The discovery of mutations in the BRAF signaling molecule in a large proportion of cutaneous melanomas immediately suggested the prospect of effective therapies for this disease. The most appealing initial target has been BRAF itself, as most mutations involve a single residue in the kinase domain of the protein. But the identification of the high mutation rate in this signaling intermediate also suggests that other molecules up- and downstream of BRAF might be productively targeted. Indeed, several receptor tyrosine kinases, as well as RAS, are mutated in a small number of melanoma cases. Moreover, genetic alterations in the phosphotidylinositol-3-kinase (PI3K) pathway, especially in PTEN, suggest that this route also poses opportunities for therapeutic exploitation. We will review here the genetic evidence suggesting the utility of targets on these pathways. We will also summarize the recent clinical data that have accumulated from initial trials designed to test BRAF inhibition and targeting of other molecules. Finally, we provide an overview of molecules entering the clinic and soon to be tested in clinical studies, as well as strategies for their employment as monotherapy and in combinations.

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Whatever are the cellular, biochemical or molecular genetic features of metastatic cutaneous melanoma that explain why the disease is so very refractory to systemic drug therapy, we do not understand them. The melanocyte is a unique and interesting cell type, widely distributed throughout the body, and embryologically migratory and perhaps therefore easily deprogrammed to the point of metastasis. But although this fact may engender hypotheses concerning the malignancy's propensity for distant metastases that arise from small primary tumors, it seems to offer us no insight into its lack of therapeutic response. Functionally, though, the melanocyte is charged with neutralizing perhaps the environment's most severe mutagenic and carcinogenic stress, solar irradiation, and maybe this fact will eventually inform our understanding of its resistance to essentially mutagenic therapy.

The translation of melanocyte biology into our practical oncologic experience is that most drugs that work in other malignancies do not work in melanoma. Dacarbazine is approved by the US Food and Drug Administration for use in melanoma, but its response rate is low and randomized data demonstrating its efficacy are lacking. However, no other cytotoxic drug or combination of drugs has been shown to be superior to dacarbazine. Single-arm studies, resulting in a small number of durable complete responses, have supported using interleukin-2. But no therapy has effectiveness in disseminated disease if one takes the measure of efficacy to be an improvement in a treated population's median survival, or even disease-free survival, in a randomized trial.

Thus the demonstration several years ago of a very high mutation rate in the signaling kinase BRAF in cutaneous melanoma led to great anticipation. The identification of a mutated target in a large fraction of melanoma cases offered the immediate potential for pharmacologic inhibition of the mutated protein and the prospect for impressive results like those that had been attained in gastrointestinal stromal tumors (GISTs) carrying KIT mutations treated with imatinib, or in non-small cell lung cancers harboring epidermal growth factor receptor (EGFR) mutations treated with gefitinib.

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Moreover, the availability of such a target generated renewed focus on the genetics of cutaneous melanoma, on the importance of the biochemical pathways on which BRAF is situated to melanomagenesis, and on the potential for providing a rational basis for designing new therapies that inhibit these pathways.

Yet monotherapy of melanoma with the first clinically available inhibitor of BRAF, sorafenib, has not yielded significant response rates, and combination studies employing this agent with chemotherapy are under way. Maybe the most important challenge facing this field is to understand whether the inability of sorafenib to cause dramatic anti-tumor responses is consequent to the pharmacological characteristics of this particular agent, or to the biology of BRAF mutation in the melanocyte setting in general. Here we will consider this issue, and provide an overview of the involvement of these signaling pathways in the pathogenesis of melanoma, and of the prospect for inhibition of these targets efficaciously.

### Alterations in the Upstream Compartment: RTK and RAS Targets

The relevant signaling pathways in melanoma are best considered as targetable compartments (Fig 1). There are two reasons to compartmentalize the potential targets. The first is conceptual. A major branch point in the transduction of extracellular signals into the cell is found at RAS. RAS plays into multiple downstream pathways, including the BRAF-ERK and phosphotidylinositol-3-kinase (PI3K)-AKT pathways that we will consider in more detail. Thus, targets upstream or inclusive of the RAS branch-point are theoretically likely, when inhibited, to result in the downregulation of parallel downstream elements without much regard to the details of the pathways. The second reason takes into account recent experience with kinase inhibitors. The most striking successes with dramatic responses with targeted therapies are those that involve inhibition of mutated upstream elements of signaling cascades (such as those already mentioned, as well as inhibitors of BCR/ABL, and other inhibitors of KIT). Thus in this review, we will concentrate on targets that either have been shown to be genetically altered in melanoma, or that directly interact with mutated effectors.

The most prevalent mutations in cutaneous melanoma involve BRAF, a downstream molecule. Nonetheless, we will begin with the upstream compartment (Fig 1A) as recent data implicate several upstream signaling molecules in melanoma pathogenesis, especially KIT and fibroblast growth factor receptor-1 (FGFR-1).

#### KIT Mutations in Melanoma

KIT was first identified as the cellular counterpart of the v-kit viral oncogene coding for a receptor tyrosine kinase and was soon shown to be implicated in the melanocyte developmental pathway when it was discovered that the W locus in mice, conferring white coat color and hematopoietic defects among other developmental abnormalities, encoded KIT, and that the phenotype was due to missense and loss of function mutations. The human counterpart of the mouse condition, piebaldism, also is due to kinase domain missense loss of function.

The events of KIT signaling are partly understood. Activation of KIT by its ligand results in dimerization of the receptor and activation of its kinase. Downstream consequences of KIT activation include mitogen-activated protein kinase (MAPK) dependent phosphorylation of the microphthalmia (Mitf) transcription factor, and the association of p300/CBP with Mitf. Moreover, KIT activation results in the phosphorylation of two residues on Mitf, which result in activation of Mitf and concurrently it proteosome-mediated degradation.

KIT is implicated in the pathogenesis of melanoma. Several studies suggest that KIT activation inhibits melanoma proliferation or, in a mouse model, stimulates not proliferation but migration of melanocytes. Others suggest that progression of melanoma occurs with loss of KIT expression. Recently it has become clear that the involvement of KIT in the pathogenesis of melanoma may be specific to certain subsets of melanoma. Curtin et al examined genomic alterations in several distinct melanoma types. They found differences in BRAF mutation rates that varied with subsets: melanomas without sun damage had mutations in BRAF or NRAS in 81% of cases. Sun-associated melanomas, or mucosal or acral melanomas, carried frequent mutations in these genes. Instead, in a subsequent study, the authors found amplification of the chromosome 4q12 region that carries KIT in these tumor types, and demonstrated activating mutations in KIT in 28%
to 39% of subjects. Similar data demonstrating KIT mutations in melanoma subsets have been found by others. These data, taken in the context of our understanding that KIT plays an important role in melanocyte development, imply KIT as an oncogene in a subset of melanoma types. Thus there is an appealing potential for using inhibitors of KIT as therapy in these subtypes.

**Fibroblast Growth Factor Receptor**

A second cell surface receptor tyrosine kinase (RTK) molecule that has been intensively studied in melanoma and that recently has been shown to carry mutations is FGFR-1. This may be a potential target in a small subset of melanomas.

Initial studies on basic fibroblast growth factor (bFGF) suggested it was a growth factor for melanocytes. The growth factor functions in a paracrine manner, elaborated from keratinocytes or dermal fibroblasts, or in an autocrine loop. Moreover, bFGF cooperates with ultraviolet (UV) irradiation in human skin to transform melanocytes to melanoma. bFGF is one of several growth factor mRNAs induced in melanocytes with transformation, and pathological studies indicate that bFGF is expressed in melanoma but not in precursor lesions, although expression levels are not prognostic. FGFR-1 in particular is abundantly expressed in melanoma cells.

There are little data on FGFR mutations in melanoma, but recently Thomas et al in a high-throughput assay of multiple oncogenes in several cancers demonstrated FGFR-1 mutations (S125L in two samples) in melanomas. Similarly, FGFR-2 is mutated frequently in endometrial cancer and an FGFR-4 polymorphism correlates with survival in melanoma. This again raises the possibility of therapeutically targeting FGFR-1 or the FGFR family in subsets carrying the mutations. Indeed, several studies suggest that introduction of a kinase-deficient FGFR-1 or antisense targeting of FGFR-1 or the FGFR family in subsets carrying the mutations. Therefore, several studies suggest that introduction of a kinase-deficient FGFR-1 or antisense targeting of FGFR-1 inhibit melanoma growth.

Further work on the characterization of the subsets of melanoma patients harboring mutations in FGFR-1 is awaited before clinical trials can be planned using this approach. However, it is likely that this subset of tumors also will lack BRAF mutations.

**RAS**

Mutations in RAS are among the most frequently observed genetic alterations in a variety of tumor types. The biology of the family of RAS proteins is beyond the scope of this discussion. But briefly, the RAS family of proteins are approximately 21 kd guanosine triphosphatases (GTPases) that are positioned centrally in the signal transduction cascade. Mutations in positions 12, 13, or 61 of the proteins render them resistant to the action of GTPase-activating proteins (GAPs) and thus constitutively active. Human melanomas carry mutations in primarily NRAS and 90% of mutations localize to codon 61 in the protein. Early reports suggested rates of approximately 24% in cultured lines and 12% in primary melanomas, and most other studies agree, although some groups report frequencies as high as 30%. Experimentally, several groups have demonstrated that the introduction of an activated RAS into melanocytes in vivo in mice engenders the formation of melanomas in several backgrounds deficient in the tumor-suppressors p16INK4A, p53, and p14ARF. This is true for HRAS and, in a model more faithful to the human genetic findings, for NRAS Q61K. In vivo, the extinguishing of HRAS expression in an inducible model leads to melanoma regression.

Thus there is abundant evidence to suggest that RAS is an appropriate upstream candidate for targeting in melanoma as it is in many other tumor types.

**Downstream Compartments**

The first of the downstream compartment significantly altered in melanoma is the RAS-RAF-MEK-ERK cascade (Fig 1B). Attention was focused on this pathway as a consequence of the discovery of mutations in BRAF.

**BRAF**

Presently, BRAF is the most important signaling molecule downstream of RAS in melanoma. In search of novel oncogenes, BRAF somatic missense mutations were identified in 66% of malignant melanomas with a single base substitution, V600E (formerly reported as V599E due to a sequence error), accounting for 80% of the mutations. A genome-scanning approach identified somatic mutations in the signaling gene BRAF that were particularly prevalent in nevi.

RAF is a cytosolic serine-threonine-specific protein kinase that is activated downstream of RAS. There are three mammalian RAF isoforms, ARAF, BRAF, and CRAF (or Raf-1), that share three conserved regions, two N-termini regions (negative-charge regulatory) and one C-terminus region that contains the kinase domain. RAF activates the MAPK extracellular signal–regulated kinase (denoted MEK), which in turn activates the extracellular signal–regulated kinase (ERK or MAP-ERK).

In contrast to ARAF and CRAF, which not only require phosphorylation of the N-termini but also activation by RAS and SRC, the N-terminus in BRAF is constitutively activated (via constitutive phosphorylation of S338), necessitating activation only by RAS. This suggests that since BRAF requires fewer steps for activation, it can be activated under a greater variety of conditions and may be the primary isoform responsible for signaling between RAS and MEK in the majority of cells. Nevertheless, this appears to impart a greater susceptibility for carcinogenic mutational events.

V600E, glutamic acid for valine substitution at amino acid position 600, accounts for more than 90% of the BRAF mutations in melanoma. This mutation imparts an approximate 480-fold increase in the activity of BRAF over the wild type. Approximately 80% of benign nevi harbor the V600E mutation, suggesting that constitutively activated BRAF alone is not sufficient for malignant transformation. Furthermore, analysis of 126 primary tumors found that 81% of melanomas that occurred on skin lacking chronic sun-induced damaged carry either BRAF or NRAS mutations.
Analysis of melanoma cell lines by several groups showed that cell lines harboring BRAF mutations usually do not have NRAS mutations.1,35,40 Although complete reciprocity is not universal, these findings suggest that activation is required at only one point in the RAS/RAF/ERK cascade to activate downstream targets and initiate cell proliferation and/or tumorigenesis.44,50 This relationship also has been supported in nevi, as well as primary and metastatic melanoma samples.37,51,52

Moreover, PTEN, a tumor-suppressor gene discussed below, is frequently altered in melanoma.49 PTEN negatively regulates the PKB/AKT pathway controlling apoptosis. Published data on the genetic characterization of a large panel of primary melanomas and melanoma metastases, and cell lines corroborate the mutual exclusivity of BRAF and NRAS mutations35,37,53 but also demonstrate the usual activation of the PTEN-AKT pathway in parallel, suggesting that an activating mutation at one of the points in the MAPK pathway is in itself not sufficient for tumorigenesis.

The role of BRAF in melanoma biology was poorly understood before the frequency with which it is mutated in melanoma was discovered. Inhibition of BRAF activity inhibits melanocyte transformation and tumor progression. Several laboratories have demonstrated that RNA interference of mutant BRAF abrogates the transformed phenotype.54,55 Thus the most active area of clinical investigation concerns the potential for inhibition of this mutated protein.

MEK
Immediately downstream of RAF (see Fig 1B) lies MEK (MAPK/ERK kinases 1 and 2). With the discovery of the importance of RAS and BRAF mutations in melanoma, the centrality of the RAS-BRAF-MEK-ERK cascade was established. Yet there are few data that implicate MEK as having a specific role in melanocyte or melanoma biology. In particular, no mutations in MEK have been detected. Nonetheless, it is an attractive kinase target insofar as it directly receives upstream signals through BRAF and we discuss its pharmacological targeting below.

PI3K-AKT
The second important downstream compartment that is altered in melanomas is the RAS-PI3K-AKT pathway (Fig 1C). The importance of this compartment in melanoma is underscored by the genetic alterations in PTEN. But it is important to note that both of the downstream compartments transduce signaling from RAS. Genetic studies have demonstrated that one consequence of this is that tumors usually carry a single upstream mutation (in KIT, or RAS, for example), or concurrent activation of the downstream pathways.

PTEN and AKT
PTEN is another crucial element in signal transduction that is altered in melanoma, and provides strong evidence of the importance of the PI3K-AKT pathway in its pathogenesis. Shortly after its cloning, PTEN mutations were reported in approximately 30% to 40% in melanoma cell lines and about 10% in primary melanomas.53,56 In vitro expression of ec-topic PTEN in melanoma cells lacking PTEN reduced melanoma tumorigenicity and metastasis, thus implicating PTEN as a critical tumor-suppressor gene in melanoma genesis.37

PTEN encodes a lipid and protein phosphatase, with extensive homology to dual specificity protein phosphatases, and, like RAS, it is implicated in the pathways that control apoptosis via protein kinase B/AKT. PTEN regulates intracellular levels of the lipid phosphatidylinositol phosphate (PIP3). The phosphatase domain is encoded by exon 5, mutations of which comprise 20% to 30% of both germline and somatic PTEN mutations.58 The role of PTEN on cell survival has been attributed to its lipid phosphatase activity as well.

Mutation or deletion of PTEN is found in up to 60% of melanomas and about 10% of uncultured tumor material has been found to harbor genetic alterations. PTEN loss was reported in 37% of all melanomas while expression of PTEN was found in almost all benign and dysplastic nevi.59,60 Speculation about PTEN inactivation through other mechanisms led to investigations of epigenetic silencing of the gene. Quantitative examination of peripheral blood and melanoma samples of patients for methylated PTEN revealed a significant level of PTEN promoter methylation resulting in low PTEN transcription levels.61 Patients with metastatic melanoma harbored a higher frequency of PTEN methylation than patients with primary melanoma and methylation levels in blood were generally lower than those in tissue specimens.

Investigation of the expression of the two subunits of PI3K, p85 and p110, in melanocytic lesions revealed that although loss of PTEN expression is involved in melanoma progression, there is no upregulation of PI3K.62 Some evidence suggested that downregulation or loss of PTEN caused upregulation of PI3K and thus stimulation of the PI3K/AKT pathway.63,64 Nor do any data exist showing PI3K mutations in melanoma, despite the identification of mutations in other tumor types (breast, ovarian, and colon cancers). Thus, PI3K itself does not exhibit features of an attractive target. However, expression of the downstream target, AKT, has been shown to increase dramatically with melanoma progression and invasion.59,66 Moreover, the AKT3 isotype in particular is specifically involved in melanomagenesis.67 This raises the possibility that in melanomas lacking clear-cut PTEN abnormality, the pathway may nonetheless be deregulated at the level of AKT3.

As is true for NRAS and BRAF, NRAS mutations and PTEN inactivation are reciprocal in a subset of melanoma cell lines and primaries.35 One would expect not to observe concurrent mutation of NRAS and PTEN if they act in series in the PI3K/AKT signaling pathway. Thus, mutations of the PTEN/AKT pathway render NRAS mutation redundant and likely explain the reciprocal distribution of PTEN and NRAS mutations in melanoma.

Taking Advantage of the Targets
BRAF and Sorafenib
When BRAF mutations were discovered in melanoma, a small molecule inhibitor with significant anti-RAF activity was already in early phases of development in the clinic. Sorafenib, initially known as BAY 43-9006,68,71 was devel-
opposed as an agent with nanomolar inhibitory characteristics against Raf-1 (or CRAF) with an $IC_{50}$ of 12 nmol/L. Early clinical work demonstrated the drug to be well tolerated. The inhibitor has activity against the vascular endothelial growth factor receptor (VEGFR) family, platelet-derived growth factor receptor B (PDGFRB), flt-3 and KIT, and specifically is active against wild-type BRAF and the V600E mutant. Its crystal structure bound to BRAF is shown in Fig 2. Karasarides et al demonstrated that BAY 43-9006 and siRNA blocked mutant BRAF activation of ERK in vitro and in xenografts. However, inhibition of the VEGFR pathway is also likely to contribute to its clinical activity.

Recently, data on the four published phase I studies of sorafenib in advanced cancer patients have been reviewed. A total of 173 patients were treated on the four studies but only three were melanoma patients. The drug is well tolerated but led to only two objective partial responses. ERK phosphorylation was reduced in peripheral blood studies in treated patients.

The phase I monotherapy randomized discontinuation trial of sorafenib in melanoma was disappointing. One of 37 patients had a response of greater than 25% tumor shrinkage; 19% of patients had stable disease, but there was no relationship between BRAF mutation status and patient outcome. This is in contrast to a similar study performed in renal cell cancer patients. In this study, of 202 patients, 73 had greater than 25% tumor shrinkage and 65 had stable disease. Of those with stable disease, progression-free survival was significantly improved by sorafenib. These two studies demonstrate monotherapy activity in renal cell cancer but no significant activity in melanoma, and suggest perhaps non-BRAF inhibition (specifically of VEGFR) may be accountable in renal cell carcinoma.

Combination therapy using chemotherapy with sorafenib has been tested as well. Unfortunately, most of this work is only available in abstract at this time. Flaherty and colleagues initially reported response rates of approximately 30% and stable disease rates of 60% in the first populations treated. These data engendered two randomized studies. The first involved randomizing 270 patients to chemotherapy with carboplatin and paclitaxel alone or with sorafenib. The median progression-free survival time was approximately 17.9 weeks versus 17.4 weeks with sorafenib, and the response rate was 11% versus 12% with sorafenib. This study suggests that the addition of sorafenib to chemotherapy offers no benefit in previously treated patients. One explanation for the disparity between the phase I and phase III studies is the effect of patient selection on the phase I results.

However, these results leave open the possibility of therapeutic effect in untreated patients. E2603 is an 800 patient randomized phase III ongoing study that will test the comparison of carboplatin and paclitaxel with or without sorafenib in previously untreated patients. Relevant to this study is the experience using sorafenib with dacarbazine or temozolomide. Phase II results of temozolomide with sorafenib (response rate 19% without prior treatment, 0% with prior treatment) are similar to those with the already discussed chemotherapy, as are results with dacarbazine (10% response rate). A randomized study of dacarbazine with or without sorafenib in 101 patients demonstrated response rates of 12% versus 24% and progression-free survival of 11.7 versus 21.7 weeks with sorafenib ($P = .07$). Although statistically negative, the data suggest a trend toward improvement in outcome. The difference between these data and those from Agarwala et al are likely to be due to the difference in patient population, ie, to the recruitment of untreated patients, rather than to the difference in chemotherapy regimen.

Thus the data on effectiveness of sorafenib have been relatively disappointing. Few anti-tumor responses have been observed, as for example are commonplace when GISTs with KIT mutations are treated with imatinib. One potential explanation for the findings is that sorafenib, very active against Raf-1 and against the VEGFR family, is less so against mutated BRAF, and that better agents are more likely to be effective. To this end, several new agents are in development. RAF265, formerly CHR265, is a Chiron/Novartis (Basel, Switzerland) agent in early clinical trials. Plexxikon (Berkeley, CA) is undertaking phase I studies of PLX4032 as well. No data on efficacy are as yet published. Other companies such as Ambit Biosciences, Deciphera, and Exelixis are developing BRAF antagonist programs.

A potential second explanation for the lack of marked efficacy is the difference between the biology of upstream targets and those downstream, like BRAF—already alluded to. It is possible that inhibition of BRAF alone in vivo is insufficient. Potentially concurrent inhibition of the PI3K-AKT pathway is necessary; this proposition is supported by the distribution of pathway alterations we discussed. One example of such a strategy is the concurrent use in a phase I trial of sorafenib with temsirolimus, an inhibitor of the mammalian target of rapamycin (mTOR) immediately downstream of AKT. The development of this and similar studies will shed light on the potential for combination targeted therapy of BRAF and other molecules.
MEK Inhibition

As noted already, there are no mutations in MEK in melanoma and no specific function that suggests its special relevance to melanoma therapy, but it is the receiver of BRAF activation and thus an attractive potential target. Sensitivity of melanoma cell lines to MEK inhibition has been demonstrated with mutation of BRAF conferring selective sensitivity to MEK inhibition.85 This work provides a rationale for the clinical investigation of MEK inhibitors in melanoma.

Several such inhibitors have been tested in patients. CI-1040 (also known as PD184352) has been tested in a phase I trial.86 Although the drug was well tolerated when given orally, only one of 77 patients enrolled demonstrated a partial response (in pancreatic cancer). A multicenter phase II trial of CI-1040 showed no responses in 67 patients.87 Still newer agents are being tested. Studies of AZD 6244 (formerly ARRY-142886) show that it is inhibitory in cell lines and xenografts carrying BRAF and RAS mutations.88 Recently, a randomized phase II trial of AZD 6244 was completed, although no results are yet available. A phase I/II trial of the second-generation MEK inhibitor PD-0325901 also is under way.

The PTEN-PI3K-AKT Pathway

PTEN is frequently altered in melanoma, but it is a classic tumor suppressor. That is, it exerts its tumorigenic effect not by gain-of-function but by loss. Consequently a pharmacologic treatment strategy would involve replacement of function, not inhibition. No agents are being tested at this time with this characteristic.

Inhibitors specifically designed to target PI3K are soon to be tested. LY294002 and wortmannin are used in vitro, but new agents are being developed for clinical use. For example, Aziz et al89 used tissue microarrays to show that PI3K expression was increased in melanoma, and suggest that the inhibitor SF 1126 (Semafore Pharmaceuticals, Indianapolis, IN) may have utility in this setting. However, PI3K expression data in melanoma are discrepant, and no mutations in the target have been reported.

AKT inhibitors may have a role, as suggested by AKT activation in melanoma.67 As yet, few specific agents are being tested. RX-0201 is one: an antisense oligonucleotide to AKT1 that is being tested in a phase I study.80 Downstream of AKT lies mTOR, and a phase II study of temsirolimus (CCI-779) demonstrated one partial response in 33 enrolled patients.91

Thus little success in this disease has been achieved to date in targeting the downstream compartment that parallels BRAF.

Upstream: KIT Inhibition

The discovery of KIT mutations in melanoma raises the possibility of therapy directed at this molecular target. Several clinical trials were undertaken initially. However, they were performed prior to the understanding of the association between KIT alterations and melanoma histology. Consequently, the studies included a broad population of melanoma patients and probably few with molecular alterations of the target.

One phase II trial enrolled 26 patients with metastatic melanoma and treated them with 800 mg daily of imatinib. Patients with metastatic disease were eligible for enrollment, regardless of whether their tumor displayed expression of KIT. During analysis, three patients had moderate staining for KIT on immunohistochemistry (IHC) and five patients had weak staining, suggesting few patients had KIT alterations. No sequencing was performed. No patients were found to be free of disease progression 6 months after treatment started.92 In another phase II trial, 21 patients, who had been previously screened by IHC for greater than 25% expression of KIT, PDGFR, c-abl, or ARG, were treated with imatinib. Twenty of 21 patients experienced disease progression by 12 weeks; however, the one patient with the strongest expression of KIT in more than 75% of tumor cells achieved a near complete response.93 Intriguingly, this was the only patient with acral melanoma in this cohort. Also, with further study, the patient was demonstrated to have a deletion affecting a splice site, resulting in an aberrant transcript of KIT. Thus, it is thought that a select group of patients having abnormal KIT may be able to derive significant benefit from this drug.

Several clinical trials are now under way that incorporate molecular genetic assessment as eligibility criteria. Two studies are employing imatinib; a third is using dasatinib. Both of these agents have significant activity against KIT. The use of imatinib in GIST-carrying KIT alterations is well established. But the spectrum of genetic alterations of KIT in melanoma, which includes mutations of the juxta-membrane and kinase domains as well as copy number increases of the wild-type gene, makes dasatinib a promising drug to treat melanomas with activation of the KIT pathway as well. Dasatinib potently inhibits wild type kit, juxta-membrane domain mutant KIT autophosphorylation, and kit-dependent activation of downstream pathways.94

These trials are all in very early stages. However, by analogy with successes that have been achieved in other tumors when mutated RTK molecules have been inhibited, this treatment strategy seems to hold promise.

Future Directions

In this discussion, we have made an assumption: genes and their protein products most likely to be targeted successfully, and consequently, those most central to our discussion, are those that are mutated in melanoma. There is a large body of molecular and clinical trial evidence that distinguishes the inhibition of mutated or translocated kinases by their clinical responses. However, at the same time, there are numerous pharmacologic successes directed at either abnormal but nonmutated targets (for example, HER2 in breast cancer, and nonmutated EGFR in a variety of cancers), or molecules that appear to play a pivotal transduction role (such as mTOR), or even relative surprises (such as the proteosome). So, although we have chosen to concentrate on mutations in these
signaling pathways, there are many other opportunities for therapy. In fact, recent genomic studies have shown that most cancer cells have a large cohort of mutations. Sjoblom et al showed in their analysis of colon and breast cancers that 189 different genes were mutated, an average of 11 per tumor.95 One of the most frequently mutated genes in melanoma is one that codes for a large structural protein very poorly characterized in its function, titin.96 The future of therapy for this disease, at least in the area of targeted inhibition, thus will be the better elucidation of the biochemical functions of the known important targets. Very likely we will also need to develop methods for choosing combination therapy. One powerful tool that will aid this development is RNA interference, and RNAi screens are already being put to use to choose therapeutic combinations.97,98 In short, the future appears promising for targeting these and other pathways in melanoma.

References
36. F. Haluska et al


