule against Bcl-2, was developed and tested in preclinical xenograft models of a wide variety of tumors. Besides hematologic malignancies, melanoma xenografts regressed dramatically with this agent, and this finding provided rationale for conducting clinical trials of oblimersen in patients with metastatic melanoma.⁴⁶ A phase I trial of oblimersen in combination with DTIC established the safety of this combination and confirmed in serial tumor biopsies of lymph nodes in patients with non-Hodgkin lymphoma that Bcl-2 expression was decreased by 15–47% with oblimersen treatment.⁴⁷ Whether or not this degree of target inhibition is sufficient to render melanoma cells more sensitive to chemotherapy is not known. Interestingly, preclinical models suggest that oblimersen may have cytotoxic effects against cancer cells that are unrelated to its antisense activity, as antisense Bcl-2 had a growth inhibitory effect on prostate cancer

cells both in the presence and absence of RNA interference (RNAi) directed against Bcl-2.⁴⁸ A phase II trial of oblimersen and DTIC found that 6 of 14 patients had a response, and that oblimersen resulted in a 40% decrease in Bcl-2 expression in melanoma samples.⁴⁹ The results of a randomized phase III trial of oblimersen with DTIC versus DTIC alone in patients with metastatic melanoma was recently reported.⁵⁰ Patients receiving oblimersen plus DTIC had a significantly increased response rate of 13% versus 7% and progression-free survival of 74 versus 49 days compared to patients receiving DTIC plus placebo. However there was no significant difference in overall survival observed between the two arms. Combining the phase II and phase III results of oblimersen, although this targeted therapy was able to modulate its target to some degree in vivo, its failure to affect survival may relate to incomplete suppression of a target that is present only in a minority of the tumors of a minority of patients. We are left to speculate on this point, as archival tumor specimens were not collected in the context of this trial. Notably, in some published pathologic series examining Bcl-2 expression in clinical samples of nevi, primary melanomas, metastatic cutaneous melanomas, and uveal melanoma, Bcl-2 expression was found to be highest in benign nevi and uveal melanoma.^{51,52} Furthermore, in uveal melanoma, high Bcl-2 expression was found in 67% of tumors but conferred an improved survival compared to low Bcl-2 expression.⁵¹ These findings raise concern that Bcl-2 may not be the appropriate antiapoptotic protein to target in melanoma.⁵³

Targeting MAPK Signaling

Sorafenib (Nexavar, Bayer/Onyx) was the first drug with in vitro activity against *BRAF* to be tested in patients with metastatic melanoma. Preclinical support for targeting the *BRAF* kinase came from studies in which *BRAF* depletion in melanoma cells, either through RNAi directed against *BRAF* in melanoma cells harboring the *BRAF* V600E nodal mutation,⁵⁴ or through sorafenib treatment of wild-type and mutant *BRAF* melanoma cell lines,⁵⁵ led to rapid apoptosis. Initial phase I trials established a dose of 400 mg orally twice daily for sorafenib, which was associated with frequent but manageable hand-foot syndrome, rash, diarrhea, and hypertension. A phase II randomized discontinuation trial of sorafenib in 37 patients with metastatic melanoma found a 3% partial response rate, 16% stable disease rate, and a median progression-free survival of 2.8 months.⁵⁶ *BRAF* mutational status did not predict which patients achieved stable disease. Despite these disappointing outcomes for single-agent sorafenib, the toxicity profile suggested this agent had nonoverlapping toxicities with cytotoxic chemotherapy. Increased tumor regression

was observed in preclinical models when sorafenib was added to various cytotoxic chemotherapy agents, providing the rationale for combining sorafenib with cytotoxic chemotherapy in patients with metastatic melanoma. The first trial to test this concept in melanoma was a large phase I/II trial of carboplatin, paclitaxel, and sorafenib including 105 patients with metastatic melanoma. The majority of these patients had American Joint Committee on Cancer stage M1c disease and had progressed despite prior therapies for metastatic disease. This combination of cytotoxic chemotherapy and a targeted therapy yielded a response rate of 27%, stable disease rate of 58%, and a median progression-free survival of 8.8 months. These results were superior to previously reported phase II trial results for carboplatin and paclitaxel,⁵⁷⁻⁵⁹ and provided the necessary evidence to launch two phase III clinical trials (first- and second-line therapy).

In the phase I/II trial, dosing of carboplatin and paclitaxel was limited by neutropenia. To determine if a more tolerable chemotherapy regimen could be substituted for carboplatin and paclitaxel and yield similar results, a phase II trial evaluating the combination of temozolomide and sorafenib, and a separate phase II trial testing DTIC and sorafenib, have been undertaken. Preliminary results of the phase II trial of temozolomide and sorafenib confirmed the phase I/II experience with carboplatin, paclitaxel, and sorafenib, as observed response rates and progression-free survival were improved compared to previously reported outcomes for temozolomide alone.⁶⁰ The early results of the trial with temozolomide and sorafenib are especially promising in patients with brain metastases, for which limited therapeutic options are available.

While the paradigm of sorafenib in combination with cytotoxic chemotherapy is being tested clinically, more potent and specific inhibitors of *BRAF* are being developed. In vitro, sorafenib inhibits *BRAF* at concentrations that are achieved in humans. However, in animal models and in the tumors of patients treated on sorafenib trials, the evidence is less robust.⁶¹ To determine if more complete inhibition of *BRAF* could lead to better clinical outcomes, more potent and specific RAF kinase inhibitors are now entering phase I clinical trials. Raf-265 (Novartis), a compound that was created by augmenting the chemical structure of sorafenib, and PLX4032 (Plexxicon), a compound that selectively inhibits mutant *BRAF*, are being tested in patients with metastatic melanoma.

Targeting other components of MAPK signaling has proven more difficult. Farnesyl transferase inhibitors (FTIs) are compounds that have been developed to inhibit the membrane localization of Ras through the attachment of the lipid farnesyl moiety to the Ras protein, thereby inhibiting its activation.⁶² Phase II and III trials of the FTI tipifarnib (Johnson & Johnson) as a single agent in a wide

variety of malignancies have not demonstrated increased progression-free or overall survival, and have found low response rates to this drug despite reproducible inhibition of farnesyl transferase activity.⁶³ A recent phase II trial conducted by Cancer and Leukemia Group B in patients with metastatic melanoma also observed no responses among 14 patients despite potent farnesyltransferase inhibition observed in tumor samples taken before and during treatment.⁶⁴ Additional studies of FTIs in combination with chemotherapy are underway. Inhibition of MEK, the kinase downstream of *BRAF*, has been shown to be an especially effective strategy for inhibiting the proliferation of melanoma cells harboring a *BRAF* mutation in preclinical models.⁶⁵ The first clinically tested MEK inhibitor, CI-1040, was not developed further because of poor pharmacokinetic properties and toxicity limitations, despite evidence of robust MEK inhibition.⁶⁶ A second-generation MEK inhibitor, PD-0325901, also produced effective MAPK inhibition in a phase I trial, with a 7% partial response rate in the melanoma patients distributed across multiple dose levels.⁶⁷

Targeting VEGF Signaling and Angiogenesis

VEGF is a secreted ligand that binds to its receptors, VEGFR1 and VEGFR2, on the membranes of endothelial cells stimulating endothelial cell proliferation and angiogenesis.³² Melanoma tumors were one of the first types used to demonstrate the tumor-induced growth of blood vessels from normal tissues.⁶⁸ Interestingly, sorafenib, besides inhibiting Raf kinase, is a potent inhibitor of VEGF receptors 1, 2, and 3. Using dynamic contrast-enhanced magnetic resonance imaging, we have demonstrated a change in the permeability of tumor vasculature in melanoma patients taking sorafenib, suggesting that some of sorafenib's antimelanoma efficacy may be due to its antiangiogenic properties.⁶¹ Depletion of *BRAF* in melanoma cells containing mutant *BRAF* also decreases VEGF secretion.⁶⁹ Sorafenib may therefore inhibit angiogenesis by decreasing the secretion of VEGF from tumor cells, while simultaneously blocking VEGF signaling through inhibition of VEGFR2 on endothelial cells. These findings have prompted investigation of other antiangiogenic drugs in melanoma. The most effective antiangiogenic drug used in oncology to date is bevacizumab (Avastin, Genentech), and current clinical trials incorporating bevacizumab in combination regimens for metastatic melanoma are underway. Another drug with antiangiogenic properties that has more mature clinical trial data in patients with metastatic melanoma is thalidomide (Thalomid, Celgene). Thalidomide has been tested in multiple phase II trials as a single agent and in combination with temozolomide. Although one group

of investigators demonstrated a 32% response rate and 9.5 month overall survival for the combination of thalidomide and extended daily dosing of temozolomide,⁷⁰ subsequent studies of this combination in patients with or without brain metastases reported response rates of 0–12%. Correlative studies have not convincingly demonstrated that thalidomide modulates tumor angiogenesis. In addition, high rates of deep vein thrombosis and pulmonary embolism as well as reports of *Pneumocystis carinii* pneumonia and aspergillosis in patients receiving extended daily dosing temozolomide and thalidomide have decreased the enthusiasm for further development of this combination.⁷¹

Targeting Growth Factor Signaling: Akt and mTOR

As described above, a number of molecular defects cooperate to drive constitutive activation of Akt and mTOR signaling in metastatic melanoma. Because of the success of the Abl/c-KIT/PDGFR multikinase inhibitor imatinib mesylate in the treatment of chronic myelogenous leukemia and gastrointestinal stromal tumors, the role of activated PDGFR signaling upstream of Akt and mTOR in melanoma has been investigated preclinically and in phase I and II trials. Imatinib has demonstrated no clinical activity as a single agent in patients with metastatic melanoma, and at a dose of 800 mg daily resulted in a level of myelosuppression that limits combining this agent with many chemotherapy regimens.⁷² We are currently conducting a phase I/II trial of imatinib and temozolomide, which is associated with more modest myelosuppression than other chemotherapy agents. We are also investigating imatinib in combination with bevacizumab, and others have initiated trials in combination with other targeted agents.

Development of potent and specific drugs that target the Akt family of kinases has proven difficult and has been reviewed previously.⁷³ A phase II trial of perifosine (Keryx), a nonenzymatic inhibitor of Akt, in patients with metastatic melanoma also found no objective responses and a high rate of nausea and gastrointestinal side effects.⁷⁴ Clinical oncology awaits the development of better Akt inhibitors as our appreciation of the importance of activated Akt as a driving force in multiple cancers has increased.⁷⁵ Downstream of Akt, mTOR serves as a nutrient sensor and directs the translation of key proteins including VEGF. Temsirolimus (Wyeth), an mTOR inhibitor which was recently found in a randomized phase III trial to improve overall survival as a single agent compared to interferon in renal cell carcinoma,⁷⁶ has been tested in melanoma patients in a phase II trial.⁷⁷ There were no responses, but preliminary results from a phase II trial of

the mTOR inhibitor everolimus (Novartis) suggest there may be disease stabilization.⁷⁸ Preclinical evidence suggests that mTOR inhibitors may potentiate the efficacy of chemotherapy.⁷⁹ Combination regimens of mTOR inhibitors and chemotherapy are currently being tested in clinical trials. In addition, the mTOR inhibitor sirolimus (Rapamune, Wyeth) combined with sorafenib led to synergistic killing of melanoma cells in vitro.⁸⁰ This combination will be studied in multiple phase II trials.

Besides disrupting protein translation, disruption of protein degradation through the action of specific proteasome inhibitors such as bortezomib (Velcade, Millennium) can reproducibly induce cell death in melanoma cells in vitro.³⁵ A phase II trial of bortezomib in patients with metastatic melanoma yielded no responses and a median progression-free survival of 1.5 months.⁸¹ The development of combination regimens involving bortezomib for metastatic melanoma is limited by the high rate of grade 3 toxicities including sensory neuropathy and thrombocytopenia observed in this phase II trial. However, a phase II trial combining bortezomib and temozolomide has been undertaken.

Targeting Cyclin-dependent Kinases

One of the most commonly dysregulated pathways in metastatic melanoma is Rb-mediated control of the cell cycle. As described above, mutations or homozygous deletion of the *p16CDKN2A* or *p14ARF* tumor suppressor genes or an activating mutation in the *CDK4* gene can lead to constitutive activation of cyclin-dependent kinase (CDK), phosphorylation of the Rb protein, and unhindered cell cycle progression. Replacing a deleted gene is not yet achievable clinically, but using small-molecule CDK inhibitors has been a focus of drug development. Flavopiridol, a natural compound, has been reported to have relative selectivity for *CDK4* and *CDK6* over other CDKs. A phase II trial of flavopiridol found no objective responses but a 44% disease stabilization rate.⁸² Toxicity was primarily gastrointestinal. The effect of flavopiridol on the cell cycle in tumors was not evaluated. Recently two compounds have been identified that specifically inhibit *CDK4* and *CDK6* and are now in phase I clinical trials.⁸³

Summary and Conclusion

Although the first generation of targeted therapies opened new possibilities for the treatment of metastatic melanoma, to date no targeted therapy has yielded promise as a single agent. This lack of success emphasizes the need for continued preclinical work to establish the significance of new drug targets. Early clinical trial results suggest that combining chemotherapy with sorafenib is feasible and

the results of the phase III trial testing the combination of sorafenib with carboplatin and paclitaxel are awaited to determine if this combination could be a new reference regimen in the treatment of metastatic melanoma. The combination of temozolomide and sorafenib will be further tested in multi-institution clinical trials in patients with brain metastases. While these trials are ongoing, second-generation Raf kinase inhibitors are entering phase I trials. Looking to the future, the possibility of combining multiple targeted therapies deserves special attention in melanoma. As inhibitors of parallel signaling pathways such as Akt/mTOR and MAPK are tested individually to establish safety, combination regimens involving an Akt inhibitor and a Raf kinase inhibitor, or an mTOR inhibitor and a Raf kinase inhibitor, should be evaluated carefully in the phase I setting with relevant pharmacodynamic endpoints to validate targeted inhibition. Similarly, the combination of these inhibitors of survival signaling and MAPK signaling with cell cycle inhibitors such as CDK inhibitors may one day replace chemotherapy-based strategies for the treatment of metastatic melanoma. These strategies could also potentially be combined with advances in immunotherapy to create simultaneous or sequential treatment paradigms that cripple melanoma progression at multiple points, ultimately providing the most sustainable impact on the natural history of this disease.

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