New Strategies in Metastatic Melanoma: Oncogene-Defined Taxonomy Leads to Therapeutic Advances

Keith T. Flaherty and David E. Fisher

**Abstract**

The discovery of BRAF and KIT mutations provided the first basis for a molecular classification of cutaneous melanoma on therapeutic grounds. As BRAF-targeted therapy quickly moves toward regulatory approval and incorporation as standard therapy for patients with metastatic disease, proof of concept has also been established for targeting mutated KIT in melanoma. NRAS mutations have long been known to be present in a subset of melanomas and represent an elusive subgroup for targeted therapies. Matching patient subgroups defined by genetic aberrations in the phosphoinositide 3-kinase and p16/cyclin dependent kinase 4 (CDK4) pathways with appropriate targeted therapies has not yet been realized. And, an increasing understanding of lineage-specific transcriptional regulators, most notably MITF, and how they may play a role in melanoma pathophysiology, has provided another axis to approach with therapies. The foundation has been established for individual oncogene targeting, and current investigations seek to understand the intersection of these susceptibilities and other described potential targets and pathways. The melanoma field stands poised to take the lead among cancer subtypes in advancing combination therapy strategies that simultaneously target multiple biologic underpinnings of the disease.

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**Introduction**

Melanoma is cured in a high proportion of patients when diagnosed at early stages. Despite efforts to improve primary and secondary prevention, the incidence of primary melanoma has increased dramatically over the past several decades, and the death rate has followed, albeit much more gradually (1). Melanoma gains metastatic potential at a remarkably early point in its progression, when one considers the fatality rate from metastases spawned by primary melanomas with only a few millimeters of dermal invasion (2). In fact, this large subpopulation of early-stage patients contributes the largest number of deaths of any stage grouping because of the vastly greater frequency at which melanomas are found in the early stages. Thus, systemic therapy approaches are needed to address the possible presence of microscopic disease at the time of initial diagnosis for many patients. With high-dose interferon being the only U.S. Food and Drug Administration (FDA)–approved therapy in the adjuvant setting and having a marginal impact on disease recurrence, there is clearly room for movement (3). In patients with overt metastatic disease, available therapies have a very limited ability to alter disease course. A small percentage of patients treated with high-dose interleukin 2 (IL-2) or conventional cytotoxic chemotherapies obtain durable responses that represent clear clinical benefit (4, 5).

The rate at which somatic genetic alterations have been cataloged in melanoma has accelerated greatly in recent years. The ability to modulate genes and proteins of interest, even when pharmacologic agents are not available, has provided preclinical evidence that many putative oncogenes represent potential therapeutic targets. Although recent genetic discoveries have been in uveal melanoma, a relatively rare subtype in comparison with cutaneous melanoma, therapeutic strategies have not yet been clearly established, and thus, this review does not cover this topic (6, 7). However, no case of melanoma has been identified in which only one oncogenic event has been thought to be responsible, and thus, it now becomes critical to understand the hierarchy of genetic alterations in order for rational treatment strategies to be devised. Melanoma signaling contains both lineage-specific and canonic and/or shared intermediates and targets (Fig. 1). It seems that some genetic alterations affect molecules that cannot be directly targeted with drugs, which creates a need to understand the downstream consequences of key genetic events (such as resistance to apoptosis or escape from immune surveillance) and how those might be targeted with drugs (Table 1). Significant gains have been made in understanding the molecular pathophysiology of melanoma and have created the hypotheses that will be tested...

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in clinical trials over the next several years. It is hoped that assembling rational combinations of therapies that are matched to the abnormalities identified in an individual’s tumor will transform patient outcomes beyond what can be achieved with single-agent approaches. And, this strategy may result in therapies that are sufficiently effective and safe to be offered to the large population of patients at risk for having microscopic metastatic disease.

On the Horizon

**BRAF**

Activating mutations in BRAF are found in approximately 50% of melanoma cases (8). More than 95% of the mutations affect a valine residue at the 600 amino acid position, resulting in a constitutively active kinase that hyperstimulates mitogen activated protein (MAP)/extracellular signal regulated kinase (ERK) kinase (MEK). Introducing this single altered gene can transform p16 null melanocytes (9). And despite the presence of other concomitant oncogenic events, potent growth suppression and some degree of apoptosis are also induced in subpopulations of patient-derived melanoma cells that harbor a BRAF mutation and are exposed to short interfering RNA (siRNA) sequences that specifically deplete the mutated mRNA (10, 11). Potent and specific pharmacologic inhibitors took several years to develop. In the meantime, sorafenib was tested in this patient subpopulation because of its known activity against RAF kinases. Phase II clinical trials failed to show efficacy, and pharmacodynamic analyses suggested that only partial inhibition of BRAF signaling was achievable at maximum tolerated doses (12, 13).

Selective BRAF inhibitors, including PLX 4032 and GSK2118436, have provided a means for more completely inhibiting BRAF kinase activity and have shown unprecedented clinical activity in patients with metastatic melanoma harboring a BRAF mutation (14, 15). More than 80% of patients show some degree of tumor regression early in
Mechanisms of secondary (or acquired) resistance are beginning to be elucidated; however, results are so far based on a small number of patient tumor samples characterized. One reported mechanism is the appearance of an activating NRAS mutation with persistence of BRAF mutation in the same tumor (19). This mutation is rarely observed in untreated melanomas and could plausibly execute phase II clinical trials with these agents, as well as novel KIT inhibitors such as nilotinib and dasatinib, in patients with metastatic melanoma harboring KIT mutations. The preliminary results from the studies suggest that single-agent efficacy can be observed, including both partial and complete responses in some patients, but that the majority of patients treated do not achieve objective responses (23, 24). Trials that have included patients with KIT amplification only have observed a low response rate in that subpopulation. And, it is becoming clear that certain KIT amplifications are not found in GIST, whereas others seem to be less responsive. Precedent for this heterogeneity in outcome exists in GIST, in which a large proportion of GISTs are also found in a small subpopulation of melanomas for which the etiology is not thought to be related to sun exposure (specifically, melanomas arising on acral and mucosal surfaces; ref. 22).

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Given the validated role of KIT inhibitors such as imatinib and sunitinib in KIT mutant GIST, it has been possible to execute phase II clinical trials with these agents, as well as novel KIT inhibitors such as nilotinib and dasatinib, in patients with metastatic melanoma harboring KIT mutations. The preliminary results from the studies suggest that single-agent efficacy can be observed, including both partial and complete responses in some patients, but that the majority of patients treated do not achieve objective responses (23, 24). Trials that have included patients with KIT amplification only have observed a low response rate in that subpopulation. And, it is becoming clear that certain mutations confer sensitivity, at least to certain KIT inhibitors, whereas others seem to be less responsive. Precedent for this heterogeneity in outcome exists in GIST, in which a number of mutations have been described as imatinib sensitive or insensitive (25). Notably, some mutations have been described in melanoma that are not found in GIST, including the emergence of elevated platelet derived growth factor (PDGF) receptor and insulin like growth factor (IGF) receptor signaling associated with acquired resistance to BRAF inhibitors, though genetic alterations in these receptors have not been described (19, 20). Lastly, activation of the MAPK pathway downstream of BRAF by COT seems to confer resistance to BRAF inhibitors in vitro, and increased expression of COT has been described in vivo when comparing patient tumor samples biopsied early or late in the course of PLX4032 treatment and compared with baseline tumor samples (21). The diversity of resistance mechanisms described to date suggests that multiple adaptations can confer resistance, and thus, the available data do not imply a unifying treatment strategy for BRAF inhibitor-resistant patients. A unifying explanation would be the possibility that BRAF/MAPK suppression triggers homo- or hetero-dimerization of multiple RTK signaling pathways, either ligand dependent or independent, thus limiting efficacy or durability of single-agent–targeted therapy.

**KIT**

The same activating mutations in KIT that can be found in a large proportion of GISTs are also found in a small subpopulation of melanomas for which the etiology is not thought to be related to sun exposure (specifically, melanomas arising on acral and mucosal surfaces; ref. 22). Given the validated role of KIT inhibitors such as imatinib and sunitinib in KIT mutant GIST, it has been possible to execute phase II clinical trials with these agents, as well as novel KIT inhibitors such as nilotinib and dasatinib, in patients with metastatic melanoma harboring KIT mutations. The preliminary results from the studies suggest that single-agent efficacy can be observed, including both partial and complete responses in some patients, but that the majority of patients treated do not achieve objective responses (23, 24). Trials that have included patients with KIT amplification only have observed a low response rate in that subpopulation. And, it is becoming clear that certain mutations confer sensitivity, at least to certain KIT inhibitors, whereas others seem to be less responsive. Precedent for this heterogeneity in outcome exists in GIST, in which a number of mutations have been described as imatinib sensitive or insensitive (25). Notably, some mutations have been described in melanoma that are not found in GIST,
and insufficient evidence exists of their responsiveness to KIT inhibitors. It remains to be seen whether mechanisms of resistance to KIT-targeted agents will overlap those operating in BRAF-inhibitor resistance.

**NRAS**

NRAS mutations have been known about in melanoma for more than 25 years (26). Twenty percent of melanomas have activating NRAS mutations, and they represent a subpopulation distinct from BRAF mutant tumors (27). NRAS mutations disable the GTPase activity of RAS and thus keep it in the GTP bound and, therefore, active state. This condition creates a technical challenge in that pharmacologic agents would need to either displace GTP, given that the dissociation constant for GTP binding to RAS is in the picomolar range and in an intracellular environment in which GTP concentrations are high, or restore GTPase activity. Such properties are quite distinct from the type of inhibitors that have been developed for oncogenic kinases.

Extensive study of RAS effector pathways has identified not only the MAPK and PI3K pathways but also others that seem to play an important role (28, 29). However, it is clear that there is heterogeneity with regard to the signal transduction dependencies in the NRAS mutant subset of tumors. In cell culture, a minority of NRAS mutant melanoma lines show sensitivity to MEK inhibitors (30) and would, presumably, also be sensitive to selective ERK inhibitors, RAF inhibitors, and particularly CRAF inhibitors, might be useful agents in this setting if agents can be developed that can block RAF dimerization, while also inhibiting the kinase activity. Such small molecule inhibitors seem to be plausible (31). The two BRAF inhibitors that have proven to be clinically effective, PLX4032 and GSK2118436, both induce dimerization of CRAF and BRAF and, consequently, increase MEK and ERK activation in the setting of upstream RAS mutations and other genetic contexts (32, 33). The majority of NRAS mutant melanoma cell lines characterized seem to tolerate MAPK pathway suppression far better than BRAF(V600E) mutant melanomas (34). Some of these tumors seem to depend on the PI3K pathway. There is preclinical evidence that MEK inhibitors combined with PI3K, AKT, or mTOR inhibitors act synergistically in some NRAS mutant tumors (29). The agents used to inhibit these constituents of the MAPK and PI3K pathway via *in vitro* studies do not represent pharmacologically viable entities and, thus, have so far only established this strategy preclinically. As pharmacologic inhibitors targeting PI3K itself, AKT, and mTOR continue to mature in clinical development, the potential utility of these agents in combination with MAPK pathway inhibitors will be a high priority for clinical evaluation in NRAS mutant melanoma.

A broad-based exploration of additional downstream signaling mediators and pathways has been undertaken in NRAS mutant cancers to identify novel points of intervention. As it is suspected that there will not be a single pathway or node of signaling that these tumors rely on, current approaches are investigating the simultaneous knockdown of pairs of signaling molecules using siRNA libraries to nominate "synthetic lethal” combinations.

**MITF**

An additional concept that has been advanced in melanoma is that lineage-specific pathways that are critical in melanocyte development might also play a role in melanogenesis and survival of fully established tumors. This notion shares conceptual (though not pharmacologic) features with the successful targeting of estrogen and androgen receptors in breast and prostate cancers. The best studied of these factors for melanoma is the transcription factor MITF, the master regulator of melanocyte differentiation. It seems that MITF itself can function as an oncogene, at least in a subset of tumors, as high-level and focal amplification of the MITF locus can be found in approximately 20% of melanomas (35). And, knockdown of MITF with an siRNA induces apoptosis in these MITF-amplified tumors, as well as in many melanomas lacking MITF amplification. It seems that MITF can contribute to melanoma pathophysiology even when it is not highly expressed.

MITF expression seems to be partially under the control of oncogenic BRAF (36). Specifically, it has been observed that BRAF inhibition results in increased MITF levels. This observation was predicted from prior evidence that MAPK directly phosphorylates MITF, leading to its ubiquitin-dependent proteolysis (37, 38). BRAF inhibition thus boosts the expression of melanocyte lineage antigens that have previously been described to be under the transcriptional control of MITF (39). It has been hypothesized that, through this mechanism, BRAF inhibition may make melanoma more immunogenic and possibly render it more susceptible to treatment with immunotherapies that activate antitumor T cells.

Modulating MITF in a direct way with pharmacologic inhibitors would be challenging, particularly if the interaction of MITF with certain promoter regions on specific genes is desired. One therapeutic strategy is to target one or more of the post-translational processes that determine MITF activity, stability, or degradation. Another approach is to target the melanocyte-specific mechanisms controlling MITF expression. Non-specific histone deacetylases seem to function in such a manner (40).

In addition, MITF target genes clearly mediate a number of vital effects, not only during melanocytic development but also within melanomas. An emerging, vast list of MITF transcriptional targets is coming to light, and it is plausible that their identification may inform therapeutic strategies on the basis of lineage-specific addictions. One candidate is cyclin dependent kinase 2 (CDK2), which seems to contribute to dysregulated cell cycle control via its transcriptional control by MITF, which is unique in the melanocyte lineage due to its genomic location adjacent to a pigment gene (41). BCL2 also seems to be regulated by MITF and may contribute to resistance to apoptosis in melanoma (42).
detailed understanding of such critical MITF target genes might identify a combination pharmaceutical approach that would circumvent the technical challenge of targeting MITF directly.

**PI3K**

A primary mechanism of PI3K pathway activation in melanoma is PTEN loss through inactivating missense mutations or allele deletion (43). There is some evidence that AKT3 is amplified in an additional number of melanomas, though the functional consequences of this have not been fully established (44). Lastly, rare cases of activating mutations in AKT3 have been described that further corroborate the importance of this pathway in melanoma pathophysiology (45). A transgenic mouse model with inducible Braf mutation and PTEN loss in melanocytes develops invasive and metastatic melanoma (46), with very rapid kinetics that suggest a 2-hit carcinogenesis model. In human melanomas, it is common to find to these 2 genetic alterations concomitantly (27). In the experimental setting, PI3K inhibition does not seem to have stand-alone efficacy in melanoma. Limited clinical investigations have been undertaken in this area, but a rapamycin-analog mTOR inhibitor failed to show single-agent activity in a cohort of genetically unselected metastatic melanoma patients (47). A significant amount of evidence suggests that targeting the PI3K pathway is a potential adjunct to MAPK pathway inhibition in Braf mutant melanoma (48). Given the established role of Braf inhibition as monotherapy and the clinical development of numerous PI3K and AKT inhibitors, combinations of these inhibitors will soon be ready for clinical testing.

**p16/CDK4**

Germline mutations in p16 predispose to development of melanoma, and mutations or allele deletions are also common somatic events in melanoma (49). Activating mutations in CDK4 have been described in familial melanoma, as well as in sporadic cases of melanoma, has CDK4 amplification (50). Intact p16 inhibits CDK4 activity, so genetic inactivation of p16 is thought to primarily result in aberrant CDK4 activity. Lastly, amplification of cyclin D, which cooperates with CDK4 to drive cell cycle progression, is observed in a subset of melanoma and provides further genetic evidence that CDK4 activity is a fundamental element in melanoma transformation (51). The potential therapeutic value of CDK4 inhibition has been less thoroughly explored compared with the other targets discussed herein, in part because of the relatively recent emergence of selective CDK4 inhibitors for clinical development. Previously available CDK inhibitors were broad spectrum and did not provide a tool for establishing the unique role of CDK4 in cell cycle progression in melanoma. Preclinical experiments are now possible with potent and selective pharmacologic inhibitors, and their investigation in melanoma is warranted on the basis of the genetic evidence.

**p53**

Unlike common epithelial cancer, p53 is nearly always intact in melanoma, and it is p53 function that is lacking because of genetic or epigenetic alterations in afferent and efferent messengers in the p53 signaling cascade. MDM2 amplification represents an example of such a somatic genetic alteration that has been described in melanoma and seems to inhibit p53 activity (52). MDM2 antagonists have recently been developed and are of interest in both the subset of tumors with MDM2 amplification, as well as potential components of combination therapy with therapies targeting other oncogenes in melanomas without MDM2 alterations.

**Conclusions and Prospects**

The successful targeting of Braf and Kit in melanomas that harbor activating mutations in those oncogenes has created the need to characterize these genes as part of the pathologic classification of melanoma. Although a large proportion of melanomas lack genetic alterations in either Braf or Kit, other oncogenic events have been identified in these tumors. The next generation of clinical investigations will seek to identify points of intervention in Braf and Kit wild-type melanomas. And for Braf and Kit mutant melanoma, the field is moving rapidly toward combination targeted therapy as a strategy for further improving outcomes. The simultaneous targeting of multiple key survival pathways seems like an attractive strategy to boost efficacy and/or suppress treatment resistance. Yet, it is unknown to what degree this approach will retain a sufficient therapeutic index to produce important clinical benefit at tolerated drug doses. Clearly, a key component of Braf- and Kit-targeted therapies is the mutant-selective or lineage-restricted dependency features, aspects that may change when canonic pathways (such as PI3K) are targeted. Another important challenge in application of combination therapies involves the circumstance of collaboration between pharmaceutical companies. Such collaboration is happening, though at a slower pace than optimal. Recent formation of the Melanoma Breakthrough Consortium under the auspices of the Melanoma Research Foundation aims to facilitate such combination trials, including those requiring multiple pharmaceutical collaborations. An understanding of resistance mechanisms for those agents that are producing initial clinical benefit is of major importance. This information may help in the development of improved strategies for avoiding both initial and acquired resistance, such as identification of experimental drug combinations. It may also permit stratification of patients on the basis of biomarker discoveries that predict clinical behaviors. Along these lines, the increasing use of molecular genotyping, as well as deep genomic annotation of melanomas (and other tumors), serves to make molecularly based diagnostics a reality within the coming years.

The list of oncogenes and tumor suppressors that are subject to somatic genetic changes that contribute to melanoma pathophysiology may not be complete. Several
groups are undertaking whole genome sequencing of melanoma to complete the catalog. And, deep sequencing methods will generate a greater understanding of genetic heterogeneity within a given tumor. High-resolution genetic sequencing may be a particularly powerful tool for understanding mechanisms of resistance to targeted therapies, characterizing individual patients’ tumors before therapy and at the time of clinical progression.

The incorporation of immunotherapy, which has shown significant activity in patient subpopulations, may also provide additive or synergistic benefit to patients. Some combination "immunotherapy plus targeted therapy" approaches may exploit the reported upregulation of melanocytic antigens by BRAF suppression (39). Others may provide benefit because of nonoverlapping mechanisms of action and noncross-reactive toxicities. An example is the SNaphshot diagnostic platform (Massachusetts General Hospital; ref. 53), which rapidly identifies several hundred oncogenic mutations whose presence may confer specific treatment opportunities.

Finally, the advent of targeted therapies with major activity against specific subgroups of melanoma patients raises the prospect of applying these drugs when tumor burden is significantly lower. Treatment in the adjuvant setting for patients with a significant risk of micrometastatic disease may plausibly represent the first clinical setting in which these agents may provide cures, albeit "statistical" ones. Several requirements should be met for this opportunity to come to light: stratification of the correct patient-to-tumor subpopulation and delivery of treatments that meet acceptable safety standards, because not all treated patients would actually have a tumor. The pace with which progress has been made in the past several years is extraordinary in comparison to previous decades. It is hoped that this trajectory will continue to improve over the years to come.

Disclosure of Potential Conflicts of Interest


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