Targeted Therapy in Advanced Melanoma

32

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Contents

Abstract

Molecularly targeted therapy, specifically small molecule therapeutics against particular oncogenes, has transformed the treatment landscape in melanoma and other cancers. Activating mutations in *BRAF*^{V600} produce constitutive activation of the mitogen-activated protein kinase (MAPK) pathway, causing unrestrained growth in nearly half of all

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melanomas. In turn, therapeutic blockade of this pathway through BRAF inhibitors produces dramatic clinical responses and improved survival compared to traditional cytotoxic chemotherapy. The addition of downstream MAPK blockade via MEK inhibition has further improved clinical outcomes. Although initial responses are impressive in most patients, and durable responses occasionally occur, acquired resistance remains a major barrier to long-term efficacy with these agents. A number of other potential therapeutic targets have been identified among other subsets of melanoma, including those with *NRAS* mutations, *KIT* mutations, and atypical *BRAF* alterations. Further, combinatorial regimens targeted MAPK and other pathways (including CDK4/6 and PI3K/AKT pathways) have shown early promise. This chapter reviews the development, current clinical activity, and future development directions for targeted therapy in melanoma.

Keywords
BRAF · Targeted therapy · NRAS · KIT · Vemurafenib · Dabrafenib · Trametinib

Introduction

Historically, advanced melanoma has been associated with a poor prognosis and a median survival of 6–9 months (Balch et al. 2009). This was, in part, explained by a notorious lack of efficacy of traditional cytotoxic chemotherapy in patients with this disease. As such, a powerful drive to identify novel therapeutic strategies for advanced melanoma has persisted among the melanoma community.

Targeting specific genetic alterations that fuel cancer cell growth and division has led to major advances across several cancer types, and is now a cornerstone of melanoma therapeutics. A prerequisite for effective targeted therapy, however, is the identification of an appropriate "target." Several characteristics make particular molecular changes attractive for targeting by anticancer agents. First, it should be cancer specific, with limited or no expression on healthy tissues. This issue surfaces more frequently when targeting cell surface molecules, and less often presents a problem when targeting cancer-specific mutations. Second, it needs to contribute to cancer growth and progression. Genomic alterations that are fundamental to tumor growth are preferable than those that arise following malignant transformation or metastatic spread. Third, it should occur in a "targetable" protein. Transcription factors and GTPase proteins have proven difficult to target, whereas kinases are more amenable to therapeutic modulation. Fourth, the cancer cell would be highly dependent on the target, with a dearth of co-occurring, functionally redundant mutations. Finally, it would occur at a high frequency in a particular cancer type or across cancers. While frequency is not necessary for successful targeting, it provides enormous advantages in terms of detection, characterization, and drug development. A recurrent mutation in the 600th codon of BRAF was identified in 2002 in approximately half of melanoma tumors, fulfilling all of the above criteria except the fourth (as melanoma is among the most highly mutated of human cancers) (Alexandrov et al. 2013).

While early targeted therapy efforts predated 2002, the discovery of these recurrent $BRAF^{V600}$ mutations sparked the first sustained and successful rational targeted therapy approaches in this disease (Davies et al. 2002). A number of comprehensive sequencing efforts have subsequently identified numerous potential genetic and non-genetic candidates for targeted therapies beyond *BRAF* mutations (discussed in "Melanoma Genetics" below). With the description of these recurrent genetic alterations, a novel classification schema was born, derived not from histologic or anatomic features, but from the genetic makeup of the tumor. The development of active targeted therapies has highlighted the clear clinical applications of this novel classification scheme.

Melanoma Genetics

A review of the genetics of melanoma as described by The Cancer Genome Atlas (TCGA) provides an ideal basis for a discussion of targeted

therapy of melanomas (Cancer Genome Atlas Network 2015). The TCGA is based on melanoma tissue obtained from regional lymph nodes and primary melanomas that were all cutaneous in origin and included no melanomas originating from mucosal surfaces, acral surfaces, or uveal origin. The TCGA provides an overall framework for melanoma genomic classification based on presumed driver mutations. This approach has classified melanoma by the predominant driver, *BRAF*, *NRAS*, *NF1* or "Triple Wild Type" (TWT). The major driver oncogenes and several of those present within the TWT population strongly support mitogen-activated protein kinase (MAPK) pathway inhibition as an important component of any targeted therapy, since over 90% of melanomas in the TCGA have driver genes activating this pathway (Cancer Genome Atlas Network 2015).

As mentioned, recurrent mutations in the serine threonine kinase BRAF at the 600th codon offered a previously unrecognized approach in treating this disease. Most commonly, this mutation involved a valine to glutamine substitution (V600E), but also included other changes (most often valine to arginine – V600 K) (Davies et al. 2002). Other, non-V600 mutations were also identified in a smaller proportion of melanomas (roughly 5%), primarily in exons 11 and 15, and most commonly codons 466, 469, 597, and 601 (Wan et al. 2004). These mutations lock BRAF into its active conformation, inducing constitutive downstream MAPK signaling through MEK1/2 and ERK independent of upstream RAS signaling. Other alterations in *BRAF* have also been identified at lower frequencies, including fusions, gene amplifications, and kinase domain duplications. The discovery of recurrent *NRAS* mutations at codons 12, 13, and 61 actually predated the identification of recurrent *BRAF* mutations. These changes are present in 15–20% of melanomas, with codon 61 mutations predominating, and less frequent codon 12 and 13 mutations. Less than 5% of the RAS-mutated melanomas involve *KRAS* or *HRAS*. This contrasts with mutation patterns in *KRAS* in other cancers (colon, lung adenocarcinomas), where codon 12 and 13 mutations predominate. Loss of function and truncating mutations in the tumor suppressor gene *NF1* were also identified in approximately 15% of melanomas (Hodis et al. 2012; Krauthammer et al. 2012). *NF1* mutations often co-occur with other MAPK activating mutations, suggesting that although they promote MAPK signaling, additional alterations are required for oncogenic pathway activation. Other low frequency mutations that promote MAPK signaling were also identified, including those in *KRAS*, *HRAS*, *MAP2K1*, *CRAF*, and various receptor tyrosine kinase encoding genes. Notably, these "driver" type mutations are largely mutually exclusive (with the exception of *NF1*). Thus, MAPK signaling is dysregulated and promoted in nearly all melanomas, suggesting that targeting this pathway could be effective in multiple genetically defined subtypes. See Fig. 1 for potential therapeutic targets and agents.

The other cohort of cutaneous melanomas has been identified as triple wild type (TWT) melanomas. Oncogenic drivers in this group are diverse, and include *KIT*, *GNAQ,* and *GNA11* (drivers of uveal melanoma); *PDGFRα* amplifications; *CTNNB1*; and *EZH2* mutations (Cancer Genome Atlas Network 2015). The overall frequency of a UV signature (C to T inversion or CC to TT inversion) is seen in less than 30% of TWT melanomas while >90% of those from the other genetic classifications demonstrate a UV signature. Finally, copy number alterations (CNA) and other structural events such as TERT, CCND1, CDK4, MDM2, MITF, and PDL1 amplifications may also occur. Despite the somewhat distinct genetic makeup of this group, evidence of MAPK activation is still present in most TWT melanomas.

The stereotypic oncogenic "driver" mutations such as *BRAF* and *NRAS* were often found to coexist with cooperating genetic alterations that promote an invasive phenotype. These include *CDKN2A* loss or mutations, *TP53* mutations, *TERT* promoter mutations, *PTEN* loss, or other alterations in the PI3K-AKT pathway (Cancer Genome Atlas Network 2015; Hodis et al. 2012). A large, elegant study performed sequencing of numerous pre-malignant and primary melanoma tumors, and showed that these changes

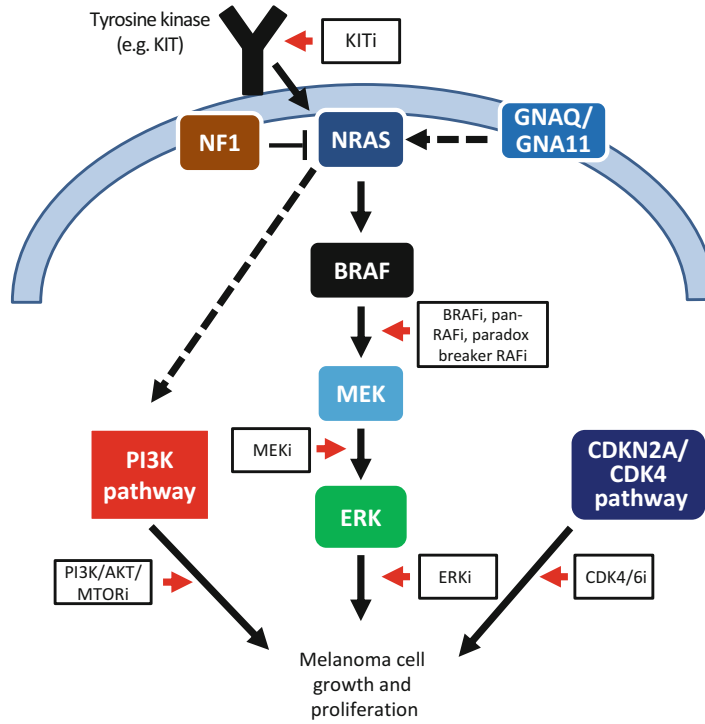

Fig. 1 Simplified schematic of relevant cell signaling pathways in melanoma and approved or experimental therapeutic approaches

accumulate in a predictable, stepwise fashion as melanomas evolve from pre-neoplastic lesions (Shain et al. 2015). *BRAF*^{V600E} mutations occurred in benign nevi, whereas *NRAS* mutations and several other drivers occurred in intermediate lesions. These intermediate lesions and melanoma in situ also frequently harbored *TERT* promoter mutations. *CDKN2A* deletions, *PTEN* loss, and *TP53* mutations occurred only in invasive melanomas. Furthermore, total mutational burden, the total number of somatic nucleotide variants identified in the tumor, accumulated with malignant progression. These co-occurring mutations may provide additional therapeutic targets.

Oncogenic driver mutations are also associated with unique clinical patterns. *BRAF* mutations occur more commonly in melanomas from skin with intermittent sun exposure, and are infrequent in melanomas arising from skin with chronic sun damage (CSD) (Curtin et al. 2005). If *BRAF* mutations are identified in skin with CSD, they frequently are V600 K rather than V600E. *NRAS* mutations occur in a relatively predictable 15–20%, regardless of anatomic location (with the exception of uveal melanoma). These alterations are also correlated with an inferior overall prognosis and thicker primary tumors. *KIT* mutations are present in 15–20% of acral and mucosal melanomas and 2% of CSD melanomas, but rarely in other cutaneous melanomas. *GNAQ* and *GNA11* mutations occur in nearly 90% of uveal melanomas, but rarely in other subtypes (Van Raamsdonk et al. 2009, 2010). *NF1* mutations also commonly occur in skin with CSD and are associated with a high overall burden of somatic mutations (Krauthammer et al. 2012).

BRAF Inhibitors

BRAF is a canonical member of the mitogen activated protein kinase (MAPK) signaling pathway. Mutations in codon V600 produce constitutive activation and MAPK signaling independent of upstream RAS stimulation. Since this mutation is present in nearly half of all melanomas, this represented a potential high frequency target with plausible biologic rationale. Initial trials of the putative BRAF inhibitor sorafenib showed disappointing clinical activity despite

promising preclinical results in *BRAF* mutant cell lines. The infrequent responses observed were thought to arise from the anti-angiogenic and multikinase inhibitory effects of sorafenib, rather than to direct inhibition of mutant *BRAF*.

A novel set of small molecules that directly target BRAF were subsequently developed. The first of these with preclinical validation and to enter clinical trials was vemurafenib (also known as PLX4032 and RG7204). The first few patients were treated with a crystalline formulation that inhibited ERK in on-treatment tumor biopsies, but failed to cause tumor regression and had unfavorable pharmacokinetics. The agent was reformulated as an amorphous formulation that enabled higher drug exposures and clinical responses (Bollag et al. 2010). Initially, several patients without *BRAF* mutations were included in the phase I portion, none of whom responded to treatment. Thereafter, only patients with $BRAF^{V600}$ mutations received vemurafenib.

The phase I study of vemurafenib produced remarkable clinical activity comparable to the most effective targeted therapies of the time (Flaherty et al. 2010). Many patients experienced dramatic clinical improvement even within hours to days of commencing treatment. In a small expansion cohort, the objective (unconfirmed) response rate was 81%, with a median progression-free survival (PFS) of approximately 7 months. Furthermore, nearly all patients experienced some degree of tumor regression, including those that did not achieve a true partial response.

Follow-up studies were similarly encouraging. A large phase III study was conducted, comparing vemurafenib with the alkylating agent dacarbazine in 675 patients with $BRAF^{V600E}$ mutant melanoma. This study demonstrated a dramatic improvement in clinical outcomes with vemurafenib, including confirmed response rates (48% vs. 5%), PFS (hazard ratio [HR] for progression = 0.26; median 5.3 months vs. 1.6 months), and OS (HR for death = 0.37; 6 month OS 84% vs. 64%, all p values <0.001) (Chapman et al. 2011). Benefits were observed in essentially all subgroups, including in both patients with poor traditional prognostic features (elevated lactate dehydrogenase [LDH], visceral disease involvement, advanced age) and those with favorable clinical prognosis (normal LDH, nonvisceral metastases, younger age). The phase II study of vemurafenib was subsequently reported with a longer duration of follow-up. This study demonstrated a 53% ORR and a median overall survival of 15.9 months among previously treated patients (Sosman et al. 2012). The median duration of response in this study was 6.7 months, although some patients remained on study for a longer duration (>12 months). In context, the historical median survival in advanced melanoma ranged from 6 to 9 months in prior series. See Table 1 for clinical activity of approved and experimental targeted therapies.

Subsequently, dabrafenib, another BRAF inhibitor, entered clinical development. A phase III clinical trial was the first large published experience with this agent, and confirmed clinical efficacy that was comparable with vemurafenib (Hauschild et al. 2012). This study randomized 250 patients at a 2:1 ratio to receive either dabrafenib or dacarbazine, and noted that dabrafenib produced superior ORR (50% vs. 7%) and PFS (median 5.1 months vs. 2.7 months, $p < 0.001$). Crossover to dabrafenib was permitted after progression on chemotherapy, decreasing the likelihood of observing an overall survival difference (which was not observed on this trial).

The original vemurafenib studies limited treatment to patients specifically with the *BRAF* V600E mutation. While V600E is the most common BRAF mutation, approximately 20% of mutations at the same codon involve alternative substitutions, most often valine to lysine (V600 K). Most subsequent studies included both mutations, however, found similar benefit for V600E and V600 K mutations. Several case series later showed that less common *BRAF* V600 mutations could also respond to treatment, including V600R and V600 M (Klein et al. 2013). Thus, many clinical trials (and clinical guidelines) now consider alternate V600 mutations as likely to benefit from BRAF (+/− MEK) inhibition. By contrast, non-V600 mutations appear to be insensitive to mutant-specific BRAF inhibitors (see "MEK Inhibitors" section below).

Table 1 Available targeted therapy options for melanoma

Agent	Indication	Response rate	PFS (median)	OS (median)	Reference
Vemurafenib	*BRAF*-mutant	48%	5.3 months	13.6 months	
Vemurafenib + cobimetinib	*BRAF*-mutant	68%	9.9 months	81% at 9 months	
Dabrafenib	*BRAF*-mutant	50%	5.1 months	Not reported	
Trametinib	*BRAF*-mutant	22%	4.8 months	81% at 6 months	
Dabrafenib + trametinib	*BRAF*-mutant	64%	11.4 months	72% at 12 months	
Binimetinib[a,b]	*NRAS*-mutant	20%	3.7 months	Not reported	
Imatinib[a]	*KIT*-mutant	16–29%[c]	2.9–3.5 months	10.7 months	
Nilotinib[a]	*KIT*-mutant, imatinib refractory	11% (2/19 patients)	3.3 months	9.1 months	
Trametinib[a]	Atypical *BRAF*-mutant	Case reports, but numerous responses reported for $BRAF^{L597}$ and $BRAF^{K601}$ mutations			

[a]Not FDA approved for this indication
[b]Not clinically available at the time of publication
[c]Responses appear be in the 30–50% range for exon 11 and 15 mutations, <10% for amplifications

Most initial studies also excluded patients with active brain metastases. Separate phase II studies were performed specifically for patients with brain involvement. In the BREAK-MB study, 172 patients with at least one asymptomatic brain metastasis between 0.5 and 4 cm were treated with dabrafenib (Long et al. 2012). Patients were divided into those naïve to local treatment (e.g., radiation; cohort A) or patients with prior treatment (cohort B). Among those with $BRAF^{V600E}$ mutations, 39% and 31% of patients in cohorts A and B experienced intracranial disease responses. Concordance between intracranial and extracranial responses was generally high, although some patients did experience intracranial disease progression despite systemic control. Retrospective data also suggest that vemurafenib is also active in the setting of brain metastases (Harding et al. 2015). Thus, BRAF inhibitors can be an important therapeutic tool when patients develop melanoma brain metastases.

BRAF inhibitors were generally well tolerated but were associated with a characteristic toxicity profile. Chronic effects included arthralgias, fevers, gastrointestinal symptoms, and numerous cutaneous toxicities (Chapman et al. 2011; Hauschild et al. 2012; Sosman et al. 2012). The skin effects included various hyperproliferative skin lesions, including papular rashes, papillomas, and most notably, cutaneous squamous cell carcinomas (cuSCCs). In most cases, patients were able to continue therapy following surgical resection. When sequenced, these cuSCCs were found to harbor RAS mutations (Su et al. 2012a). Interestingly, BRAF inhibitors appeared to neither initiate nor promote carcinogenesis in these tumors. Instead, they paradoxically promoted MAPK signaling, thus hastening tumor growth in these RAS-mutated cells. Several other rare cases of other RAS mutant cancers arising on BRAF inhibitors have also been described.

Although vemurafenib and dabrafenib had relatively similar toxicity profiles, several key differences were observed. Vemurafenib was generally associated with higher rates of phototoxicity, whereas dabrafenib caused more pyrexia. Overall, these agents are generally thought to have equivalent efficacy, and can thus be chosen based on tolerability and physician or patient preference. At this time however, BRAF inhibitors are rarely used as single agents, and are generally combined with MEK inhibitors.

Other BRAF Inhibitors

Encorafenib (LGX818) is a highly potent BRAF inhibitor currently under clinical development.

While early trials demonstrated clinical activity in small numbers of patients, further studies are primarily focusing on combining this agent with the MEK inhibitor binimetinib (see BRAF + MEK Inhibitors). PLX8394 is a structurally distinct agent termed a "paradox-breaker" BRAF inhibitor. This agent, in preclinical models, inhibits BRAF without inducing paradoxical MAPK signaling in RAS-mutant cells (Zhang et al. 2015). PLX8394 is currently in phase I/II clinical trials. Other multikinase inhibitors with some degree of specificity for BRAF, including RAF265, are also undergoing clinical development (Su et al. 2012b).

MEK Inhibitors

MEK is immediately downstream of RAF in the MAPK pathway. As such, it was hoped that inhibition of this signaling node would have activity in both *RAF* and *RAS* mutant cancers. A phase I/II study was conducted that included patients with a variety of malignancies. A large subset of melanoma was enrolled, consisting of 36 patients with *BRAF* mutations, 10 with *NRAS* mutations, and 29 with *BRAF*/*NRAS* wild-type disease (Falchook et al. 2012). Among the *BRAF* mutant cohort, the response rate was 33% and the median PFS was 5.7 months. Notably, no responses were observed among the six patients who previously received a BRAF inhibitor. No patients with *NRAS* mutations responded to therapy, and 10% of the *BRAF*/*NRAS* WT group responded. Two of these patients were later found to have atypical non-V600 *BRAF* mutations, suggesting that only a small proportion of truly *BRAF* wild-type melanomas would benefit from trametinib.

To follow up this study, a phase III study comparing trametinib to investigator's choice chemotherapy was performed in 322 patients (Flaherty et al. 2012b). The median PFS was substantially higher in patients treated with trametinib (4.8 vs. 1.5 months, $p < 0.001$). In addition, OS was superior, despite allowing for crossover to trametinib for patients who progressed on chemotherapy (6 month OS rate 81% vs. 67%, $p = 0.01$). Based on these data, trametinib received FDA approval for advanced, *BRAF*-mutated melanoma. Given the marginally inferior outcomes compared to BRAF inhibition (albeit comparing across trials), trametinib has not been extensively used as a single agent in this setting. Several other MEK inhibitors have undergone clinical development (see "*NRAS* Mutant Melanoma" and "Uveal Melanoma" sections).

The toxicities observed with trametinib were quite distinct from those of BRAF inhibitors. Cutaneous events were also present, but stemmed from hypoproliferative effects on keratinocytes. Clinically, this manifests as an acneiform rash similar to that observed with EGFR inhibitors (e.g., erlotinib, gefitinib). Ocular toxicities, including central serous retinopathy and retinal vein occlusion, were observed occasionally, but at much lower rates than earlier generation MEK inhibitors. Cardiomyopathy, which usually reversed following treatment, was also observed infrequently. Gastrointestinal symptoms, edema, and joint pain also occurred and were generally of low-grade and clinically manageable.

BRAF + MEK Inhibition

A number of sequencing efforts demonstrated that most tumors resistant to BRAF inhibitors had incomplete MAPK blockade (see BRAF resistance, below) (Shi et al. 2014). Further, both BRAF and MEK inhibitors demonstrated substantial clinical activity as single agents at distinct nodes in the MAPK pathway. Thus, combining these agents was thought to be a rational therapeutic strategy for the *BRAF* mutant cohort. A phase I/II trial was conducted with dabrafenib and trametinib. In a randomized portion of this study, dabrafenib and trametinib were compared with dabrafenib monotherapy (Flaherty et al. 2012a). Improvements were noted in ORR (76% vs. 54%, $p = 0.03$) and median PFS (9.4 vs. 5.8 months, $p < 0.001$) with the highest dose of the combination (dabrafenib 150 mg twice daily and trametinib 2 mg daily). Based on these data, the combination of dabrafenib and trametinib received FDA approval in 2014.

Several follow-up phase III studies have verified and extended these findings in larger

populations. Dabrafenib and trametinib were compared with dabrafenib (COMBI-D study) in 423 patients with advanced, *BRAF*-mutant melanoma (Long et al. 2015). The combination resulted in superior PFS (median 11 vs. 8.8 months, $p < 0.001$) and OS (median 25.1 vs. 18. months, $p = 0.01$). Survival at 1 year (74% vs. 68%) and 2 years (51% vs. 42%) were also improved in the combination arm. In parallel, the COMBI-V study compared the same combination with vemurafenib in 704 patients (Robert et al. 2014). Similar findings were reported in this study, with improved 12 month OS (72% vs. 65%, $p = 0.005$), median PFS (11.4 vs. 7.3 months, $p < 0.001$), and response rates (64% vs. 51%).

Vemurafenib has also been evaluated in combination with another MEK inhibitor, cobimetinib. After promising response rates and PFS were observed in early phase trials, a phase III study comparing this combination with vemurafenib monotherapy was conducted (Larkin et al. 2014). Similar to the other BRAF + MEK inhibitor regimen, the combination produced superior PFS (median 9.9 vs. 6.2 months, $p < 0.001$) and response rate (68% vs. 45%, $p < 0.001$). Vemurafenib and cobimetinib received regulatory approval in November 2015 for treatment of *BRAF* V600 mutant melanoma. Based on these data, combined BRAF and MEK inhibition with either dabrafenib + trametinib or vemurafenib + cobimetinib is preferred over single-agent therapy and is now widely used.

Combined BRAF/MEK inhibition produced a unique toxicity profile. Stereotypic MEK inhibitor effects were observed at expected rates (cardiac, ocular) (Larkin et al. 2014; Long et al. 2015; Robert et al. 2014). Fevers also emerged as the most problematic, stereotypic toxicity with dabrafenib + trametinib. In most cases, these were manageable with nonsteroidal anti-inflammatory drugs (NSAIDs) and/or corticosteroids. Intriguingly, the cutaneous effects of either single agent were attenuated by combining agents. This apparent "cancelling out" of toxicities is attributed to blockade of paradoxical MAPK activation by the concurrent MEK inhibition in BRAF WT skin cells. The combination of vemurafenib and cobimetinib was not associated with pyrexia, but photosensitivity, diarrhea, and elevated creatinine phosphokinase levels were observed. Overall, the toxicity profiles of either combination are generally viewed as equivalent or potentially even superior compared to BRAF or MEK inhibitor monotherapy.

Another BRAF and MEK inhibitor combination is also undergoing clinical development: encorafenib and binimetinib. Early results suggest that response rates, progression-free survival, and incidence of toxicities are relatively comparable to the approved combinations. A randomized phase III study is currently comparing encorafenib and binimetinib with vemurafenib.

Therapy Selection

The data presented above clearly demonstrates superiority for combined BRAF + MEK inhibitors compared to single agent BRAF inhibitors. Therapy selection between BRAF + MEK inhibitors and immune therapy, however, is not so clear. In fact, this decision presents a common conundrum for patients with $BRAF^{V600}$ mutations. The clinical efficacy of immune checkpoint inhibitors extends to patients with and without *BRAF* mutations, providing a compelling alternative clinical option for these patients. A cooperative group trial sponsored by the US National Cancer Institute is currently evaluating dabrafenib + trametinib compared to ipilimumab + nivolumab with crossover to the alternative group at the time of progression (https://clinicaltrials.gov/ct2/show/NCT02224781). This study will provide more definitive guidance for clinicians about the most appropriate first-line selection. In the interim, there are several principals to guide this decision.

In general, BRAF-directed therapies are associated with high response rates and clinical benefit in nearly all patients. Therefore, for patients who are highly symptomatic and "need a response," many clinicians will choose BRAF + MEK inhibition as the initial therapy over immune checkpoint inhibition. Unfortunately, this group of patients generally has poor outcomes overall,

with a limited duration of benefit from BRAF + MEK inhibitors. By contrast, immune therapies may provide durable benefit for a sizable minority of patients (or perhaps even a majority for ipilimumab + nivolumab). Thus, many clinicians prefer an immune approach for most patients initially and reserve BRAF-directed therapies for the salvage setting.

Recent long-term data from early dabrafenib + trametinib studies add complexity to this decision (Long et al. 2016). Patients with low LDH or <3 sites of disease had outstanding outcomes to targeted therapy, with 3-year overall survival rates of 62% and 58%, respectively. This is a population that typically performs well with immune therapy as well. By contrast, patients with high LDH and those with ≥3 sites of disease involvement had dismal long-term outcomes with dabrafenib + trametinib, with 3-year OS of 5% and 19%, respectively. Thus, the traditional melanoma risk factors (metastatic stage, LDH) appear to correlate with outcomes for both immune and targeted therapies. Better therapies and improved selection markers are needed, particularly for the poor-prognostic subgroups.

Long-Term Outcomes

The traditional dogma has been that BRAF-directed therapy produces responses of limited duration with inevitable onset of acquired resistance. The long-term outcome data has challenged these assumptions, with extended follow-up from BRAF inhibitor monotherapy and BRAF+MEK combination studies. Particularly with the combination, a 3–4 year survival of approximately 20% appears to be emerging, with a "tail of the curve" appearance to the PFS figures (i.e., very few patients have disease progression between 2.5 and 4 years) (Long et al. 2016). While it is unclear whether these patients will have truly long-lasting responses, the lack of delayed progression argues for durability. Our own clinical experience mirrors these studies, with a small but definite patient population still receiving treatment many years after beginning therapy.

BRAF Inhibitor Resistance

Unfortunately, long-term PFS with BRAF-directed therapy is the exception rather than the rule. While this topic is comprehensively covered in another chapter, the core signaling nodes and concepts that underpin resistance are briefly reviewed here. Although nearly all patients receive clinical benefit and tumor regression from BRAF inhibitors, most ultimately develop acquired resistance to therapy causing disease progression. Posttreatment biopsies, obtained at the time of disease progression, revealed that MAPK signaling was reactivated in most progressing tumors despite ongoing BRAF inhibition. Intensive sequencing efforts by a number of groups revealed numerous and recurrent genetic and transcriptomic changes that reinvigorate MAPK signaling (Rizos et al. 2014; Shi et al. 2014; Van Allen et al. 2014). These included *NRAS* mutations, *BRAF* amplification, alternate splicing of *BRAF* (causing dimerization and efficient signaling), *MEK1/2* mutations, and *COT* overexpression. A smaller set of resistant samples displays MAPK-independent resistance mechanisms, including PI3K-AKT pathway changes or receptor tyrosine kinase upregulation. Overall, though, the majority of these changes leading to BRAF inhibitor resistance involved MAPK pathway reactivation. Hence, combined BRAF and MEK inhibition was considered to more completely extinguish MAPK signaling. Several studies have also shown, however, that genetic changes promoting MAPK signaling drive combination therapy resistance as well. More recently, a number of nongenetic and immune correlates of resistance have been identified which may further complicate attempts to target resistant pathways and impact immune therapy approaches (Hugo et al. 2015). Further, substantial heterogeneity within and across progressing tumors has been described. This diverse and complex landscape of resistance has decreased our optimism that targeting canonical signaling pathways can reverse or overcome resistance. A number of different approaches, however, have been attempted or are ongoing.

Targeting BRAF Inhibitor Resistance

One initial trial assessed MEK inhibition with trametinib following resistance to dabrafenib or vemurafenib. In a phase II study, 40 patients who failed BRAF inhibition received trametinib; none responded to therapy, and the median PFS was a dismal 1.8 months (Kim et al. 2013). Combined BRAF and MEK inhibition, which clearly delayed the onset of acquired resistance and improved clinical outcomes compared to monotherapy, was assessed in the BRAF inhibitor resistant setting. As part of the initial phase II study of dabrafenib + trametinib, a cohort of patients who failed vemurafenib or dabrafenib was included. These patients had markedly inferior outcomes to patients treated in the naïve setting, with median PFS of 3.6 months and a response rate of 12% (Johnson et al. 2014). Similar results were observed with vemurafenib and cobimetinib (Ribas et al. 2014). Thus, this approach is not commonly used.

Other single agents or combinations of targeted therapies have produced promising preclinical results, but this has yet to translate into clear clinical efficacy. ERK inhibitors may have a role in overcoming BRAF inhibitor resistance. The final canonical member of the MAPK pathway, however, has been challenging to target. Several inhibitors are now in clinical trials, with some early and modest signs of efficacy. Various combinations with preclinical support are also being tested to prevent or overcome resistance, including combinations of BRAF +/− MEK inhibitors with inhibitors of the PI3K/AKT pathway, heat shock proteins, MDM2 (a protein that interacts with p53), and autophagy (hydroxychloroquine).

Another intriguing strategy to delay resistance is intermittent dosing of BRAF inhibitors. While this approach has not been confirmed clinically, preclinical data suggests that drug dependence develops with continuous dosing of vemurafenib (Das Thakur et al. 2013). Discontinuous dosing, by contrast, exploits this dependency and forestalls the onset of resistance. A US National Cancer Institute-sponsored cooperative group study is currently testing this strategy with dabrafenib and trametinib. (https://clinicaltrials.gov/ct2/show/NCT02196181). In this study, patients receive continuous dosing for 8 weeks. Nonprogressing patients are then randomized to a 5 weeks on and 3 weeks off treatment schedule or continuous dosing. While the intermittent dosing schedule has not been validated for routine use, many experts prefer this strategy to repeated dose decreases in the presence of toxicities.

Finally, combining targeted and immune therapies, the two active therapeutic classes in melanoma, remains of great interest. Several elegant preclinical studies have suggested that BRAF and/or MEK inhibitors have various effects on the tumor microenvironment (preclinical and clinical data reviewed in Robert et al. 2016). Specifically, BRAF inhibitors have been associated with upregulation of melanoma differentiation antigens, major histocompatibility complexes, and immunogenic cytokines. MEK inhibitors have been shown to dampen T cell proliferation, which could either compromise immune therapy activity, or suppress toxicities. The combination has also demonstrated increased tumor infiltrating lymphocytes with increased clonality (suggesting a more specific antitumor response), and increase in PD-1/PD-L1 expression.

Clinically, combined BRAF-directed and immune therapies have had mixed results. The combination of vemurafenib and ipilimumab produced unacceptable hepatotoxicity, and the initial experience with the triple combination of dabrafenib, trametinib, and ipilimumab was complicated by frequent colitis and bowel perforations. While these initial studies have demonstrated the unpredictable nature of these toxicities, several subsequent studies have been more promising. Dabrafenib and ipilimumab appear to have a tolerable side effect profile in early results. Several other studies combining anti-PD-1/PD-L1 directed agents (atezolizumab, pembrolizumab) with BRAF+MEK inhibitors have shown signs of efficacy without substantial additive toxicity. Interestingly, a run-in period of vemurafenib and cobimetinib (for 28 or 56 days) appeared to produce superior activity (responses in 14 of 16 patients vs. 1 of 3 patients) and toxicity (grade 3/4 toxicities in 5 of 14 vs. 2 of 3) compared to concurrent administration with

atezolizumab. The median PFS in these few patients was 10.9 months, however, which is similar to vemurafenib + cobimetinib alone. Further study is needed to assess these novel approaches.

Therapy of Melanomas Without *BRAF*^{V600} Mutations

Genetics of *BRAF* V600 Wild-Type Melanomas

Following *BRAF*, *RAS* is the next most frequent driver mutation making up approximately 20% of the cases of cutaneous melanoma in numerous studies (Cancer Genome Atlas Network 2015; Hodis et al. 2012; Krauthammer et al. 2012). *NF1* loss of function (LOF) mutations make up 14% of melanomas analyzed with some overlap with BRAF-non-V600 mutations. The tumors which have *NF1* mutations also have the highest overall somatic mutation burden, likely reflecting their primaries on chronically sun-exposed sites from older patients (Krauthammer et al. 2012). Other recurrent alterations in *RAC1*, *PPP6C*, *ARID2*, *IDH1, RB1*, and *DDX3X* have been identified. Finally, there are a number of candidate fusion drivers in cases involving *BRAF* and other genes (Hutchinson et al. 2013). Many of these fusions have intact kinase domains without the regulatory sequences, leading to constitutive kinase activation.

Another way of looking at the TCGA would be the class of the genetic alteration in terms of the strength of evidence for them to be actionable targets. **Class 1** would be those which are known to be **clinically targetable** including those responsive to BRAF inhibition or MEK inhibition, CDK inhibition, MDM2/p53 inhibition, PI3K/Akt, mTOR inhibitors; or **class 2 translational actionable**, ERK inhibitors, MEK inhibitors, IDH1 inhibitors, EZH2 inhibitors and even Aurora kinase inhibitors (PPP6C) and **class 3 preclinical**, chromatin remodelers inhibition, BH3 mimetics, and others. The TCGA report also included an extensive gene expression and protein expression analysis (Cancer Genome Atlas Network 2015). This was most revealing for the presence of a strong immune RNA expression signature with overexpression of genes associated with T cells, B cells, NK cells, chemokines, cytokines, and immune signaling molecules and inhibitory checkpoint proteins in melanomas from all of the genetically classified TCGA melanoma cohorts. The presence of an immune signature was associated with an improved prognosis independent of any treatment. This likely is of greatest relevance to the responsiveness to immunotherapy, although it may also have implications in targeted therapies. The remainder of this chapter will focus on defined subsets of *BRAF* V600 WT melanomas including those with mutations in *NRAS*, *NF1*, TWT and followed by uveal, mucosal, and acral, subtypes not represented in the TCGA.

NRAS-Mutated Melanomas

NRAS mutant melanomas present with several specific clinical features (Thomas et al. 2015). Primary tumors have been associated with regions of chronic sun damage, increase in number of mitoses, decrease in tumor infiltrating lymphocytes, and frequently upstaged primaries. At diagnosis of stage IV (M1) disease *NRAS* melanomas have a worse prognosis to TWT melanomas and more likely involvement of the central nervous system.

Directly targeting *NRAS* has been a challenge, since it is not a kinase and because of the very tight RAS-GTP binding (Stephen et al. 2014). NRAS-mediated activation involves the reversible exchange of GDP for GTP whereas turning off RAS involves hydrolysis of GTP to GDP. Guanine nucleotide exchange factors (GEFs) accelerate the activation of NRAS, while GTPase activating proteins (GAP) accelerate the off switch. The downstream effectors include ERK through the MAP kinase pathway, PI3K and PDK1, RALGDS, RAL guanine nucleotide dissociation stimulator, PLD phospholipase D (PLD), Phospholipase C (PLC), protein kinase C (PKC), and T-cell lymphoma invasion and metastasis 1 (TIAM1) (Johnson and Puzanov 2015). In preclinical studies completely knocking down

(extinguishing) PIK3CA, p110α can reduce both NRAS- and KRAS-driven tumors formation. These results highlight the need to simultaneously inhibit other pathways beyond MAPK signaling for *NRAS*-mutated melanomas. Most of the downstream pathways, however, are not tumor-specific targets which may lead to significant side effects. As always, well-designed clinical studies with both pharmacokinetics and pharmacodynamic endpoints are needed for combination therapy.

Numerous attempts have been made including preventing the binding of NRAS from the cell membrane with initially farnesyltransferase inhibitors, and following their failure, the targeting of the geranyl-geranyl-transferase, inhibition of RAS-SOS protein interaction with small molecules, and inhibiting the binding of RAS to the RAF molecule (s) (Gajewski et al. 2012). Since *NRAS* mutant melanomas predominantly have a Q61 mutation, the cysteine reactive molecules selective for *KRAS* G12C binds to another pocket and would not be effective (Burd et al. 2014). This has led to a focus on MEK inhibition downstream from RAS-RAF, which is already known to be problematic due to the greater ERK reactivation feedback that it induces. Nevertheless an oral MEK inhibitor (MEK162, Binimetinib), a non-competitive ATP inhibitor of MEK1 and MEK2, has been tested clinically in patients with metastatic melanoma harboring BRAF or NRAS mutations. Early encouraging results in NRAS-mutated patients have been noted, with an objective response rate of 20%, of which only 10% were confirmed with imaging (Ascierto et al. 2013). The median PFS was 3.7 months with a median duration of response of 7.6 weeks. This is consistent with rapid development of acquired resistance, even for responding patients. This phase I/II study led to a phase III trial comparing binimetinib versus chemotherapy enrolling 402 NRAS-mutant melanoma patients with a 2:1 randomization favoring binimetinib. The study was recently reported at the 2016 ASCO meeting. Patients were required to have a Q61 NRAS mutation and towards the completion of accrual, prior immunotherapy was allowed. Only 20% had prior checkpoint inhibitors, including 13% with ipilimumab and only a few percent (5–6%) receiving prior anti-PD1. The study met its primary endpoint with an improvement of PFS (HR = 0.62, $p = 0.001$). However, the increase in median PFS was not especially impressive, increasing from 1.5 to 2.8 months, and the ORR favored binimetinib 15% versus 7%. The PFS benefit appeared to be more pronounced in those patients with poor prognostic factors including those with stage IV M1c, more numbers of organ involved, presence of visceral disease, and elevated serum LDH. Interestingly, even though those with prior immunotherapy represented only 20% of the patients, they had the most obvious benefit in terms of median PFS, increasing from 1.6 months to 5.5 months. The median overall survival for all patients enrolled was no different (11 vs. 10.1 months, HR = 1.0). Of note 45% of both groups received immunotherapy following protocol treatment with those who received binimetinib being slightly more likely to have PD as their best response to immunotherapy. Results of this trial may lead to approval of the first targeted agent for *NRAS* mutant melanoma, but the results are below the hoped-for improvement. It may provide a component of therapy in the future. The results with those patients who have had prior immunotherapy is the most promising aspect of the trial, since nearly all patients in the future would be treated in this order, even with the availability of a MEK inhibitor.

Combination therapy with a MEK inhibitor backbone has also been a treatment strategy of interest. Generally, combined PI3K and MEK inhibitor therapy has seemed to be feasible with manageable safety and toxicity profile. The most common adverse events (AEs) of therapy include diarrhea, rash, fatigue, vomiting, and hyperglycemia. The clinical activity of GDC-0973 (MEK1/2 inhibitor) and GDC-0941 (class I PI3K inhibitor) was studied in 78 patients with advanced solid tumors (Asati et al. 2016). Daily dosing of BKM120 (pan-PI3K inhibitor) and trametinib (MEK inhibitor) was evaluated with 49 patients with advanced RAS- or B-RAF-mutant cancers. In another combination study, 49 patients were treated with the pan-PI3K inhibitor copanlisib and the MEK inhibitor refametinib (Asati et al. 2016). The combination of BYL719 (PI3Kα

inhibitor) and binimetinib (MEK inhibitor) was studied in 58 patients with advanced solid tumors with RAS or B-RAF mutations (Asati et al. 2016). None of these trials with MEK inhibitors and PI-3 kinase/mTOR inhibitors generated promising enough results to pursue phase II trials in melanoma, including in *NRAS* mutant melanoma. Toxicity of the regimen, while tolerable, never allowed dose escalation required to see the promising clinical effects in patients.

Interestingly, hyperactivation of AKT and loss of PTEN expression dominates the picture in brain metastasis versus other sites of disease (Davies et al. 2009). Brain-derived factors appear to induce hyperactivation of the AKT survival pathway and to promote the survival and drug resistance of melanoma cells in the brain. Thus, inhibition of PI3K-AKT signaling shows potential for enhancing and/or prolonging the antitumor effect of MEK inhibitors in melanoma brain metastases.

In all of non-*BRAF* ^{V600} mutant melanoma (NRAS, NF1, TWT), interest in combining MEK inhibitors with CDK4/6 inhibitors has the frequent dysregulation of the CDK4/6-RB1 pathway. This occurs through several mechanisms, including overexpression and/or amplification of D-type cyclins, mutation or amplification of *CDK4/6*, or loss of cyclin D-CDK4/6 negative regulators such as p16INK4A (Hodis et al. 2012). In mouse models of *NRAS* mutant melanoma, including xenograft and syngeneic models, combined MAPK and CDK4/6 inhibition has been promising (Kwong et al. 2012). Inhibition of MEK activates apoptosis, but not cell-cycle arrest. Therefore, cell death is balanced by continued proliferation, leading to tumor stasis in vivo. In contrast, the knock out (extinguishing) of *NRAS* induces apoptosis and cell cycle arrest. CDK4 was identified as the critical driver of this differential phenotype. The predominant cytostatic effects of CDK4/6 inhibition, when combined with MEK inhibition, led to apoptosis with blockade of continued proliferation, resulting in net tumor regression and substantial synergy in therapeutic efficacy. Consistent with these results, combined CDK4 and MEK inhibition led to increased apoptosis and/or reduced viability in colony formation assays in human melanoma and pancreatic cancer cell lines.

Combination of CDK4/6 inhibitors with RAS/RAF/MEK/ERK pathway inhibitors is a promising therapeutic approach, particularly in patients with melanoma. Identifying the optimal dose and schedule to maximally inhibit both pathways while minimizing toxicity remains an elusive goal. Treatment-related toxicities were common and included creatinine phosphokinase elevation, rash, edema, anemia, nausea, diarrhea, and fatigue. Clinical studies have included binimetinib + ribociclib, trametinib + palbociclib, and PD901+ palbociclib (Sosman et al. 2014). There is the most experience with the combination of binimetinib + ribociclib, first with a 28-day cycle of ribociclib (3 weeks on and 1 week off) and continuous binimetinib. Twenty-two patients were enrolled with some significant toxicities including renal, creatinine phosphokinase elevations, anemia, atrial fibrillation with five DLTs including an intracranial bleed. Only well below single-agent MTD could be assessed for ribociclib and binimetinib, but still clinical activity was extremely encouraging with five confirmed PR, four unconfirmed PR, and eight SD – 9/22 (41%) ORR. The duration of response was 56–351 days and overall median PFS was 6.7 months. Due to toxicity, a 21-day cycle of ribociclib (14 days on, 7 days off) was assessed enrolling 22 patients with 4 objective responses and a median PFS of 4 months. An additional 7 patients were enrolled to receive 200 mg ribociclib daily for 14 of 28 days/45 mg BID binimetinib. Ultimately, this was the recommended phase 2 dose. Cumulatively, 6 responses were seen out of 16 treated patients at this selected dose and level with an overall RR of 35% (10/29), median DOR of 5.0 months, and median PFS of 6.4 months. All of these metrics appeared superior to binimetinib alone. While trametinib plus palbociclib has completed a phase I trial, the heterogenous composition of patients did not allow any assessment of clinical activity in NRAS mutant melanoma.

There have been numerous approaches that are currently being tested in clinical trials, but too early to provide a clear idea of the clinical activity. One approach of great interest is ERK inhibition

which would conceptually prevent the ERK-induced feedback seen with MEK inhibitors. Three ERK inhibitors have entered clinical trial development. SCH772984 and RG7842 (GDC0994; Genentech/Roche) are currently being tested in Phase I clinical trials (Morris et al. 2013). BVD-523 (ulixertinib; Biomed Valley Discoveries), a novel ERK1/2 kinase inhibitor, has recently entered phase I clinical trials with responses noted in three patients with BRAF mutant melanoma, including one refractory to prior BRAF/MEK inhibition. Expansion cohorts have included over 20 patients with *NRAS* mutant melanoma, but no results have been reported at this time.

For RAS-mutant tumors, effective inhibition of MAPK signaling remains a major challenge. MEK inhibitors have shown definite modest clinical activity in this setting and they may be more effective if combined with either CDK4/6 inhibitors, PI3K/mTOR inhibitors, or other approaches. ERK inhibitors or RAF inhibitors that can overcome resistance due to RAF dimerization or even potential RAS inhibitors may ultimately become a reality.

Other potential approaches include c-Met inhibition, based on ex vivo work demonstrating increased C-Met phosphorylation in *NRAS* mutant melanoma. Inhibition of NRAS decreases C-MET responsiveness to HGF, and *NRAS* mutant tumors were more sensitive to c-Met blockade (Chattopadhyay et al. 2012). RAF paradox breakers (PLX7904 and PLX8394) are RAF inhibitors that evade paradoxical MAPK pathway activation with no pERK induction in RAS mutant tumors, and pan-RAF inhibitors are in early clinical trials (Zhang et al. 2015). Finally, inhibitors of polo-like kinase (PLK1), a molecule which is overexpressed in *NRAS* ^{Q61} mutant melanoma, when combined with MEK inhibitors, lead to major reduction in cell cycle-related genes including CCND1, CDC25A, CHEK2, E2F1, causing dual cell cycle arrest (Posch et al. 2015). Results suggest that cells escaping G1 arrest induced by MEK inhibition, or cells escaping G2/M arrest from PLK1 inhibition, maintain their susceptibility to the other drugs in combination. This will have to be tested in the clinic but remains of interest. Additional targets include MDM2, through its effect on p53, BET inhibitors, where members of family of BET proteins (especially BRD4 and BRD2) are overexpressed in many melanomas, and MITF, due to the finding that MEK inhibition increased MITF expression, which in turn elevated levels of PGC1a (Fedorenko et al. 2012). A HIV1 protease nelfinavir suppresses both MITF and PAX3 and inhibits growth. Finally, the combination of metformin and trametinib has a synergistic effect in *NRAS* mutant tumors and reduces tumor size in a xenograft model (Smith et al. 2016). This is likely a result of suppressing the phosphorylation of NRAS effector proteins ERK and S6 decreasing cell viability.

Targeting of the NF1 Loss of Function (LOF) Melanomas

In the TCGA and the Yale cohort (213 melanomas), three genes are mutated with an incidence greater than 10%: *BRAF* and *NRAS*, with known recurrent activating mutations, and *NF1*. *NF1* had a high number of inactivating or damaging mutations; 90% are nonsense, splice-site variant or insertion-deletion (indel) and LOH (loss of the other allele in most of these cases) (Hodis et al. 2012; Krauthammer et al. 2012). These are cases where *NF1* was presumably the driver of the melanoma without activations through *BRAF* or *NRAS* mutations. Inactivating *NF1* mutations were present in 46% of melanomas expressing wild-type *BRAF* and wild-type *RAS*, occurred in older patients, harbored many more somatic mutations throughout the entire exome, and had an OS similar to BRAF, NRAS, and TWT melanomas. NF1 is a GTPase-activating protein, a GAP protein that suppresses RAS function. NF1 suppression leads to increased RAS activation in a large percentage of melanomas (Krauthammer et al. 2012). Loss of *NF1*, however, does not predict sensitivity to MEK or ERK inhibitors. These tumors showed a distinct pattern of co-mutation with other genes related to RAS pathways such as RASopathy gene mutations (Krauthammer et al. 2012). These are included in (15/26) 60% with RASopathy gene mutations in *RASA2* (9 cases),

PTPN11 (4), *SOS1* (2), *RAF1* (2), and *SPRED1* (2) that may enhance its role in melanoma development.

In terms of therapy specific for *NF1* mutant melanomas, there is very little information. One preclinical study suggested that MEK inhibition could be effective, although we do not yet have clinical experience demonstrating this finding (Nissan et al. 2014). However, in neurofibromatosis type 1, where the *NF1* gene is damaged in the germline, patients are predisposed to cutaneous neurofibromas, plexiform neurofibromas (PNFs), and malignant peripheral nerve sheath tumors (MPNSTs) among other neoplasias and manifestations. In these cases, MEK inhibition has shown substantial benefit (Dombi et al. 2016).

BRAF/NRAS wild-type melanomas are highly sensitive to the MEK inhibitor, trametinib, ex vivo, but the loss of *NF1* protein expression alone does not select for sensitive cell lines. In a review of "exceptional responses" (objective response or SD > 6 months) to everolimus, one patient with head and neck cancer with a LOF *NF1* mutation had PFS of nearly 10 months (Lim et al. 2016). Ultimately, the most effective targeted therapy approaches for melanomas with *NF1* mutations has yet to be defined.

BRAF-resistant cell lines are sensitive to AZ628, an inhibitor of $BRAF^{V600E}$, WT *BRAF*, and *CRAF* (a so-called pan-RAF inhibitor) (Peng et al. 2015). This inhibitor was combined with the MEK inhibitor selumetinib with near complete pERK decrease and improved responses in resistant cell lines through the loss-of-function mutations in NF1 (LOXIMVI, HCT116). It should be noted that AZ628, RAF265, and MLN2480 are all type II RAF inhibitors (pan-RAF), whereas vemurafenib and dabrafenib are type I inhibitors. These agents could potentially have activity in NRAS, NF1, and TWT melanomas.

Targeting Atypical BRAF Mutant Melanoma (Non-V600)

Approximately 5% of all melanomas harbor mutations in *BRAF* at loci other than *V600 (BRAF non-V600 mutations*) (Cancer Genome Atlas Network 2015). These mutations are often not detected by testing platforms commonly used in clinical practice that assess only codon 600 for mutations. However, they are detected by sequencing all the *BRAF* exons. A number of these *BRAF* non-V600 mutations result in increased kinase activity of the BRAF protein in vitro (i.e., L597 V, K601E, G469A). In addition, other mutations that do not increase the catalytic activity of BRAF (including G466E, D594V, G596R) appear to increase MAPK pathway activity through protein-protein interactions with CRAF or wild-type BRAF (Wan et al. 2004). This occurs in the setting of upstream activation (NRAS mutation or receptor tyrosine kinase (RTK) activation). Furthermore, BRAF fusions which activate MAPK signaling have been identified in a small percentage of melanomas (Hutchinson et al. 2013). MEK inhibitors have demonstrated activity in vitro in melanomas with high activity non-V600 BRAF mutations, and individual patients with these mutations have demonstrated significant clinical responses to treatment with MEK inhibitors. A phase II trial is currently ongoing to assess the activity of trametinib across the spectrum of atypical BRAF mutations and fusions.

Targeting KIT

KIT mutations and/or amplifications are rare in melanoma, although 5–20% of melanomas originating on mucosal, acral, and chronic sun damage (CSD) surfaces demonstrate KIT genetic abnormalities (Curtin et al. 2006). By contrast, these alterations are almost never seen originating from other cutaneous areas without CSD. The mutant allele is sometimes amplified and in some tumors the wild-type KIT locus is amplified. Given the experience with gastrointestinal stromal tumors, inhibition of KIT was thought to be an attractive therapeutic strategy. Exon 11 and 13 are the most sensitive and the most abundant mutations are at L576P and K642E. The first large cohort from the USA enrolled patients with mutations and/or amplifications in KIT (Carvajal et al. 2011). Two hundred and ninety-seven patients were screened

with melanomas originating in one of these three sites and only 51 were found to have mutations and/or amplifications in KIT, with only 28 enrolled onto imatinib treatment over 3 years. Of 25 evaluable patients treated at 400 mg daily imatinib, there were four objective responses, (2CR and 2PR; ORR 16%) and all were durable >12 months. All responders had mutations (rather than amplifications) and those were at codons L576 and K642 in two of four patients. Of the 209 actually screened, 18/84 acral melanoma, 17/93 mucosal melanoma, and 5/32 CSD had *KIT* mutations. In another trial undertaken in China, 43 patients were enrolled with either *KIT* amplification or mutation or both and received imatinib at 400 mg/day which could be increased to 800 mg q day at progression (Guo et al. 2011). In this study, there were 10 PR and no CR; 9/10 responses had mutations at exon 11 or 13 (9/26), while 1 of 3 patients with amplified *KIT* responded. More recently, Hodi and colleagues published on 25 treated patients out of 213 screened over 5 years (Hodi et al. 2013). Of 24 patients with *KIT* alterations, 8 had KIT mutations alone, 5 had mutations+ amplifications of *KIT*, and 11 had only KIT amplification. Seven patients experienced PRs, but these only occurred in *KIT*-mutated melanomas. The ORR was 29%, but in 13 patients with mutations, the ORR was over 50% (7/13), and 6 responses had exon 11 or 13 mutations (of which 4 had L576P and K642E mutations). However, only 1/7 patients had response duration >12 months with one ongoing response at 27+ months. Two other studies of nilotinib or sunitinib were performed. In patients who were refractory or with intolerable side effect on imatinib, only 2/11 had a response in second line to nilotinib (Carvajal et al. 2015). Finally, 52 patients with acral or mucosal melanoma were treated with sunitinib and only 13 of the 44 patients whose tumors were tested for *KIT* mutations. 1/13 with mutations responded while 3/31 WT responded to sunitinib (Buchbinder et al. 2015). In this study, the presence of mutations did not correlate with response rate and all responses were 5–10 months in duration. In summary, the primary drug studied in *KIT* mutant/amplified melanoma has been imatinib, and overall responses were almost always observed in those with mutations in exon 11 and exon 13 (particularly L576P and K642E). Although some responses were very durable, most lasted less than 12 months. The ORR has been in the range of 15–25%, leaving plenty of room for further improvement.

Uveal melanomas have a distinct biology from cutaneous melanoma with a low mutation burden without a UV signature and absence of mutations of *BRAF*, *NRAS*, *KIT*, or *NF1*. In over 80% of tumors, either *GNAQ* or *GNA11* is mutated in a mutually exclusive fashion. These genes appear to activate the MAP kinase pathway through PKC and the RAS-GEF RasGRP3 (Chen et al. 2017; Van Raamsdonk et al. 2009, 2010). More recently the YAP- hippo pathway has also been implicated in uveal melanomas (Feng et al. 2014). About 40–50% of uveal melanoma have LOF mutations or deletions in the *BAP1* gene, which is associated with inferior prognosis and high likelihood of metastases (Harbour et al. 2010). *SF3B1*, a splicing factor, is another recurrently mutated gene which occurs in 15% of uveal melanomas and is associated with a good prognosis (Harbour et al. 2013). Therapy with interferon in the adjuvant setting and checkpoint inhibitors in the metastatic setting have been very disappointing (Luke et al. 2013). One promising lead had been seen with a MEK inhibitor, selumetinib. A randomized phase II trial showed a RR of 14% versus 0% for dacarbazine chemotherapy and a doubling of PFS, increasing from a median of 7 weeks to 15.9 weeks (Carvajal et al. 2014). However in a follow-up study with selumetinib + dacarbazine versus dacarbazine alone, the PFS endpoint was not met (Komatsubara et al. 2016). Finally, more disappointment occurred with a recent study presentation where a MEK inhibitor, trametinib alone or with an AKTi, demonstrated only one objective response in 20 patients, and the study was closed early due to lack of efficacy. Other approaches are ongoing with a MEK inhibitor and a PKC inhibitor, and targeting BAP1 with EZH2 inhibitor, targeting of YAP, or inhibition of the hippo pathway. Certainly therapy of uveal melanoma has been stagnant without any real evidence of efficacy with either targeted or immunotherapy. New

approaches targeting the biology of uveal melanoma are desperately needed.

Conclusions

Ultimately, targeted therapy in melanoma has been a qualified success story thus far. The dramatic clinical efficacy of BRAF and MEK inhibitors in *BRAF* mutant melanoma greatly benefits patients harboring these mutations, although acquired resistance limits the duration of benefit. Novel strategies to overcome resistance in the *BRAF* cohort, to identify tractable therapeutic targets in the *BRAF* WT population, and to design effective combinatorial strategies remain urgent needs.

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