New Strategies in...

New strategies in metastatic melanoma: oncogene-defined taxonomy leads to therapeutic advances

Keith T. Flaherty & David E. Fisher*

Massachusetts General Hospital Cancer Center
55 Fruit Street
Boston, MA 02114

*Corresponding author:
Mass General Hospital & Harvard Medical School
Dermatology (MGH) & Pediatric Oncology (HMS)
Mailstop: Cutaneous Biology Research Center, Room 3232
Building 149, 13th Street
Charlestown, MA 02129
617-632-4916
617-632-2085 (fax)
dfisher3@partners.org
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 towards 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 PI3 kinase and p16/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 amongst cancer subtypes in advancing combination therapy strategies that simultaneously target multiple biologic underpinnings of the disease.
Background

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 due to 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 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 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 catalogued 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 to cutaneous melanoma, therapeutic strategies have not yet been clearly established and thus this review will not cover this topic.(6, 7) However, no case of melanoma has been identified in which only one oncogenic event has 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 canonical/shared intermediates and targets (Figure 1). It appears that some genetic alterations affect molecules that cannot be directly targeted with drugs. This 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 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 MEK. Introducing this single altered gene can transform p16 null melanocytes(9). And, despite the presence of other concomitant oncogenic events, potent growth suppression as well and some degree of apoptosis are induced in subpopulations of patient-derived melanoma cells that harbor a BRAF mutation and are exposed to short interfering RNA 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 2 clinical trials failed to demonstrate 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 demonstrated unprecedented clinical activity in patients with metastatic melanoma harboring a BRAF mutation(14, 15). More than 80% of patients demonstrate some degree of tumor regression early in the course of treatment; approximately 60% of all patients treated had sufficient regression to be counted as confirmed objective responses. Symptom burden is improved within one to two weeks of initiating therapy. Based on the precedent of other signaling oncogene directed therapies in metastatic gastrointestinal stromal tumor (GIST) and non-small cell lung cancer(16, 17), it is not surprising that clinical evidence of resistance emerges eventually. The median progression free survival for both selective BRAF inhibitors is approximately 6 to 7 months. While this compares favorably to a historical standards set by available therapies (approximately 2 month median progression free survival), the emergence of resistance clearly points to the need for understanding what additional points of intervention might delay or prevent this phenomenon. It was recently reported that overall survival was improved in a phase 3 trial comparing PLX4032 to dacarbazine in treatment naïve BRAF mutant metastatic melanoma patients (www.roche.com/media/media_releases/med-cor-2011-01-19.htm). So, the benefits of single agent BRAF inhibition had been sufficiently confirmed to incorporate this treatment approach into the management of patients with metastatic melanoma while also quickly proceeding with rational combination approaches.

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(18). This is rarely observed in untreated melanomas and could plausibly reactivate the MAP kinase pathway through CRAF, or other RAS effector pathways. In a single case, PTEN was deleted in a patient’s tumor biopsy at the time of disease progression on PLX4032, whereas it had been present at baseline(19). This provides indirect evidence that activation of the PI3 kinase pathway might be one mechanism by which BRAF inhibition can be circumvented. Some investigators have described the emergence of elevated PDGF receptor and IGF receptor signaling associated with acquired resistance to BRAF inhibitors though genetic alterations in these receptors have not been described(18, 19). Lastly, activation of the MAP kinase pathway downstream of BRAF by COT appears 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 to baseline tumor samples(20). The diversity of resistance mechanisms described to date suggest that there are multiple adaptations that can confer resistance and thus the available data does not imply a unifying treatment strategy for BRAF inhibitor resistant patients. A unifying explanation would be the possibility that BRAF/MAPK suppression triggers homeostatic upregulation 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)(21). Given the validated role of KIT inhibitors such as imatinib and sunitinib in KIT mutant GIST, it has been possible to execute phase 2 clinical trials with these agents, as well as novel KIT inhibitors such as nilotinib and dasatanib, 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(22, 23). 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 appear to be less responsive. Precedent for this heterogeneity in outcome exists in GIST, where a number of mutations have been described as imatinib sensitive or insensitive(24). Notably, some mutations have been described in melanoma that are not found in GIST and insufficient evidence exists regarding 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(25). Twenty percent of melanomas have activating NRAS mutations and they represent a subpopulation distinct from BRAF mutant tumors (26). NRAS mutations disable the GTPase activity of RAS and thus keep it in the GTP bound and, therefore, active state. This creates a technical challenge in that pharmacologic agents would need to 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 MAP kinase and PI3 kinase pathways, but others that appear to play an important role(27, 28). 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 (29), 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 appear to be plausible (30). 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 (31, 32). The majority of NRAS mutant melanoma cell lines characterized appear to tolerate MAPK pathway suppression far better than BRAF(V600E) mutant melanomas (33). Some of these tumors appear to depend on the PI3 kinase pathway. There is preclinical evidence that MEK inhibitors combined with PI3K, AKT, or mTOR inhibitors act synergistically in some NRAS mutant tumors (28). The agents used to inhibit these constituents of the MAP kinase and PI3 kinase 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 PI3 kinase itself, AKT, and mTOR continue to mature in clinical development, the potential utility of these agents in combination with MAP kinase 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 a 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 melanomagenesis 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 appears 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(34). And, knockdown of MITF with a siRNA induces apoptosis in these MITF amplified tumors as well as many melanomas lacking MITF amplification. It appears that MITF can contribute to melanoma pathophysiology even when it is not highly expressed.

MITF expression appears to be partially under the control of oncogenic BRAF(35). 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(36, 37). BRAF inhibition thus boosts the expression of melanocyte lineage antigens that have previously been described to be under the transcriptional control of MITF(38). 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. Nonspecific histone deacetylases appear to function in such a manner(39).
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 based upon lineage-specific addictions. One candidate is CDK2 which appears 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(40). BCL2 also appears to be regulated by MITF and may contribute to resistance to apoptosis in melanoma(41). A more 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 PI3 kinase pathway activation in melanoma is PTEN loss through inactivating missense mutations or allele deletion(42). 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(43). Lastly, rare cases of activating mutations in AKT3 have been described which further corroborate the importance of this pathway in melanoma pathophysiology(44). A transgenic mouse model with inducible BRAF mutation and PTEN loss in melanocytes develops invasive and metastatic melanoma(45) with very rapid kinetics that suggest a two-hit carcinogenesis model. In human melanomas it is common to find these two genetic alterations concomitantly(26). In the experimental setting, PI3 kinase inhibition does not appear to have stand-alone efficacy in melanoma. Limited clinical investigations have been undertaken in this area; but a rapamycin-analog mTOR inhibitor failed to demonstrate single-agent activity in a cohort of genetically unselected metastatic melanoma patients(46). A significant amount of evidence suggests that targeting the PI3 kinase pathway is a potential adjunct to MAP kinase pathway
inhibition in BRAF mutant melanoma(47). 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 deletion are common somatic events in melanoma as well(48). Activating mutations in CDK4 have been described in familial melanoma as well as in sporadic cases of melanoma as has CDK4 amplification(49). 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(50). The potential therapeutic value of CDK4 inhibition has been less thoroughly explored compared to the others targets discuss, in part due to 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 based on the genetic evidence.

**p53**

Unlike common epithelial cancer, p53 is nearly always intact in melanoma and it is p53 function which is lacking due to 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 appears to inhibit p53 activity(51). MDM2 antagonists have recently been
developed and are of interest in both the subset of tumors with MDM2 amplification as well as a potential component 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. While 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 which may change when canonical 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 which 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 based upon 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 in order to complete the catalogue. 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(38). Others may provide benefit because of non-overlapping mechanisms of action and non-crossreactive toxicities. An example is the SNaPshot diagnostic platform (Massachusetts General Hospital(52)) 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/tumor subpopulation, and delivery of treatments which meet acceptable safety standards, since not all treated patients would actually have 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.

Figure Legends:

Figure 1. Signaling pathways in melanoma. Key triggers, intermediates, and targets of melanoma signaling are indicated. Shown in green are documented melanoma oncoproteins. Melanoma tumor suppressors are indicated in red. Cytoplasmic or nuclear localization are indicated. See text for additional details of each factor.

Table 1. Key oncogenic targets in melanoma. Oncogenes (or their pathways), drug targets, and actual drugs in clinical development are listed (as labeled). This list is not exhaustive, but is meant to provide examples of both direct and indirect targeting strategies for advanced melanoma.
References:

22. Carvajal RC, PB; Wolchok JD; Cane, L; Teitcher, JB; Lutzky, J.; Pavlick, AC; Bastian, BC; Antonescu CR; Schwartz, GK. A phase II study of imatinib mesylate (IM) for patients with advanced melanoma harboring somatic alterations of KIT. J Clin Oncol 2009;27: 9001.
47. Smalley KS, Haass NK, Brafford PA, Lioni M, Flaherty KT, Herlyn M. Multiple signaling pathways must be targeted to overcome drug resistance in cell lines derived from melanoma metastases. Mol Cancer Ther 2006;5: 1136-44.
49. Walker GJ, Flores JF, Glendening JM, Lin AH, Markl ID, Fountain JW. Virtually 100% of melanoma cell lines harbor alterations at the DNA level within CDKN2A, CDKN2B, or one of their downstream targets. Genes Chromosomes Cancer 1998;22: 157-63.
<table>
<thead>
<tr>
<th>Oncogene/pathway</th>
<th>Drug target</th>
<th>Drugs in clinical development</th>
</tr>
</thead>
<tbody>
<tr>
<td>cKIT</td>
<td>cKIT</td>
<td>imatinib, nilotinib, sunitinib, dasatinib</td>
</tr>
<tr>
<td>NRAS</td>
<td>MEK, PI3K, AKT, mTOR</td>
<td>GSK112212, AZD6244, GDC0941, MK2206, temsirolimus</td>
</tr>
<tr>
<td>BRAF</td>
<td>BRAF, MEK</td>
<td>vemurafenib, GSK2118436</td>
</tr>
<tr>
<td>PI3K</td>
<td>PI3K, AKT, mTOR</td>
<td>GDC0941, GSK2126458, BEZ235, BKM120, XL765, XL147, MK2206, GSK690693, temsirolimus</td>
</tr>
<tr>
<td>MITF</td>
<td>CDK2, HDAC</td>
<td>SCH727965, panobinostat</td>
</tr>
<tr>
<td>p16/CDK4</td>
<td>CDK4</td>
<td>PD0332991, LY2835219</td>
</tr>
<tr>
<td>p53</td>
<td>mdm2</td>
<td>RG7112</td>
</tr>
</tbody>
</table>
Clinical Cancer Research

New strategies in metastatic melanoma: oncogene-defined taxonomy leads to therapeutic advances

Keith T Flaherty and David E. Fisher

Clin Cancer Res  Published OnlineFirst June 13, 2011.

Updated version  Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-10-2612

Author Manuscript  Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pub@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.