

MEK inhibitors for the treatment of NRAS mutant melanoma

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Abstract: Melanoma is increasing rapidly in incidence and prevalence, especially in younger females and older males. Treatment options have expanded beyond high-dose interleukin 2 and adoptive T-cell therapy to include inhibitors of immune checkpoints programmed death 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and small molecular inhibitors of pathways activated in melanoma, in particular the mitogen-activated protein kinase (MAPK) pathway. PD-1/CTLA-4 inhibitors and inhibitors of MAPK such as BRAF/MEK inhibitors have significantly improved survival in both the metastatic and, more recently, adjuvant settings. In this review, we discuss the preclinical data, clinical development, and potential use of novel MEK inhibitor binimetinib, particularly in the setting of *NRAS* mutant melanoma.

Keywords: advanced, metastatic, melanoma, BRAF, MEK, NRAS, binimetinib, MAPK, ERK

Introduction

Surveillance, Epidemiology, and End Results data estimated that in 2017 alone, 87,110 new cases of cutaneous malignant melanoma (CMM) were reported along with 9,730 deaths.^{1,2} The incidence of CMM has been increasing by 1.4% annually on average over the last 10 years – in stark comparison to the incidence of other common malignancies like female breast cancer (stable incidence), prostate cancer (declining by 5.8%), colorectal cancer (CRC; declining by 2.7%), and lung cancer (declining by 2.0%).^{1,2} This increase in incidence is disproportionally seen in young women and older men.^{1,2}

Compelling epidemiologic data suggest that primary risk factor for CMM is exposure to ultraviolet (UV) light. These include the significantly greater incidence of cutaneous melanoma in fair-skinned compared to dark-skinned individuals³ and the greater incidence of melanoma in countries further away from the equator such as the United States,^{1,2} Australia,⁴ and New Zealand.⁵ However, several factors including the occurrence of melanoma in patients with intermittent sun exposure and on body sites typically not exposed to the sun such as the trunk suggests that the relationship between melanoma tumorigenesis and UV exposure is not a simple model in which risk correlated directly with exposure.^{6,7}

This led Whiteman et al to propose the divergent pathway hypothesis: an understanding that “melanomas are a consequence of unchecked melanocytic proliferation, and that the degree of proliferation is a trait that is variably distributed in the population”.^{8–10} This model predicted that patients with “greater inherent predisposition” toward melanoma would only require modest levels of sun exposure to trigger melanoma and that these would typically occur at sites such as the trunk and/or extremities. Conversely, patients with “lower inherent predisposition” toward

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melanoma would require much greater levels of sun exposure to drive melanoma tumorigenesis, and hence, these tumors would typically occur at sun-exposed areas such as the head and neck. The differential incidence in truncal melanoma (peaking in middle-age) compared to head and neck melanoma (increasingly common with older age) supports this hypothesis.¹¹ Genetic studies supported the divergent pathway hypothesis – mutations in *BRAF* were primarily seen in younger patients with tumors arising in areas only intermittently exposed to the sun. Correspondingly, *BRAF* mutations were less commonly identified in tumors from older patients in chronically sun-exposed areas and were particularly unusual in acral skin or mucosal sites.¹²

The critical breakthrough in our understanding of the mutational landscape of CMM, and, in particular, the link between UV exposure and its effects on preexisting nevi and melanoma came through large-scale collaborative sequencing efforts from groups including The Cancer Genome Atlas (TCGA). Beyond clarifying links between UV exposure and melanoma tumorigenesis, these data provided critical insight and led to the pursuit of inhibitors of genetic drivers as a potential therapeutic option in melanoma. In this review, we dissect the genetic diversity of CMM with particular attention to the mitogen-activated protein kinase (MAPK) pathway and the role of *BRAF*/MEK inhibitors in the treatment of this disease. We describe the recent development of the selective MEK inhibitor binimetinib in the treatment of metastatic melanoma, and focus on its clinical development and describe its place in the melanoma therapeutic armamentarium.

Genetic diversity of cutaneous melanoma

Seminal studies by Curtin et al using an array-based comparative genomic hybridization approach clarified the mutational landscape of cutaneous melanoma particularly in relation to UV exposure and chronic sun damage (CSD).¹³ Evaluating acral, mucosal, and melanoma associated with CSD skin and non-CSD skin, they discovered that the majority of non-CSD melanomas exhibited mutations in *BRAF* or *NRAS* while the majority of CSD melanomas were *BRAF* wild type (WT). Although *BRAF* mutations occasionally occurred with *PTEN* loss, *BRAF* and *NRAS* mutations appeared to be mutually exclusive suggesting that each was a distinct driver. Acral and mucosal melanomas were not strongly enriched for either *BRAF* and/or *NRAS* mutations.

Subsequently, advances in next-generation sequencing permitted large-scale sequencing of hundreds of specimens

by TCGA, which provided a framework for the genomic classification of CMM: *BRAF* mutant, *NRAS* mutant, *NF1* mutant, and WT. Collectively, *BRAF* (37%–50%), *NRAS* (13%–25%), and *NF1* (12%) mutations account for the majority of mutations found in cutaneous melanoma. CSD melanomas typically arise in older (>55 years) patients on sun-exposed areas (head/neck, dorsal surfaces of distal extremities) and are associated with specific driver mutations (*BRAF*^{nonV600E}, *NRAS*, *NF1*, or *KIT*) and an increased incidence of cytosine to thymidine (C>T) transitions that is characteristic of an ultraviolet (UV) light-induced mutational signature genetic signatures.^{14–17} Conversely, non-CSD melanomas typically arise in younger (<55 years) patients on intermittently sun-exposed areas (trunk, proximal extremities), and are associated with *BRAF*^{V600E} driver mutations while lacking genetic signature of UV mutagenesis.^{14–17}

NRAS mutations are found in 15%–20% of CMM in either exon 2 (codons 12, 13) or exon 3 (codon 61).^{13,18,19} Unlike *BRAF* mutations, which are typically seen in non-CSD skin, *NRAS* mutations occur in both CSD and non-CSD skin although a predisposition for the upper extremities has been observed in multiple series.^{13,20} *NRAS* mutant primary melanomas are typically thicker with higher Breslow depth and Clark level although they are less likely to have mitoses and/or ulceration²⁰ and lower tumor-infiltrating lymphocyte (TIL) grade.²¹ Also, while *BRAF* mutations are typically present in most melanocytic nevi, *NRAS* mutations are conspicuously absent in nevi other than congenital nevi and the associated congenital nevus-derived melanoma.²² *NRAS* mutant melanomas (NEMOs) have been linked with increased risk of visceral and central nervous system metastases²³ compared to *NRAS* WT tumors. Although some reports have linked *NRAS* mutant metastatic melanoma to improved outcomes with immunotherapies including high-dose interleukin 2²⁴ and programmed death 1 (PD-1) or cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) checkpoint inhibition,²⁵ the converse has been observed in other series²⁶ and has not been conclusively validated in prospective series.

Eighty percent of *NRAS* mutations generally occur in codon 61 and typically comprise amino acid substitutions at position 61 from glutamine (Q) to arginine (R – 38%), lysine (K – 34%), leucine (L – 10%), and less commonly to glutamate (E), histidine (H), or proline (P). These mutations are typically in the switch II region of the G domain. Conversely, *NRAS* codon 12 or 13 mutations comprise 20% of all *NRAS* mutations and generally occur within the P-loop region of the G domain secondary to an amino acid

substitution from glycine (G) to most commonly aspartic acid (D). Codon 61 mutations are associated with locking of the Ras protein into its activated conformation and impaired GTPase activity.²⁷ Codon 12/13 mutations render Ras insensitive to normal physiologic inactivation by Ras-GTPase-activating proteins.²⁷ Both codon 61 or codon 12/13 mutations result in constitutively activated RAS signaling – which drives cellular transformation through a network of signal transduction pathways involved in growth, motility, and survival, thereby enhancing tumor growth.^{28–31}

Roles of BRAF and MEK inhibition in melanoma

Activation of MAPK pathway signaling starts with initiation of receptor tyrosine kinase (RTK) on the cell surface through growth factors, cytokines, and extracellular mitogens.³² In the quiescent state, inactive Ras-GDP is plasma membrane-bound while inactive Raf, MEK, and ERK are cytoplasmic. Ligand binding activates RTK auto-phosphorylation, which generates binding sites for the SHC and GRB2 adaptor molecules, which in turn recruit cytoplasmic SOS (Ras GTPase exchange factor [RasGEF]) to the plasma membrane.^{29,30,33,34} SOS/RasGEF catalyzes exchange of GDP for GTP and Ras-GTP and then recruits Raf to the membrane where it is activated.^{29,35–37} Raf activates MEK1/2 and MEK1/2 activates ERK1/2 through activation loop phosphorylation, while ERK negatively regulates SOS to negatively regulate the pathway.^{32,37–40} Terminal targets of phosphorylated ERK1/2 include cytoplasmic signaling proteins such as RSK (p90 ribosomal S6 kinase) and multiple transcription factors including ternary complex factor (TCF) transcription factors.^{38,39} TCF transcription factors are key to the regulation of immediate early gene expression, products of which include c-Fos and c-Myc.^{41,42} Thus, physiologic activation of MEK1/2 and ERK1/2 is required for multiple normal cellular survival, proliferation, motility, and metabolism, while over-activation of the pathway leads to constitutive activation and malignant transformation. Hence, inhibition of overactive signaling in MAPK pathway components such as BRAF and MEK represents an attractive strategy in controlling *BRAF* and *NRAS* mutant malignancies.

As discussed, somatic mutations in *BRAF* and *NRAS* are seen in 37%–50% and 13%–25% of CMM, where they tend to be nonoverlapping with other oncogenic mutations found in melanoma (mutations in *NF1* and *KIT*). BRAF inhibitors inhibit downstream signaling in *BRAF* mutant tumors, and consequently, BRAF inhibitors vemurafenib (Zelboraf[®]; Genentech, San Francisco, CA, USA) and dabrafenib (Tafinlar[®]; Novartis,

Basel, Switzerland) have both demonstrated profound reductions in tumor burden (~50% objective responses) that lasted 6–7 months in randomized Phase III trials of *BRAF*^{V600E/K} mutant melanoma.^{43,44} Responses to single-agent BRAF inhibition are fleeting secondary to acquired resistance. Multiple mechanisms of acquired resistance to single-agent BRAF inhibition have been described including MAPK pathway-independent and MAPK pathway-dependent methods such as acquired RAS mutations, mutant BRAF amplification, and alternative splicing, of which the MAPK pathway-dependent methods are more common and account for 70% of acquired resistance.^{37,45–58} Although the respective frequencies of each of these mechanisms of acquired resistance are not yet known, most resistance appears to functionally result in reactivation of MAPK pathway signaling. Hence, combined BRAF and MEK inhibitor therapy is an attractive strategy to delay or prevent resistance. Three pivotal Phase III studies established the superiority of combination BRAF and MEK inhibition over BRAF inhibition alone: COMBI-d (dabrafenib/trametinib vs dabrafenib/placebo), COMBI-v (dabrafenib/trametinib vs vemurafenib), and coBRIM (vemurafenib/cobimetinib vs vemurafenib/placebo). As expected, BRAF/MEK inhibitor combinations produced greater rates of response (64%–68%) and median durations of response (9.9–11.5 months) compared to BRAF inhibitor monotherapy.^{59–61} Interestingly, the rate of cutaneous squamous-cell carcinomas was much lower with combination therapy reflecting the more profound degree of MAPK pathway inhibition achieved with combined BRAF and MEK inhibition. Based on these results, US Food and Drug Administration (FDA) approval was granted for both dabrafenib/trametinib and vemurafenib/cobimetinib combinations. Separately, combined BRAF and MEK inhibition is effective after failure of BRAF inhibitor monotherapy.^{62–64} One clear role for MEK inhibitors is in the combination with BRAF inhibitors to potentiate the response of these agents in *BRAF* mutant melanoma.

Recently, a body of work has emerged that suggests that oncogenic activation of the Ras/MAPK pathway interacts with and affects tumor-associated immunity. In the *BRAF*^{V600E}/*PTEN*^{null} melanoma mouse model, MAPK pathway activation mediates T-cell exclusion, while BRAF/MEK inhibition enhances CD8⁺ T-cell infiltration into tumor microenvironment (TME) and synergizes with PD-1 blockade.⁶⁵ Within TME, while BRAF inhibition alone increases tumor-associated macrophages and T regulatory cell infiltration, combined BRAF/MEK inhibition reverses both these trends while increasing major histocompatibility complex expression and inflammatory immune-related

gene upregulation – arguing for potential synergies between BRAF/MEK inhibition and PD-1 blockade.⁶⁶

The adverse effects of MAPK pathway activation on systemic immunity are supported by clinical data from triple-negative breast cancer patients in whom MAPK pathway activation is associated with significantly decreased levels of TILs and poorer survival.^{67–69} Programmed death ligand 1 (PD-L1) expression appears to correlate with the presence of *KRAS* mutations^{70–73} although this observation has to be considered with regard to both the diversity of the methods used to evaluate PD-1/PD-L1 expression and the heterogeneity of the *KRAS* mutant lung cancer population. The immune effects of Ras signaling may be better understood given the recently reported role of tristetrarolin (TTP) in stabilizing PD-L1 mRNA. In instances where Ras signaling is constitutively enhanced, the activity of AU-rich element-binding protein TTP is decreased resulting in stabilization of PD-L1 mRNA and increased PD-L1 expression.⁷⁴ Approximately 40%–50% of *KRAS* mutant lung cancers are concurrently *TP53* or *STK11* mutant – a subgroup characterized by greater immune infiltrate and somatic mutation burden.^{75,76} However, whether this can be used as a predictive biomarker for PD-1/PD-L1 inhibition is unknown and will need to wait till this has been assessed in a prospective study. Overall, the available data suggest that *RAS* activation represents a non-cell autonomous mechanism that mediates tumor development and modulates efficacy of antitumor immunity and argues for another possible role for MEK inhibitors in *HRAS/KRAS/NRAS* mutant tumors in combination with PD-1 blockade. Although multiple clinical trials are testing the efficacy of the PD-1/MEK inhibitor combination in multiple malignancies including tumors known to harbor *NRAS* mutations (melanoma – NCT01940809; CRC – NCT03374254; advanced malignancies – NCT03182673 and NCT02586987), other than a single study in *KRAS* mutant lung cancer (NCT03004105), these studies are not focused on malignancies in which Ras signaling is overactive through one or more mechanisms.

MEK inhibitors: clinical development

The MEK protein is a typical kinase with an adenosine triphosphate (ATP) binding pocket that toggles between catalytically active (phosphorylated) and catalytically inactive (unphosphorylated) states. Three types of MEK inhibitors have been developed: ATP-competitive (type I) and ATP noncompetitive (types II and III).^{77,78} Type I inhibitors occupy the ATP-binding pocket, while type II inhibitors

bind to a binding site that includes the ATP-binding pocket and an adjacent less-conserved allosteric site. However, both type I and type II inhibitors are equally nonselective. Type III inhibitors occupy highly specific allosteric sites and are more selective.

PD098059

The first MEK inhibitor to be disclosed was PD098059. Unlike earlier agents that had variably inhibited both RAF and MEK, PD098059 was a non-ATP competitive MEK inhibitor that inhibited MEK in vitro at micromolar concentrations (half-maximal inhibitory concentration [IC₅₀] 2 μM) without inhibiting RAF.⁷⁹ In vitro testing clearly established that PD098059 inhibited MAPK activation, inhibited cell growth, and reversed phenotype of ras-transformed cells.^{80,81} However, the evaluation of PD098059 in Raf/MEK signaling cancer was truncated by data that showed that PD098059 while effectively inhibiting MEK signaling paradoxically promoted ERK1/2 phosphorylation through an Raf-1-independent/MEK-dependent mechanism mediated by reactive oxygen species.⁸² This suggested that PD098059 may not completely ablate MEK activity and may have differential activity dependent on the redox state of the target cell, and resulted in the truncation of clinical development.

Selumetinib (AZD6244,ARRY-142886)

Selumetinib (AZD6244) is a potent, highly selective inhibitor of MEK1/2 (IC₅₀ 10–14 nM) and ERK1/2 phosphorylation (IC₅₀ 10 nM). Preclinical data demonstrated activity of AZD6244 in both in vitro and human tumor mouse xenograft models of non-small-cell lung cancer (NSCLC) and melanoma.^{32,83,84} Dose-finding studies studied two separate formulations including an oral free-base suspension and a hydrogen sulfate (Hyd-Sulfate) salt formulation.^{85,86} Both studies identified separate maximal tolerated doses (MTD): 100 mg twice daily (oral free-base suspension) and 75 mg twice daily (Hyd-Sulfate). Dose-finding studies also evaluated the combination of selumetinib with cetuximab,⁸⁷ erlotinib,⁸⁸ sorafenib,⁸⁹ cisplatin/gemcitabine,⁹⁰ docetaxel,^{91,92} dacarbazine,^{93,94} and platinum-based chemotherapy⁹⁵ in a variety of tumors.

Subsequent Phase II studies across a variety of histologies were largely negative except in *BRAF* mutated cutaneous melanoma and uveal melanoma. In a Phase II study of uveal melanoma patients who were randomized to either selumetinib or chemotherapy, selumetinib modestly improved progression-free survival (PFS) although overall

survival (OS) was not improved and selumetinib was associated with considerable toxicity.⁹⁶ In *BRAF* mutant melanoma, responses observed were restricted to patients with tumors that expressed low levels of phosphorylated AKT (pAKT),⁹⁷ confirming the effect of selumetinib and vemurafenib observed in low pAKT cell lines.⁹⁸ Signaling through the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway has been implicated in intrinsic resistance to MEK (and *BRAF*) inhibitor therapy,^{39,99} and co-blockade of MEK and AKT was reported to be additive in *KRAS* mutant tumor cell lines and in vivo model systems¹⁰⁰ – providing a rationale to target both MEK and PI3K/AKT in tumors driven by oncogenic RAS signaling. Unfortunately, studies testing selumetinib in combination with AKT inhibitor MK-2206 in *KRAS* mutant cancers,¹⁰¹ *KRAS* mutant CRC,¹⁰² and *KRAS* mutant pancreatic cancer¹⁰³ yielded negative results – likely due to inability to dose-escalate selumetinib and MK-2206 to doses capable of eliciting sufficient pERK/pAKT inhibition.

More recently, selumetinib has been studied in nonmalignant conditions characterized by elevated MAPK signaling such as neurofibromatosis type 1-related plexiform neurofibromas. MTD of 25 mg/m² was ~60% of the adult dose with promising efficacy and relatively tolerable toxicity profile. Separately, based on observations linking MAPK and PI3K-AKT-mTOR pathway activity to lower tumor immune infiltrates,⁶⁵ a study of selumetinib in combination with PD-1 and CTLA-4 immune checkpoint inhibitors has been launched (NCT02586987).

Trametinib (GSK1120212)

Trametinib is an allosteric, non-ATP-competitive inhibitor of both MEK1 (IC₅₀ 0.7 nM) and MEK2 (IC₅₀ 0.9 nM) kinases with excellent efficacy and high activity in preclinical studies.^{104–106} Following initial dose-finding studies, the clinical evaluation of trametinib centered around *BRAF* mutant tumors and melanoma in particular.

In melanoma, trametinib monotherapy was initially compared to chemotherapy (dacarbazine or paclitaxel) in the Phase III METRIC study of *BRAF*^{V600E/K} mutant melanoma that accrued 322 patients in a 2:1 ratio.¹⁰⁷ Trametinib monotherapy resulted in an overall response rate (ORR) of 22% and improved PFS and OS (summarized in Table 1). These data resulted in the FDA approval of trametinib to treat *BRAF*^{V600E/K} mutant unresectable or metastatic melanoma in May 2013.

Following a successful open-label Phase III trial against dacarbazine in *BRAF*^{V600E/K} mutant melanoma, dabrafenib

independently garnered FDA approval in May 2013.⁴⁴ The dabrafenib/trametinib combination was studied in two separate placebo-controlled Phase III trials against dabrafenib (COMBI-d) and vemurafenib (COMBI-v) (summarized in Table 1). In both studies, dabrafenib/trametinib combination had greater ORR (COMBI-d: 67%; COMBI-v: 76%) and median PFS (COMBI-d: 9.3 months; COMBI-v: 11.4 months) with improved OS compared to *BRAF* inhibitor monotherapy.^{59,60} The significant improvement observed resulted in FDA approval for the dabrafenib/trametinib combination to treat *BRAF*^{V600E/K} mutant melanoma in January 2014.

Given the success of dabrafenib/trametinib in treating metastatic patients, its use in the high-risk resected setting has been explored in stage III patients.¹⁰⁸ In a blinded Phase III trial of dabrafenib/trametinib against matched placebo in stage III A–C *BRAF*^{V600E/K} mutant melanoma (COMBI-AD), risk of relapse was 53% lower in the treatment group (hazard ratio for relapse or death, 0.47). Relapse-free survival rates were similarly greater in the treatment group: 1-year 88% (vs 56%), 2-year 67% (vs 44%), and 3-year 58% (vs 39%). The combination was granted breakthrough therapy status.

Given possible synergies between *BRAF*/MEK inhibition with PD-1 inhibitors, the triple combination of dabrafenib, trametinib, and pembrolizumab has been studied in *BRAF*^{V600E/K} mutant melanoma. A Phase I/II study of the triple combination (KEYNOTE-022) reported ORR of 67% with no toxicity signals.¹⁰⁹ A Phase III trial (COMBI-i) is comparing the triple combination of dabrafenib, trametinib, and investigational PD-1 inhibitor PDR001 to dabrafenib, trametinib, and placebo (NCT02967692).

Cobimetinib (GDC-0973, XL518)

Cobimetinib is a potent and highly selective inhibitor of MEK1 (IC₅₀ 0.9 nM) with greater than 100-fold selectivity for MEK1 over MEK2 (IC₅₀ 199 nM). Preclinical data demonstrated broad in vivo efficacy in xenograft models utilizing *BRAF* or *KRAS* mutant cell lines.¹¹⁰ Unlike trametinib, cobimetinib was not evaluated as a single agent but rather in combination with vemurafenib in *BRAF* mutant melanoma.

In melanoma, cobimetinib was initially evaluated in the Phase III coBRIM study that compared vemurafenib/cobimetinib combination with vemurafenib/placebo in 495 *BRAF*^{V600E} mutant melanoma patients (summarized in Table 1). As expected, combination therapy resulted in greater ORR (68% vs 45%), PFS (9.9 months vs 6.2 months), and OS

Table 1 NRAS inhibitors in the treatment of metastatic cutaneous melanoma

Study (reference)	Patient population and primary endpoint(s)	Intervention(s)	Number of patients	ORR (%)	mPFS (months) (95% CI)	mOS (months) (95% CI)	Grade 3/4 adverse events
Trametinib							
METRIC ⁰⁷	Metastatic melanoma with V600E or V600K BRAF mutation Primary endpoint(s): PFS	2:1 randomization to: • Trametinib 2 mg daily • Intravenous dacarbazine 1,000 mg/m ² or paclitaxel 175 mg/m ² every 3 weeks Patients in the chemotherapy arm were permitted to switch to trametinib arm if they had disease progression	322	Trametinib: 22% Chemotherapy: 8%	Trametinib: 4.8 months Chemotherapy: 1.5 months HR 0.45, 95% CI, 0.33–0.63	At 6 months: Trametinib: 81% Chemotherapy: 67% HR for death: 0.54, 95% CI, 0.32–0.91	All: • Trametinib: 15% • Chemotherapy: 15% Rash: • Trametinib: 8% • Chemotherapy: 0% Hypertension: • Trametinib: 12% • Chemotherapy: 3% Fatigue: • Trametinib: 4% • Chemotherapy: 3% Vomiting: • Trametinib: 1% • Chemotherapy: 2%
COMBI-d ⁵⁹	BRAF V600E or V600K mutated, previously untreated, advanced melanoma Primary endpoint(s): OS	1:1 randomization to: • Dabrafenib 150 mg twice daily + trametinib 2 mg daily • Dabrafenib + placebo	423	Dabrafenib + trametinib: 69% Dabrafenib + placebo: 53%	Dabrafenib + trametinib: 11 months (8.0–13.9) Dabrafenib + placebo: 8.8 months (5.9–9.3) HR 0.56 (95% CI, 0.46–0.69)	All: • Dabrafenib + trametinib: 48% • Dabrafenib+ placebo: 50% Pyrexia: • Dabrafenib + trametinib: 59% • Dabrafenib + placebo: 25% Fatigue: • Dabrafenib + trametinib: 29% • Dabrafenib + placebo: 37% Nausea: • Dabrafenib + trametinib: 36% • Dabrafenib + placebo: 27% Cutaneous SCC: • Dabrafenib + trametinib: 4% • Dabrafenib + placebo: 12%	
COMBI-v ⁶⁰	BRAF V600E or V600K mutated, previously untreated, advanced melanoma Primary endpoint(s): OS	1:1 randomization to: • Dabrafenib 150 mg twice daily + trametinib 2 mg daily • Vemurafenib 960 mg twice daily	704	Dabrafenib + trametinib: 64% Vemurafenib: 51%	Dabrafenib + trametinib: 11.4 months Vemurafenib: 7.3 months HR 0.61, 95% CI, 0.52–0.73	All: • Dabrafenib + trametinib: 52% • Vemurafenib: 63% Pyrexia: • Dabrafenib + trametinib: 53% • Vemurafenib: 21%	

<p>Cobimetinib coBRIM^{61,111}</p>	<p>BRAF V600 E/K positive disease previously untreated</p> <p>1:1 randomization to:</p> <ul style="list-style-type: none"> Cobimetinib (60 mg daily, 21 days on/7 days off) + vemurafenib 960 mg twice daily Vemurafenib 960 mg twice daily + placebo 	<p>495</p>	<p>Cobimetinib + vemurafenib: 70% Vemurafenib + placebo: 50%</p> <p>Cobimetinib + vemurafenib: 12.3 months Vemurafenib + placebo: 7.3 months</p> <p>Cobimetinib + vemurafenib: 22.3 months Vemurafenib + placebo: 17.4 months</p>	<p>Rash:</p> <ul style="list-style-type: none"> Dabrafenib + trametinib: 22% Vemurafenib: 43% <p>Nausea:</p> <ul style="list-style-type: none"> Dabrafenib + trametinib: 35% Vemurafenib: 36% <p>Arthralgia:</p> <ul style="list-style-type: none"> Dabrafenib + trametinib: 24% Vemurafenib: 51% <p>All:</p> <ul style="list-style-type: none"> Cobimetinib + vemurafenib: 62% Vemurafenib + placebo: 58% <p>Diarrhea:</p> <ul style="list-style-type: none"> Cobimetinib + vemurafenib: 6% Vemurafenib + placebo: 0% <p>Cutaneous SCC:</p> <ul style="list-style-type: none"> Cobimetinib + vemurafenib: 2% Vemurafenib + placebo: 11% <p>Keratoacanthoma:</p> <ul style="list-style-type: none"> Cobimetinib + vemurafenib: 1% Vemurafenib + placebo: 18%
<p>Binimetinib NEMO¹¹⁸</p>	<p>NRAS mutated melanoma previously untreated, or progressed on immunotherapy</p> <p>Primary endpoint(s): PFS</p> <p>2:1 randomization to:</p> <ul style="list-style-type: none"> Binimetinib 45 mg twice daily Dacarbazine 1,000 mg/m² intravenously every 3 weeks 	<p>402</p>	<p>Binimetinib: 15% (11%–20%) Dacarbazine: 7% (3%–13%)</p> <p>Binimetinib: 2.8 months (2.8–3.6) Dacarbazine: 1.5 months (1.5–1.7) HR 0.62 (95% CI, 0.47–0.8)</p>	<p>All:</p> <ul style="list-style-type: none"> Binimetinib: 5% Dacarbazine: 5% <p>Hypertension:</p> <ul style="list-style-type: none"> Binimetinib: 7% Dacarbazine: 2% <p>Anemia:</p> <ul style="list-style-type: none"> Binimetinib: 2% Dacarbazine: 5% <p>Neutropenia:</p> <ul style="list-style-type: none"> Binimetinib: 1% Dacarbazine: 9% <p>All:</p> <ul style="list-style-type: none"> Encorafenib + binimetinib: 58%
<p>COLUMBUS¹¹⁹</p>	<p>Locally advanced or metastatic melanoma with BRAF V600 mutation</p> <p>1:1 randomization to:</p> <ul style="list-style-type: none"> Encorafenib 450 mg daily + binimetinib 45 mg twice daily 	<p>921</p>	<p>Encorafenib + binimetinib: 63% Encorafenib: 51% Vemurafenib: 40%</p> <p>Encorafenib + binimetinib: 14.9 months (11.0–18.5)</p> <p>Not reported</p>	<p>All:</p> <ul style="list-style-type: none"> Encorafenib + binimetinib: 58%

(Continued)

Table 1 (Continued)

Phase III studies of MEK inhibitors in advanced melanoma							
Study (reference)	Patient population and primary endpoint(s)	Intervention(s)	Number of patients	ORR (%)	mPFS (months) (95% CI)	mOS (months) (95% CI)	Grade 3/4 adverse events
	Prior therapy with immunotherapy was allowed Primary endpoint(s): PFS	<ul style="list-style-type: none"> Encorafenib 300 mg daily Vemurafenib 960 mg twice daily 			Encorafenib: 9.6 months (7.5–14.8) Vemurafenib: 7.3 months (5.6–8.2) HR for combination vs encorafenib: 0.68 (95% CI, 0.52–0.90) HR for combination vs vemurafenib: 0.68 (95% CI, 0.52–0.90)		<ul style="list-style-type: none"> Encorafenib: 66% Vemurafenib: 63% Nausea: <ul style="list-style-type: none"> Encorafenib + binimetinib: 41% Encorafenib: 39% Vemurafenib: 34% Pyrexia: <ul style="list-style-type: none"> Encorafenib + binimetinib: 18% Encorafenib: 15% Vemurafenib: 28% Fatigue: <ul style="list-style-type: none"> Encorafenib + binimetinib: 29% Encorafenib: 25% Vemurafenib: 31% Rash: <ul style="list-style-type: none"> Encorafenib + binimetinib: 14% Encorafenib: 21% Vemurafenib: 29%

Abbreviations: HR, hazard ratio; mPFS, median progression-free survival; mOS, median overall survival; ORR, overall response rate; SCC, squamous-cell carcinoma.

(22.3 months vs 17.4 months) compared to vemurafenib monotherapy,^{61,111} resulting in FDA approval for the combination in November 2015.

Concordantly with the development of dabrafenib/trametinib, cobimetinib has also been evaluated alongside PD-1 inhibitors across a variety of histologies. Separate Phase Ib studies evaluated the cobimetinib/atezolizumab combination in microsatellite stable (MSS) CRC and *BRAF* WT melanoma with ORR of 17% (MSS CRC) and 45% (melanoma).¹¹² Further evaluation in MSS CRC is ongoing in the randomized Phase II ATELIER study (NCT03340558) that is comparing the cobimetinib/atezolizumab combination with atezolizumab monotherapy in CRC with liver metastases. In melanoma, the triple combination of vemurafenib, cobimetinib, and atezolizumab was initially studied in a Phase Ib study in *BRAF*^{V600} mutant melanoma with encouraging results (85% ORR). These results will be formally assessed in the Phase III TRILOGY trial of the triple combination compared with vemurafenib/cobimetinib/placebo (NCT02908672).

Binimetinib (MEK162, ARRY-162, ARRY-438162)

Binimetinib is an orally bioavailable, highly selective, ATP noncompetitive allosteric MEK inhibitor that was initially discovered by Array BioPharm., and subsequently codeveloped by Array BioPharm. and Novartis. Binimetinib inhibits MEK1/2 at nanomolar concentrations (IC₅₀ 12 nM) with significant antitumor activity in preclinical studies involving cell lines and murine xenograft models.^{113,114}

In a Phase I study of 93 patients, 19 patients with a variety of solid tumors were enrolled during dose escalation with a further 74 patients during dose expansion.¹¹⁵ The expansion cohort primarily enrolled patients with gastrointestinal malignancies including biliary cancer (28 patients) and CRC (31 *KRAS* mutant and 15 *BRAF* mutant patients). MTD was 60 mg twice daily although after greater than expected incidence of ocular toxicities was observed at MTD during dose-expansion phase, 45 mg twice daily was selected as the recommended Phase II dose (RP2D). Common adverse events (AEs) across all dose levels included rash (81%), nausea (56%), vomiting (52%), diarrhea (51%), peripheral edema (46%), and fatigue (43%), with the most common grade 3 events including anemia (11%), abdominal pain (4%), and dehydration (4%). The incidence of grade 4 AEs was low at 6% with no grade 5 AE reported. AE-related treatment discontinuation occurred in 15% (60 mg twice daily) to 25% (80 mg twice daily) of patients, most commonly due

to ocular events. Of the 26 patients with evaluable biliary cancer, one complete response and one partial response were observed. A separate Phase I study in Japanese patients also identified 45 mg twice daily as RP2D.¹¹⁶

Binimetinib at 45 mg twice daily monotherapy was subsequently evaluated in a Phase II study of 71 patients with *NRAS* (30 patients) or *BRAF*^{V600E/K} (41 patients) mutant melanoma.¹¹⁷ Most patients had previously been treated with either chemotherapy or cytokine-based immunotherapy, but relatively few had received prior immune checkpoint inhibitors (30% of *NRAS* mutant; 15% of *BRAF* mutant).¹¹⁷ Although no complete responses were observed, ORR was 11% in *NRAS* mutant patients and 5% in *BRAF* mutant patients. Latter was substantially lower than the 22% ORR observed in the METRIC study with trametinib in *BRAF* mutant melanoma although 17% of these had received prior BRAF inhibitor therapy.^{107,117} Toxicity profile was relatively favorable with relatively low incidence of grade 3/4 AEs except for serum creatine phosphokinase elevations (23% of *NRAS* mutant; 17% of *BRAF* mutant). The incidence of ocular events was low (27% of *NRAS* mutant; 12% of *BRAF* mutant) with no grade 3/4 events.

Following Phase II data, separate Phase III studies in metastatic NEMO and *BRAF*^{V600E/K} mutant melanoma (COLUMBUS) were launched (data summarized in Table 1). In NEMO, binimetinib monotherapy was compared with dacarbazine.¹¹⁸ Binimetinib resulted in ORR 15% with median PFS 2.8 months that compared favorably to dacarbazine (ORR 7%, median PFS 1.5 months). Although statistically significant, these results have to be interpreted within the context of a relatively good-risk patient population (69% normal lactose dehydrogenase) with minimal prior exposure to effective immune checkpoint inhibitor therapy (prior ipilimumab 13%; prior PD-1/PD-L1 6%). Although a new drug application (NDA) for binimetinib was filed for this indication, this was subsequently withdrawn based on feedback from the FDA during a preplanned review meeting.

In COLUMBUS, binimetinib combined with BRAF inhibitor encorafenib was compared with both vemurafenib and encorafenib separately.¹¹⁹ Encorafenib/binimetinib combination was unsurprisingly better than either encorafenib or vemurafenib monotherapy with 63% ORR (encorafenib 51%; vemurafenib 40%) and median PFS of 14.9 months (encorafenib 9.6 months; vemurafenib 7.3 months). Although the ORR in the combination arm was similar to the dabrafenib/trametinib and vemurafenib/cobimetinib combinations, the median PFS of encorafenib/binimetinib was greater than what was observed with the BRAF/MEK inhibitor combination

arms in COMBI-d, COMBI-v, and coBRIM.^{59–61} Incidence of grade 3/4 AEs was lower in the encorafenib/binimetinib group (58%) than in either encorafenib (66%) or vemurafenib (63%) group. Concordantly, the treatment discontinuation rate of the combination was lower than in either monotherapy arm. An NDA for the use of encorafenib/binimetinib in this indication has been submitted to the FDA, results of which are pending.

The further development of binimetinib is along a similar trajectory to that described for trametinib and cobimetinib. Combinations with PD-1/CTLA-4 immune checkpoint inhibitors (NCT03271047 and NCT03374254 – MSS CRC); CDK4/6 inhibitor palbociclib in *KRAS* mutant NSCLC (NCT03170206); MET inhibitor PF-02341066 in *RAS* mutant/WT CRC with aberrant C-MET signaling (NCT02510001); chemotherapy in *KRAS* mutant NSCLC (NCT02185690), CRC (NCT02928224, NCT02613650), and biliary tract cancer (NCT02773459); erlotinib in *KRAS*/EGFR mutant NSCLC (NCT01859026); imatinib in gastrointestinal stromal tumors (NCT01991379); and as monotherapy in NF1 plexiform neurofibromas (NCT03231306).

Conclusion

Following the initial approval of trametinib to treat *BRAF* mutant melanoma in MEK inhibitors in May 2013, the clinical development of MEK inhibitors has proceeded rapidly. Initial success in *BRAF* mutant melanoma in combination with BRAF inhibitors has been parlayed into other indications where BRAF is an oncogenic driver including NSCLC¹²⁰ and papillary/anaplastic thyroid cancer.¹²¹

Separately, preclinical studies have demonstrated that MEK inhibitors either singly or in combination with BRAF inhibitors exert immunomodulatory effects on the tumor and the TME and may enhance immune activation.^{65,66} These observations are buttressed by clinical trials demonstrating efficacy of the PD-1/MEK inhibitor combination in CRC and *BRAF* WT melanoma while the PD-1/MEK/BRAF inhibitor triple combination has produced startling response rates in early-phase studies, although confirmatory studies are ongoing.

Finally, data regarding the clinical benefit of dose-adjusted selumetinib in treating children and adults with highly symptomatic plexiform neurofibromas associated with neurofibromatosis type I, a common genetic tumor syndrome caused by germline mutations in NF1, are encouraging. These reports argue for expanding the role of NRAS inhibitors beyond traditional oncologic indications to RASopathies and other conditions associated with dysregulation of the RAS/MAPK pathway.

Acknowledgment

The authors have not received any funding or benefits from the industry to conduct this review.

Disclosure

Diwakar Davar has received the following funding: Checkmate Pharmaceuticals, Merck, Bristol-Myers Squibb (research support). The other author reports no conflicts of interest in this work.

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