Achievements and Challenges of Molecular Targeted Therapy in Melanoma

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OVERVIEW

The treatment of melanoma has been revolutionized over the past decade with the development of effective molecular and immune targeted therapies. The great majority of patients with melanoma have mutations in oncogenes that predominantly drive signaling through the mitogen activated protein kinase (MAPK) pathway. Analytic tools have been developed that can effectively stratify patients into molecular subsets based on the identification of mutations in oncogenes and/or tumor suppressor genes that drive the MAPK pathway. At the same time, potent and selective inhibitors of mediators of the MAPK pathway such as RAF, MEK, and ERK have become available. The most dramatic example is the development of single-agent inhibitors of BRAF (vemurafenib, dabrafenib, encorafenib) and MEK (trametinib, cobimetinib, binimetinib) for patients with metastatic BRAFV600-mutant melanoma, a subset that represents 40% to 50% of patients with metastatic melanoma. More recently, the elucidation of mechanisms underlying resistance to single-agent BRAF inhibitor therapy led to a second generation of trials that demonstrated the superiority of BRAF inhibitor/MEK inhibitor combinations (dabrafenib/trametinib; vemurafenib/cobimetinib) compared to single-agent BRAF inhibitors. Moving beyond BRAFV600 targeting, a number of other molecular subsets—such as mutations in MEK, NRAS, and non-V600 BRAF and loss of function of the tumor suppressor neurofibromatosis 1 (NF1)—are predicted to respond to MAPK pathway targeting by single-agent pan-RAF, MEK, or ERK inhibitors. As these strategies are being tested in clinical trials, preclinical and early clinical trial data are now emerging about which combinatorial approaches might be best for these patients.

Traditionally, melanoma has been a challenging disease to treat because chemotherapy is typically not effective and cytokine therapy, such as high-dose interleukin-2 (HD IL-2), only helps a small percentage of patients.1 Highlighting the futility of early approaches to the development of effective therapies for melanoma, from 1976 to 2011 the U.S. Food and Drug Administration (FDA) approved only dacarbazine and HD IL-2 for the treatment of metastatic melanoma, and neither agent was demonstrated to be associated with an overall survival advantage. Over the past decade, however, great advances in immune-targeted and molecular-targeted therapies have led to a therapeutic revolution. Since 2011, six agents, including three immunotherapies (ipilimumab, pembrolizumab, nivolumab) and three molecular targeted therapies (vemurafenib, dabrafenib, trametinib), have received regulatory approval.2 The following section summarizes genomic approaches for molecular subtyping, outlines the rationale for molecular targeted therapy in melanoma, and reviews the successes and challenges of this strategy.

Melanoma is a malignancy that arises from melanocytes, the pigment producing cells in the body that may be derived from a number of different anatomic sites including skin, mucosal surfaces, conjunctivae, and uveal structures. With the emergence of powerful molecular diagnostic tools, a number of genetic mutations and amplifications/deletions have been identified that appear to drive tumor growth and survival signaling and render these cells sensitive to small-molecule inhibitors.3 The great majority of these genetic aberrations lead to constitutive activation of the MAPK pathway (Fig. 1A).4 Mutations in the serine-threonine kinase BRAF, particularly at the 600 position with substitution of valine with either glutamate (V600E) or lysine (V600K), drive signaling through the MAPK pathway and are present in more than 50% of patients with cutaneous melanoma, 10% to 20% of melanomas arising in mucosal or acral locations, and 0% of uveal melanomas.5-7 Mutations upstream of BRAF, in particular at the 600 position with substitution of valine with either glutamate (V600E) or lysine (V600K), drive signaling through the MAPK pathway and are present in more than 50% of patients with cutaneous melanoma, 10% to 20% of melanomas arising in mucosal or acral locations, and 0% of uveal melanomas.5-7 Mutations upstream of BRAF, in particular activating NRAS mutations and loss-of-function mutations of neurofibromatosis 1 (NF1, a major regulator of NRAS activation), are the MAPK pathway drivers in nearly 30% of melanomas and are typically mutually exclusive of BRAFV600 mutations.3,8-9 Interestingly, 20% to 30% of mucosal melanomas harbor either a mutation or genomic amplification of CKIT, which is rarely aberrant in cutaneous melanoma, and more than 80% of uveal melanomas contain a mutation in one of two G-protein subunits, GNAQ and GNA11, also rarely seen in cutaneous melanoma.10-13

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aberrations lead to MAPK signaling. Recently, a number of small-molecule inhibitors have been developed that inhibit many of the key mediators of the MAPK pathway including RAF (pan-RAF and BRAF-specific), MEK, and ERK, as well as upstream activators of the pathway such as CKIT.

GENOMIC APPROACHES TO MOLECULAR DIAGNOSIS AND TARGET DISCOVERY

Early in the development of potent and specific BRAF inhibitors it became clear that only patients with \( \text{BRAF} \) mutations were benefiting from these therapies. As a result, both Roche-Genentech and GlaxoSmithKline (GSK) developed companion diagnostics that were used to prescreen patients for the pivotal trials of vemurafenib and dabrafenib, respectively. Whereas the first generation of mutation detection analytics used direct sequencing, both the Roche assay (known as the cobas 4800 BRAF V600 Mutation Test) and the assay developed by GSK (THxID-BRAF test, licensed by Quest Diagnostics and bioMerieux) involve polymerase chain reaction (PCR) techniques—real-time PCR for the cobas assay and amplification refractory mutation system (ARMS)-PCR for the THxID-BRAF assay—using primers designed to quantify predominantly V600E (cobas) or both V600E and V600K (THxID-BRAF). Both assays have been shown to be superior to Sanger sequencing, although the cobas assay only detects 70% of V600K mutations whereas the THxID-BRAF test is able to detect these with great accuracy, and both received FDA approval concurrently with vemurafenib (cobas) and dabrafenib (THxID-BRAF). Both assays have been shown to be superior to Sanger sequencing, although the cobas assay only detects 70% of V600K mutations whereas the THxID-BRAF test is able to detect these with great accuracy, and both received FDA approval concurrently with vemurafenib (cobas) and dabrafenib (THxID-BRAF). However, as described above, a number of additional mutations have been identified that might similarly be targeted with small-molecule inhibitors, including non-V600 \( \text{BRAF} \), \( \text{NRAS} \), \( \text{CKIT} \), and \( \text{NF1} \) loss; thus molecular analysis platforms that can accurately and cost-effectively analyze multiple mutations are required.

A number of novel techniques to target mutant epitopes across relevant genes have been developed, including those that use mass spectrometry (Sequenom) and florescent tags (SNaPshot), but these are now being replaced by massively parallel sequencing techniques that can detect single nucleotide variants, insertions, deletions, and gene rearrangements with deep coverage. As these assays become more widely available, the challenge for providers will be to determine how best to utilize the vast amount of information. It is expected that these types of effort will identify small subsets of patients with novel genetic aberrations that predict response to agents not previously used in melanoma.

**KEY POINTS**

- The great majority of patients with melanoma have mutations in genes (oncogenes and/or tumor suppressor genes) that drive MAPK pathway activation.
- Potent and specific inhibitors of MAPK pathway mediators have been discovered and include BRAF inhibitors (vemurafenib, dabrafenib, encorafenib), MEK inhibitors (trametinib, selumetinib, cobimetinib, binimetinib), ERK inhibitors (BVD-523, GDC-0994), and pan-RAF inhibitors (LY3009120).
- Combination BRAF/MEK inhibition is superior to single-agent BRAF inhibition in patients with \( \text{BRAF}^{V600E} \)-mutant melanoma; a number of trials of BRAF/MEK inhibition plus a third targeted agent are underway to determine whether triplet therapy is superior to BRAF/MEK doublet therapy.
- Single-agent pan-RAF, MEK, and ERK inhibitor therapies may be effective strategies for the treatment of other molecular subsets of melanoma, including uveal melanoma (typically either \( \text{GNAQ} \) or \( \text{GNA11} \) mutant), \( \text{NRAS} \)-mutant, MEK-mutant, non-V600 \( \text{BRAF} \) mutant, and \( \text{NF1} \)-loss function.
- The use of novel molecular targeted therapies, alone or in combination, holds great promise for the treatment of melanoma, but is not without challenges.
BRAF\textsuperscript{V600} TARGETING, RESISTANCE, AND OVERCOMING RESISTANCE

With the original observation that oncogenic mutations in BRAF were present and driving tumor growth in more than 65% of melanomas (although later the prevalence was determined to be closer to 50%), efforts to therapeutically target BRAF were undertaken.\textsuperscript{3,5} Although the initial focus was on small molecules that blocked a wide range of kinases including RAF (sorafenib, RAF265), eventually more potent and specific inhibitors that preferentially targeted mutant isoforms of RAF (particularly at the 600 position) were tested in the clinic and have revolutionized the treatment of BRAF-mutant melanoma.\textsuperscript{23-27} Both vemurafenib and dabrafenib were determined to be superior to chemotherapy in phase III trials, as was the MEK1/2 inhibitor trametinib, although unfortunately disease progression typically occurred within 5 to 7 months with single-agent BRAF and MEK inhibitors.\textsuperscript{28-30}

More recent strategies have demonstrated the safety of combined BRAF/MEK targeting (dabrafenib/trametinib, vemurafenib/cobimetinib, encorafenib/binimetinib), as well as the superiority of the combination with respect to response rate, progression-free survival (PFS), and overall survival (OS) in three randomized trials (COMBI-d, COMBI-v, co-BRIM).\textsuperscript{31-36}

Unfortunately, innate (primary) and acquired (secondary) resistance remain central obstacles to optimal BRAF-targeting therapy (Fig. 1B).

Tumor Heterogeneity and Innate Resistance

Melanoma primary tumors and metastases are highly heterogeneous with regard to their genomic, epigenomic, and transcriptional profiles. This heterogeneity is hard to explain by the cancer stem cell model. Moreover, many studies suggest that melanomas are not hierarchically organized, but suggest an extraordinary plasticity in several tumor subpopulations that are supported by the microenvironment.

Slow-cycling tumor cell populations (such as JARID1B-positive melanoma cells) and tumor cells with an epithelial mesenchymal transition (EMT)-like phenotype (i.e., an invasive mesenchymal phenotype) contribute significantly to tumor maintenance and growth. This plasticity may facilitate a nonhierarchical organization of the tumor that supports adaptation and survival during a major stress situation as the sudden interruption of MAPK signaling.\textsuperscript{37} This may explain why BRAF inhibitors cause only limited cell death in vitro and in vivo during targeted therapy. Pretreatment overexpression of antiapoptotic BCL2 family members, namely BCL2 and BCL2A1, has also been associated with resistance to BRAF inhibitors and may be an alternative explanation for the limited apoptosis associated with BRAF inhibitors.\textsuperscript{38,39}

Other mechanisms of primary resistance include genetic aberrations that lead to cell cycle activation (including cyclin D amplification/expression, p16 loss, and CDK4 activation) or parallel growth factor signaling (PTEN loss).\textsuperscript{40-42}

Tumor Heterogeneity and Acquired Resistance

Even after an impressive clinical response, most patients experience progression of disease after months, and sometimes years, of successful tumor control by mono- or combination therapy with single-agent BRAF and/or MEK kinase inhibitors. The range of response duration is quite broad, ranging from weeks to years, and the resistance mechanisms causing the treatment failure have been the subject of intense scientific scrutiny. Based on high-throughput analysis of transcriptional profiles of many primary melanoma cell cultures from different laboratories worldwide, the phenotype-switching model was established.\textsuperscript{43} In this context, the transcriptional adaptation induced by interruption of the MAPK pathway by RAF or MEK kinase inhibitors can be interpreted as a shift from the proliferative to the invasive (stem-like, EMT-like) phenotype associated with reduced glucose metabolism.\textsuperscript{44} In doing so, melanoma cells will gain time to acquire resistance by the activation of alternative signaling pathways, such as through the selection of tumor cells with additional activating mutations.

Detailed analyses of biopsies before therapy, during tumor regression, and during relapse have provided evidence that adaptive mechanisms, as shown by elevated ERK1/2 phosphorylation levels in progressive lesions, and genomic alterations, such as activating mutations in MEK and NRAS, contribute to reactivation of the MAPK pathway.\textsuperscript{45} The first report on this phenomenon described a de novo MEKI mutation (P124L) in a regrowing metastasis in a patient with BRAF-mutated cancer that was successfully treated with the MEK inhibitor selumetinib.\textsuperscript{46} This MEKI mutation conferred strong resistance to MEK inhibitors and mild resistance to the BRAF inhibitor vemurafenib. Although less frequent, the appearance of a MEK mutation that results in resistance against both BRAF and MEK inhibitors has also been reported.\textsuperscript{47} Several investigations have identified concomitant NRAS mutations with the persistence of V600E BRAF.\textsuperscript{47-49} This finding is of special interest because BRAF inhibitors may cause paradoxical pathway activation in this molecular context, as demonstrated for cutaneous epithelial malignancies associated with BRAF inhibitors.\textsuperscript{50,51} Many of these issues are reflected by the results of deep exome sequencing approaches that compare the genetic landscape before and after targeted therapy.\textsuperscript{47} In our understanding, the presence of an NRAS mutation at the time of progression is important because, if it is active, treatment beyond progression might result in a detrimental effect as a result of paradoxical activation of the pathway.

To date, so-called gatekeeper mutations in the kinase domain of BRAF have not been identified; however, BRAF amplification and truncated BRAF variants have been reported to mediate resistance to BRAF inhibitor therapy. Initially, BRAF amplifications were preferentially seen in BRAF-mutated melanoma cell cultures.\textsuperscript{52} However, a comparison of pre- and post-treatment biopsies by deep exome sequencing demonstrated that BRAF amplification is associated with progressive disease.\textsuperscript{53} Additionally, alterations in BRAF splicing result in a truncated BRAF protein that lacks exons 4 to 8, a region that encompasses the RAS-binding domain and contributes to resistance to BRAF inhibitors through enhanced protein dimerization and activation of the pathway.\textsuperscript{54}
Resistance to BRAF inhibitors also has been described to occur through upregulation of parallel signaling pathways, such as the phosphoinoside-3-kinase (PI3K) pathway, or the MAPK pathway itself through alternative kinase activation.55-57 One example of this latter scenario is resistance mediated through activation of the kinase COT.58 Bypass signaling can also be facilitated by the tumor microenvironment. Cells derived from the stroma of resistant tumors secrete a number of growth and viability factors that are able to rescue melanoma cells from BRAF inhibition. Among them, hepatocyte growth factor (HGF) appears to a secreted factor that is able to sustain tumor cell growth via activation of the HGF receptor MET in a paracrine manner, leading to tumor upregulation of the PI3K pathway.59,60 Interestingly, simultaneous amplification of HGF and MET, which suggests an autocrine activation loop, was detected in melanomas with rapid progression during MEK inhibition (Reinhard Dummer, MD, unpublished data, November 2014). Other growth factor receptors can also maintain proliferation during BRAF inhibition. For example, epidermal growth factor receptor (EGFR) is expressed by melanoma cells although the expression level is quite low compared to that in colon or squamous cell carcinomas. In cases of innate or acquired resistance, melanoma cells are able to secrete EGF and activate EGFR by an autocrine loop.61,62 These observations are convincing examples of how the microenvironment and the secretome of stromal and tumor cells cooperate to create resistance.

Reports that allow to us to link various biochemical mechanisms such as maintenance of an eIF4F complex that is associated with reactivation of MAPK signaling, persistent ERK-independent phosphorylation of the inhibitory eIF4E-binding protein 4EBP1, or increased proapoptotic BCL-2-modifying factor (BMF)-dependent degradation of eIF4G are of special interest because they open new avenues for intervention.63 Recently, secretion of interferon beta was found to be associated with BRAF inhibition and tumor regression. In cases of resistance, PKCζ-phosphorylated ATF2 downregulates interferon beta-1 expression (and signaling), which promotes the resistance of melanoma cells to chemotherapeutic agents.64

**Strategies to Overcome Resistance**

Today, the combination of BRAF and MEK inhibitors is the backbone of targeted therapy in BRAF-mutated melanoma. Depending on resistance mechanisms present in the individual patient, the addition of a third inhibitor might overcome resistance. Candidates for third agents in “triplet regimens” include a cyclin-dependent kinase inhibitor, an receptor tyrosine kinase (RTK) inhibitor (including MET, fibroblast growth factor receptor, or vascular endothelial growth factor inhibitor), an inhibitor targeting a key mediator of a parallel growth factor pathway (e.g., PI3K, AKT, mTOR inhibitors), or an agent targeting apoptosis.65,66 A major challenge in this context is how reliably the principal mechanism of resistance can be identified in one individual and whether this mechanism is relevant for all metastases in a given patient. In addition, there could be safety issues as the simultaneous delivery of multiple drugs might be associated with intolerable toxicity.

Moving forward, the integration of antibody therapy with targeted therapy also holds promise for overcoming resistance to current targeted therapy regimens.67 For example, resistance to BRAF inhibition has been associated with increased MAPK signaling as well as increased expression of programmed death-ligand 1 (PD-L1), enabling melanoma cells to avoid destruction through immune suppression.68 The combination of immunotherapy against PD-L1 with inhibitors of MAP kinase pathway targets downstream of BRAF, such as MEK, represents a promising novel strategy for overcoming such resistance and improving patient outcomes.69,70

Another possible approach to overcome resistance is intermittent therapy with drug holidays. In a xenograft model of primary human melanoma the proliferation of vemurafenib-resistant melanomas became drug dependent, implying that interruption of drug administration could cause regression of established drug-resistant tumors. As a consequence, intermittent dosing delayed resistance to vemurafenib.70 It remains to be seen whether this observation is relevant for combination kinase inhibitor therapy. This strategy needs to be investigated as an approach to cope with resistance in carefully designed clinical trials.

**TARGETING ANYTHING BUT BRAF**

As described above, the landscape of mutations, deletions, and amplifications identified in patients with metastatic melanoma has been analyzed as part of The Cancer Genome Atlas. Although the functional significance of each specific mutation has not been determined, there are a number of relatively common mutations that appear to be critical drivers of oncogenic signaling and may be targetable (either directly or indirectly) with small-molecule inhibitors.3 These include NRAS mutations, GNAQ/GNA11 (detected in less than 1% of all melanomas but more than 80% of uveal melanomas, CKIT (detected in less than 6% of all melanomas but enriched in acral and mucosal melanomas), and loss of NF1. Not surprisingly, MEK inhibitors have been, and are currently being, studied extensively in these patients. Additionally, newer agents such as pan-RAF and ERK inhibitors have shown preclinical activity in melanoma and have now have entered the clinic and are currently being evaluated in phase I trials (NCT02014116, NCT01781429, NCT01875705).71-78

**Targeting Uveal Melanoma**

Uveal melanoma is a deadly disease that is often associated with metastatic involvement of the liver and lungs despite successful treatment of the primary tumor with surgery or stereotactic radiation techniques. Mutations in GNAQ and GNA11 are present in the great majority of cases and predict signaling through protein kinase C (PKC), the MAPK pathway, and the PI3K pathway. In fact, preclinical evidence supports the use of inhibitors of MET, MEK, PKC, and PI3K/
AKT/mTOR, either as single agents or in combination.79-87 In the clinic, single-agent MEK inhibition has been tested in a randomized phase II trial compared to chemotherapy (either dacarbazine or temozolomide) in 120 patients with metastatic uveal melanoma and was associated with an improved response rate (14 vs. 0%) and PFS (15.9 vs. 7 weeks, hazard ratio [HR] 0.46, p < 0.001) compared to chemotherapy, but showed no significant improvement in OS (11.8 vs. 9.1 months, HR 0.66, p = 0.09).88 Building on the single-agent MEK inhibitor data, a randomized phase II trial of the MEK inhibitor trametinib and the AKT inhibitor GSK2141795 (NCT01979523) is currently ongoing. Targeting PKC signaling with the PKC inhibitor ABE007, either as a single agent (NCT01430416) or in combination with a MEK inhibitor (NCT01801358) or PI3K inhibitor (NCT02273219), is another strategy that is being tested in the clinic. Last, a number of trials of MET inhibitors are ongoing and currently enrolling patients with uveal melanoma, including the randomized phase II trial in the Alliance intergroup comparing the MET inhibitor cabozantinib with chemotherapy (NCT01835145).

**Targeting CKIT**
A number of inhibitors of CKIT have been tested in melanoma, with varying results. The first was a phase II trial of imatinib that enrolled 26 patients with metastatic melanoma independent of mutation or amplification status.89 In retrospect, it is not surprising that there were no responses to therapy in this initial study; however, in three subsequent clinical trials of imatinib in patients with mucosal or acral melanoma, responses were seen in 17% to 29% of patients.90-92 Responses were observed in nearly 30% of patients with CKIT mutations or amplifications, particularly in either exon 11 or 13. Phase II trials of nilotinib (NCT00788775, NCT01099514, NCT01028222), dasatinib (NCT00700882), and sunitinib (NCT00577382) have either completed accrual or are ongoing.

**Targeting NRAS**
Activating mutations of NRAS are the second most common oncogenic mutations in melanoma and are present in 20% to 30% of patients with cancer associated with MAPK activation.38 As such, targeting mediators of this pathway is a logical approach that is supported by preclinical evidence; pan-RAF inhibitors, MEK inhibitors, and ERK inhibitors all have substantial preclinical activity in these tumors.71,72,77,78 The MEK inhibitor binimetinib is associated with a 20% response rate and 4.8-month PFS in NRAS-mutant patients.93 Based on these data, a randomized phase III trial comparing binimetinib with dacarbazine is underway (NCT01763164) and positive results would change the standard of care for this patient population. A randomized phase II trial of the MEK inhibitor pimasertib compared to dacarbazine has completed enrollment (NCT01693068).

Despite promising early clinical data, it is clear that the majority of patients with NRAS-mutant melanoma will either not benefit, or will only transiently benefit, from treatment with MEK inhibitors.93 Thus, strategies to overcome either intrinsic or acquired resistance to MEK inhibitors are needed. In a seminal preclinical experiment, Kwong et al performed comparative analysis of NRAS-mutant melanoma tumors treated with vehicle, MEK inhibitor, and NRAS extinction. They determined that CDK4 was the top pathway regulator in the MEK-treated model, but not in the NRAS extinction model.94 In other words, CDK4 expression was identified as a top candidate for mediating MEK inhibitor resistance in NRAS-mutant melanoma. Proving this point, dual MEK and CDK4/6 inhibitor therapy was more effective than either agent alone in multiple NRAS xenograft models.94 Building on this work, a phase I trial of the MEK inhibitor binimetinib in combination with the CDK4/6 inhibitor LEE011 in patients with metastatic NRAS-mutant melanoma opened to enrollment and the preliminary data were presented at the American Society of Clinical Oncology’s 2014 Annual Meeting.95 Tumor regression was seen in most patients across all dose cohorts, responses were achieved in 7 of the 22 patients enrolled, and another 11 had stable disease. A similar trial evaluating the combination of trametinib/palbociclib is currently open for patients with all solid tumors (NCT02065063).

**Targeting Angiogenesis**
Tumor angiogenesis in melanoma has been well documented. Numerous molecules that promote angiogenesis are overexpressed in melanoma, including VEGF, PDGF, fibroblast growth factor (FGF), and interleukin 8.96-97 Furthermore, their expression is associated with invasion and metastasis in preclinical models and may be associated with worse prognosis in patients with melanoma. Based on these findings and the effectiveness of angiogenesis inhibitors in other cancers (including renal cell carcinoma, breast cancer, and colon cancer), a number of antiangiogenic agents have been tested in patients with advanced melanoma. Bevacizumab, a monoclonal antibody targeting VEGF, has been evaluated in patients with melanoma with the most promising results seen for the combination of carboplatin/paclitaxel/bevacizumab. In an initial phase II trial of 53 patients, 9 (17%) patients achieved a partial response (PR) and an additional 30 (57%) had stable disease for at least 8 weeks.98 Based on these results, a multicenter, randomized phase II trial was performed evaluating the combination of carboplatin/paclitaxel with or without bevacizumab. Specifically, 214 patients were enrolled via a 2:1 random allocation to receive carboplatin/paclitaxel plus bevacizumab (143 patients) or carboplatin/paclitaxel plus placebo (71 patients). The response rate (25.5 vs. 16.4%; p = 0.16), median PFS (5.6 vs. 4.2 months; p = 0.14), median OS (12.3 vs. 9.2 months; p = 0.19), and 1-year OS (53 vs. 39%; p = 0.06) were all better in patients receiving carboplatin/paclitaxel with bevacizumab; however, none of these findings were significant.99 The addition of bevacizumab appeared to provide particular benefit with regard to improved OS in patients with M1c disease (HR for OS 0.64; 95% CI, 0.44 to 0.95), especially among patients who had elevated levels of lactate dehydrogenase (LDH; HR 0.53; 95% CI, 0.32 to 0.88).99 The
benefit of VEGF pathway inhibition in patients with M1c disease and elevated LDH has been suggested in other trials of antiangiogenic agents, including a phase III trial of carboplatin/paclitaxel with or without sorafenib (E2603) in which patients in this high-risk category showed a trend toward improved PFS and OS.23,100

THE PROMISE AND CHALLENGES FOR FUTURE THERAPY IN MELANOMA

Although targeted therapies represent a promising weapon in the armamentarium against melanoma, their effective use in the clinic is not without challenges. As previously mentioned, multiple mechanisms of resistance to targeted therapy, including genomic and phenotypic mechanisms, are observed among different patients, as well as within or between tumors in the same individual. Advanced cancers in particular show a great deal of genetic instability, resulting in the emergence of multiple metastatic clones, each with a different genetic profile and sensitivity to specific treatments.101-103 Tumor heterogeneity and transcriptional plasticity have been implicated as drivers of resistance to targeted therapy in melanoma and represent a significant challenge to effective treatment of this disease.103 Increased use of novel targeted therapy combinations in the future may help to overcome both inter- and intratumor heterogeneity by broadening the targeting spectrum and potentially increasing the probability of therapeutic effectiveness.104 Unfortunately, partial or complete overlap of toxicities is common when taking a combinational approach, and must be taken into account when dose escalation rules are chosen. In many cases synergistic toxicity will result, perhaps caused by nonspecific targets, requiring substantial dose reductions. For example, the combination of the selective MEK 1/2 inhibitor binimetinib with the CDK 4/6 inhibitor LEE01 demonstrated promising antitumor activity in patients with advanced NRAS-mutant melanoma, but also resulted in frequent adverse events that necessitated multiple dosing reductions and interruptions.95 In general, however, combinations that target parallel pathways are less likely to have overlapping toxicity and may be better tolerated, as are agents with greater specificity.105 For example, the combination of the BRAF inhibitor dabrafenib with the MEK inhibitor trametinib in patients with BRAF-mutant melanoma showed a decrease in many class-related toxicities associated with BRAF inhibitors compared to dabrafenib monotherapy.31,105,106

As novel targeted therapies continue through development, the translation of preclinical data into the clinical setting is often difficult. Cell lines and animal models may not effectively mimic human tumors, the tumor microenvironment, or immune responses, and may not demonstrate the same mechanisms of resistance. For novel drug combinations, this translation can be fraught with complications if laboratory and animal models used for one class of agents differ from those used for another.

Identifying appropriate biomarkers is critical for the detection, treatment, and monitoring of targeted therapies. Biomarker identification and analysis has typically relied on the use of tumor tissue, which involves a great cost, logistical difficulties, and risk to the patient. The continued development of noninvasive tools such as circulating tumor DNA (ctDNA) will be important for use as a tissue surrogate for a

**TABLE 1. Clinical Trial Results of Targeted Therapy in Genetically Defined Patient Populations**

<table>
<thead>
<tr>
<th>Inhibitor(s)</th>
<th>Target</th>
<th>Trial Phase</th>
<th>CR + PR (%)</th>
<th>Median PFS/TTP (Months)</th>
<th>Reference</th>
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<tr>
<td>Imatinib</td>
<td>CKIT</td>
<td>II</td>
<td>29</td>
<td>3.7</td>
<td>Hodi et al 11</td>
</tr>
</tbody>
</table>

Abbreviations: CR, complete response; PR, partial response; PFS, progression-free survival; TTP, time to progression; N/A, not available.

*Some patients pretreated with BRAF inhibitor.
variety of different solid tumor types and clinical indications, in addition to opening the possibility of regular monitoring of patients and changing treatments as necessary. However, even if clinically relevant biomarkers and actionable mutations are discovered, there may not be a matched agent in development that effectively targets them.

The use of novel targeted combinations is also complicated by the many different motivations of the various stakeholders involved in drug development, including pharmaceutical companies, academic researchers, and regulatory agencies. All too often, these stakeholders have different priorities, such as intellectual property, conflict of interest, incentives for academics, and publication policies. Different views on these priorities can lead to distrust, a decision not to collaborate, and eventual failure of a project. Lack of collaboration can lead to duplication of efforts and lack of progress, resulting in inefficient use of limited patient and financial resources. Finding a way to satisfy these different priorities is critical in order to move therapies forward in the most efficient manner possible.

CONCLUSION

Although not without challenges, the use of novel targeted therapies in the context of molecular testing has opened new avenues for a precision medicine approach for metastatic melanoma, including a significant benefit already realized for this patient population. In some cases impressive tumor regressions have been demonstrated (Table 1); however, responses are not seen in the majority of patients with non-BRAF mutated cancer who are treated with targeted therapy and relapse is frequent in such cases despite combination therapy with BRAF and MEK inhibitors. Intensive translational research has highlighted the complexity of the resistance mechanisms involved and offers opportunities for interventions and improved patient outcomes. There is a need for additional clinical trials accompanied by high-throughput biomarker analyses to further improve these outcomes. Overcoming the scientific challenges, as well as satisfying the priorities of the various stakeholders involved in the development of novel therapies, will be critical for improving the treatment of patients with melanoma.

Disclosures of Potential Conflicts of Interest


References

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