CLINICAL INVESTIGATION

Advances in targeted therapy for melanoma: a focus on MEK inhibition

Increased knowledge of the role that dysregulated MAPK signaling plays in melanoma has opened the door for novel therapeutic strategies, among them MEK-targeted approaches. With the recent approval of the selective MEK inhibitor trametinib, both as a single agent and in combination with dabrafenib for patients with *BRAF*-mutant metastatic melanoma, MEK-based therapy now has a place as a standard of care option for the treatment of this disease. Early data suggest a benefit of MEK inhibition in other melanoma subtypes as well, including *NRAS*-mutant and uveal melanoma. This review summarizes the clinical development and the currently available data regarding the role of MEK inhibitor therapy in the treatment of advanced melanoma.

Keywords: BRAF • MAPK pathway • MEK • melanoma • NRAS • targeted therapy • uveal melanoma

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While there have been numerous advances in systemic therapy for the treatment of metastatic melanoma in recent years, for most patients it remains an incurable disease [1]. Notably, since 2011, there have been US FDA approvals for four new agents used to treat metastatic disease, three of which – vemurafenib (PLX4032; RG7204), dabrafenib (GSK2118436) and trametinib (GSK1120212) – target aberrant MAPK pathway signaling. The identification of mutated *BRAF* as a major driver of oncogenesis in approximately half of all melanomas, and the resultant successful clinical development of selective BRAF inhibitors has opened the door for novel agents that target this pathway [2–4]. Among these are MEK targeted therapies, and while MEK inhibitors have been in development for some time, recent evidence of early clinical activity in many tumor types, including thyroid and ovarian cancer, as well as specific subsets of melanoma has provided a platform for the continued expansion of possible treatment options [5,6].

The RAS-RAF-MEK signaling cascade is an integral component in regulating cell growth and proliferation, and represents an attractive target for therapeutic intervention [7]. First identified in a screen of cell lines and melanoma specimens in 2002, the majority of *BRAF* mutations in melanoma result from the substitution of a glutamic acid for a valine (V600E), though other variants exist [2]. It is now known that this change results in a constitutively active kinase with resultant persistent downstream activation of the MAPK pathway [8]. The subsequent development of the selective BRAF inhibitors vemurafenib and dabrafenib represented a landmark breakthrough in the treatment of metastatic melanoma [3,4]. Vemurafenib, the first molecular targeted agent to receive regulatory approval in 2011, has demonstrated an overall survival advantage in a Phase III study when compared with chemotherapy in patients with $BRAF^{V600E}$ melanoma [3,9]. Dabrafenib has also been shown to improve progression-free survival (PFS) when compared with dacarbazine in a randomized Phase III trial [4]. High response rates coupled with the potential for a relatively rapid response make these agents particularly useful for patients with symptomatic or rapidly progressive disease. How-

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ever, like many systemic agents for the treatment of advanced cancer, limitations exist due to the emergence of resistance as well as toxicity. On average, responses with BRAF inhibitor monotherapy last 6–7 months, and most patients relapse within a year of starting treatment [4,9–11]. The development of resistance is a multifactorial process, though MAPK reactivation through different mechanisms appears to play a key role [12–21].

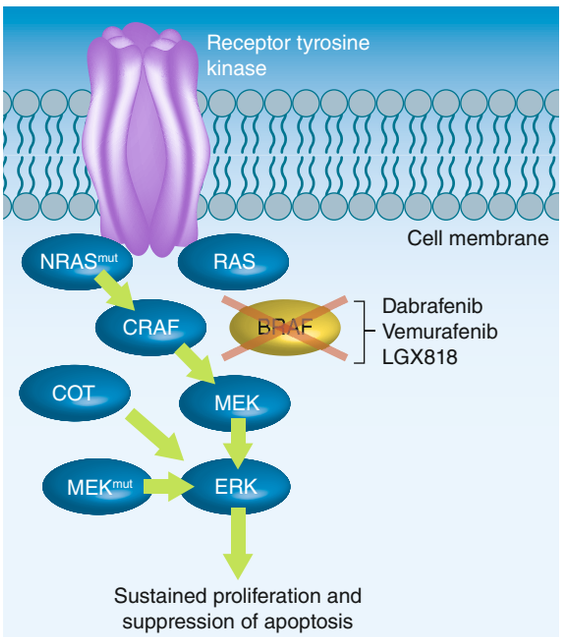

Figure 1. Potential mechanisms of resistance to selective BRAF inhibition that are dependent on MAPK pathway activation/MEK signaling.

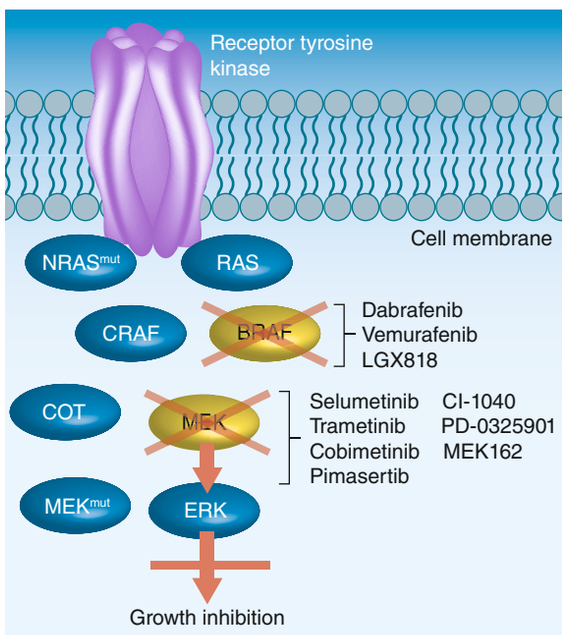

Figure 2. MEK targeted strategies provide a mechanism to overcome selected mediators of resistance.

Figures 1 & 2 highlight some of the potential mediators involved in this process. Additionally, treatment with these agents is uniquely associated with the development of hyperproliferative skin lesions, including squamous cell carcinomas (SCCs), largely attributed to persistent MAPK signaling through RAS mediated paradoxical activation of other RAF isoforms [22].

The hypothesis that MEK inhibition in melanoma has the potential to have a meaningful clinical benefit has now come full circle. In 2013, the MEK inhibitor trametinib was approved as monotherapy for the treatment of patients with metastatic $BRAF^{V600}$ melanoma, and the approval of its use in combination with dabrafenib in this same population came in early 2014. Subsequent studies have continued to build upon the understanding of the molecular pathogenesis of melanoma. In addition to having a role in *BRAF*-mutated melanoma, it is also becoming apparent that other distinct subsets, including *NRAS* mutated and uveal melanoma may also be susceptible to targeting this pathway. This review aims to summarize the history of MEK inhibitor development in melanoma, the current role in clinical practice as well as future therapeutic strategies.

Early MEK inhibitor development

As the MAPK pathway plays an integral role in regulating cell growth and proliferation, there has long been an interest in targeting this pathway for therapeutic benefit. One of the first MEK inhibitors to enter clinical testing included the non-ATP-competitive MEK 1/2 inhibitor CI-1040. CI-1040 is highly selective for MEK, and acts to form a bond at a hydrophobic binding pocket on MEK 1/2. This induces a conformational change in unphosphorylated MEK, and essentially locks the protein in its inactive state [23] An initial dose escalation study that included patients with a variety of solid tumors, including melanoma, established that CI-1040 could be safely administered, but subsequent studies failed to demonstrate clinical efficacy [23,24]. PD-0325901 is a second-generation MEK inhibitor that had a more favorable pharmacokinetic profile than CI-1040, and demonstrated promising preclinical activity [25,26]. In early phase testing, there was some signal of potential clinical activity with a few partial responses (PRs) and stable disease seen in some of the melanoma patients that were treated [27,28]. However, there were a number of concerning toxicities noted, including neurologic effects as well as retinal vein occlusion. With this concern, development of this agent was subsequently suspended.

Selumetinib

Selumetinib (ARRY-142886; AZD-6244) is a second-generation, highly selective MEK 1/2 inhibitor that

has recently shown some promising results in later phase clinical trials for patients with metastatic melanoma, particularly with a newer formulation of the drug. Initial preclinical work and molecular modeling data showed that selumetinib was able to bind the allosteric inhibitor site on MEK 1/2, rendering it catalytically inactive. Early phase testing of the free base formulation of selumetinib established 100 mg b.i.d. as the dose to pursue in subsequent studies, largely due to cutaneous toxicity seen at higher doses [29]. However, in a Phase II study using the initial formulation of the drug in which patients with advanced melanoma were randomized to receive either selumetinib or temozolomide, response rates were similar and there was no improvement in PFS in the selumetinib arm [30]. In this study, median progression free survival (PFS) was only 78 days and the response rate was 5.8% for those who received selumetinib treatment, and even among patients with *BRAF* mutant melanoma, the response rate was 11%. Subsequent studies using a hydrogen sulfate formulation, which appears to have a more favorable pharmacokinetic profile, have demonstrated more promising results [31]. Phase I studies have established 75 mg b.i.d. as the maximum tolerated dose (MTD); the most common side effects at this dose included fatigue, dermatitis, nausea, diarrhea and peripheral edema. A retrospective analysis of a Phase I study in which patients with metastatic melanoma were treated at a variety of doses using the new formulation suggested that patients with *BRAF*-mutated melanoma had higher response rates and longer PFS when compared with patients with *BRAF* wild-type melanoma [32]. Additionally, recent results from a randomized Phase II study of selumetinib plus dacarbazine versus dacarbazine in patients with *BRAF*-mutated melanoma showed a significant increase in PFS that favored the selumetinib containing arm (5.6 vs 3 months (hazard ratio [HR]: 0.63; 80% CI: 0.47–0.84; p = 0.021), though overall survival (OS) was not significantly different between the two groups [33].

Newer MEK inhibitors

Trametinib

Trametinib (GSK1120212) is among the newer MEK targeted agents, and was the first to receive regulatory approval for treatment of patients with melanoma harboring a $BRAF^{V600}$mutation, first as a single agent and most recently for use in combination with the selective BRAF inhibitor dabrafenib. Trametinib is a highly selective allosteric inhibitor of MEK1/2, and preclinical models demonstrated that *BRAF* mutant melanomas may be more sensitive to this agent, suggestive of a potential benefit in this cohort of patients [34]. Trametinib binds MEK 1/2 at the same site as earlier generation MEK inhibitors, but it is unique in that it has a favorable mean peak:trough ratio, making once daily dosing feasible [34,35]. Initial clinical trials with trametinib yielded promising results, and established 2 mg daily as the recommended dose to move forward in subsequent studies on the basis of pharmacokinetic, pharmacodymic, long-term safety and preliminary efficacy data although the MTD administered was 3 mg daily [35]. Multiple tumor types were treated in this Phase I study, with an overall response rate of approximately 10%, however, *BRAF* mutant melanoma appeared to be a particularly sensitive subset. A total of 97 patients with melanoma were enrolled, 36 of which had *BRAF* mutant tumors [36]. In a separate analysis of the cohort of melanoma patients, the 30 patients with *BRAF* mutant disease that had never received BRAF inhibitor therapy had an overall unconfirmed response rate (complete response (CR) + PR) of 40%, with a median PFS of 5.7 months [36]. Additionally, of the six patients with *BRAF* mutant melanoma that had previously received a BRAF inhibitor, one had a PR and four had stable disease. Overall, trametinib appeared to be well tolerated, with the most common adverse events being cutaneous toxicity in the form of rash/dermatitis and diarrhea. Of note, no cutaneous SCCs were seen. A two arm, Phase II study of 97 patients with *BRAF* mutated metastatic melanoma further supported these early results. In this study, patients who had previously received a BRAF inhibitor as well as those that were naïve to BRAF inhibitor therapy received trametinib 2 mg once daily. [37]. The 57 patients in the cohort that were naïve to BRAF inhibitor therapy had a confirmed response rate of 25%, with 51% having stable disease [37]. In contrast, there were no objective responses in the cohort of 40 patients that had been previously treated with a BRAF inhibitor, and only 28% of patients had stable disease [37]. PFS was 4 months in the BRAF inhibitor naïve group and was 1.8 months in the previously treated cohort [37]. The METRIC study, a randomized Phase III trial, has now confirmed the benefit of trametinib in patients with *BRAF* mutant melanoma [38]. In this study, 322 patients with $BRAF^{V600E/K}$ melanoma were randomized 2:1 to receive either trametinib or cytotoxic chemotherapy (dacarbazine or paclitaxel); patients originally randomized to receive chemotherapy were allowed to cross over to the trametinib arm at the time of disease progression. Patients that had previously been treated with a BRAF inhibitor, MEK inhibitor or ipilimumab were excluded. Median PFS was significantly improved in the trametinib arm, and was 4.8 months compared with 1.5 months in the chemotherapy group (HR: 0.45; 95% CI: 0.33–0.63; p < 0.001) [38]. While final OS analyses have not yet been reported, the 6 month

OS in the trametinib group was 81% versus 67% in the chemotherapy arm despite crossover [38]. As with the earlier studies of this agent, toxicities appeared to be manageable and no cutaneous SCCs were observed. The FDA subsequently granted regulatory approval for trametinib as a single agent for the treatment of unresectable or metastatic $BRAF^{V600E/K}$ melanoma in May 2013 based on the results of this study.

Cobimetinib

Cobimetinib (GDC-0973) is a potent, selective, noncompetitive inhibitor of MEK1 which has demonstrated clinical activity as a single agent and has also shown promise when used in combination with other therapies. Preclinical data suggested favorable pharmacodynamic properties, in that inhibition of p-ERK in tumors persisted after cobimetinib was no longer detectable in plasma [39]. Initial Phase I testing showed that cobimetinib was well tolerated as a single agent, and some responses were seen in patients with $BRAF^{V600}$ melanoma [40]. Subsequent studies have focused on using cobimetinib in combination, including vemurafenib and the PI3K inhibitor GDC-0941.

MEK 162

MEK 162 is a selective non-ATP-competitive inhibitor of MEK1 and MEK2. Preclinical data suggested that MEK162 inhibited growth of NRAS as well as BRAF driven tumorigenesis [41]. In a Phase I trial of 19 patients with advanced solid tumors, the MTD was established as 60 mg daily. MEK 162 appeared to have an acceptable safety profile consistent with other agents in this class [42]. A subsequent Phase II study in patients with *NRAS*- or *BRAF*-mutated melanoma showed further evidence of clinical activity [43]. Safety and efficacy data has been reported on two of the three cohorts, including the *BRAF* and *NRAS* mutated cohorts treated at 45 mg b.i.d. Patients were allowed to have had prior treatment with a BRAF inhibitor, but prior MEK targeted therapies were excluded. In the 41 patients included in the BRAF mutated cohort, 17% of patients had previously received a BRAF inhibitor. In this group, 8/41 (20%) of patients had a PR, with two confirmed [43]. Subsequent studies with MEK162 either alone or in combination are currently ongoing.

Emerging MEK inhibitors

A number of MEK inhibitors are still in the early phases of development, including pimasertib (AS703026) and E6201. E6201 is a novel ATP-competitive MEK inhibitor that is a derivative of a natural product, f152A1, that was found to be an inhibitor of TNF-α [44]. Preclinical data have suggested that cell lines that were *BRAF* mutated and *PTEN* wild-type appeared to have the highest sensitivity to E6201, suggesting a role for the PI3K pathway in mediating resistance [45]. Additionally, recent preclinical data have suggested that E6201 may have different properties than other agents in this class. In $BRAF^{V600E}$ melanoma cell lines with the acquired *MEK-C121S* mutation, known to confer resistance to both vemurafenib and selumetinib, retained their sensitivity to E6201 [46]. Currently a Phase I trial in patients with solid tumors, which includes an expansion cohort of patients with *BRAF*-mutated melanoma is currently underway (NCT00794781). Pimasertib is another selective, non-ATP-competitive inhibitor of MEK 1/2 that has entered early phase clinical testing. In a Phase I study, two confirmed PRs were seen in previously treated melanoma patients and subsequent studies are ongoing [47].

Role of MEK inhibitors in combination therapy

With the recent FDA approval of dabrafenib and trametinib for use in combination for the treatment of patients with $BRAF^{V600}$ metastatic melanoma, MEK targeted therapy is likely to have a central role in combination approaches for this patient population, rather than as single agents. While the development of selective BRAF inhibitors represents a landmark advance in the treatment of metastatic melanoma, average response duration is measured in months and resistance invariably develops. While the mechanisms leading to the development of BRAF inhibitor resistance are not completely understood, current data suggest that MAPK pathway re-activation likely plays a critical role [12–21]. With this in mind, early efforts focused on the potential synergy between dual BRAF and MEK inhibition. An early study of dabrafenib and trametinib consisted of three parts: a pharmacokinetic drug–drug interaction study, a dose escalation cohort of the combination and a randomized Phase II trial in patients with metastatic $BRAF^{V600E/K}$ melanoma. Pharmacokinetic analyses from the first phase of the study revealed no effect of trametinib on a single dose of dabrafenib [48]. In the second phase of the study, dose escalation began at a 50% dose reduction for each agent (75 mg dabrafenib b.i.d. and 1 mg of trametinib daily). A total of 24 patients were ultimately treated at the highest dose level of dabrafenib 150 mg b.i.d. and trametinib 2 mg daily [48]. At this dose level, one dose limiting toxicity of neutrophilic panniculitis was seen, but the combination was otherwise well tolerated. This was subsequently established as the recommended Phase II dose, successfully demonstrating that each drug could be administered concurrently at their

respective monotherapy doses. A total of 162 patients with metastatic *BRAF*$^{V600E/K}$-mutated melanoma were subsequently enrolled in the Phase II portion of the study, which consisted of three arms: a single agent dabrafenib arm, dabrafenib 150 mg b.i.d. plus trametinib 1 mg daily (150/1) and dabrafenib 150 mg b.i.d. plus trametinib 2 mg daily (150/2) [49]. In the 150/2 arm, median PFS was9.4 months as compared with 5.8 months in the dabrafenib alone group (HR: 0.39; 95% CI: 0.25–0.62; p < 0.001). In the 150/2 combination arm, there were five CRs (9%), and 36 (67%) PRs among 54 patients. Notably, the remaining 13 patients experienced stable disease at the first restaging evaluation, implying that the majority of patients treated with a combination approach are likely to experience some clinical benefit. Toxicity on the combination arm appeared to be manageable, though some adverse events, including pyrexia, chills, fatigue, nausea/vomiting and diarrhea appeared to be more common. Of note, the frequency of cutaneous SCCs and keratoacanthomas was reduced in the combination arm, consistent with the hypothesis that these lesions are due to paradoxical reactivation of the MAPK pathway caused by BRAF inhibition in keratinocytes that are wild-type for *BRAF* [22]. Based on these results, the FDA approved the use of dabrafenib and trametinib for use in combination for patients with *BRAF*^{V600}-mutated melanoma in January 2014. Two larger, randomized Phase III studies comparing single agent BRAF inhibitors with the combination of dabrafenib/trametinib have recently closed to accrual and will hopefully confirm the benefit seen in the previous studies (NCT01597908 and NCT01584648).

Cobimetinib has also been tested in combination with vemurafenib, and an updated analysis has yielded promising results. Data is available for 128 patients with BRAF^{V600} metastatic melanoma that have been treated, this included 65 patients who have previously progressed on vemurafenib and 63 who are BRAF inhibitor naïve [50]. Multiple dosing cohorts have been tested, and the two cohorts of cobimetinib at 60 mg daily on a 21 days on and 7 days off schedule (every 28 day cycle) in combination with vemurafenib at either 720 or 960 mg b.i.d. have been selected for expansion. In the BRAF inhibitor naïve group, the objective response rate (including both confirmed and unconfirmed) was 85% [50]. For patients who have previously received a BRAF inhibitor, the response rate was 15%, and 43% had stable disease. Median PFS for the previously treated group was 2.8 months, and with 10 months of follow-up, had not yet been reached for the BRAF inhibitor naïve group. Toxicities appeared to be consistent with this class of agents, and included rash, diarrhea, photosensitivity and arthralgias. Based on the activity observed in early studies a randomized Phase III trial comparing vemurafenib to the combination of vemurafenib and cobimetinib is being conducted and has recently completed accrual (NCT01689519). Additionally, other studies are investigating the strategy of dual BRAF + MEK inhibition, and a comparison of the MEK inhibitor MEK162 in combination with the selective BRAF inhibitor LGX818 versus BRAF inhibitor monotherapy is currently ongoing (NCT 01909453). Table 1 summarizes the current studies investigating the role of this combination strategy.

While there appears to be a clear benefit to dual BRAF + MEK inhibition in this patient population, testing of combinations targeting other pathways in conjunction with MEK has also shown promise. Among these is the PI3K/AKT/mTOR pathway, a crucial signaling cascade known to play a central role in regulating cellular proliferation, metabolism and survival [51,52]. A number of early signals have suggested that alterations in this pathway may contribute to melanoma growth [53]. Preclinical data have suggested that PI3K, along with BRAF, works to allow melanoma to escape apoptotic signals [54]. PTEN, which serves to regulate PI3K signaling under normal conditions, is lost in approximately 10% of all melanomas, and the loss of PTEN may be a contributor to resistance to BRAF inhibition [55]. *In vitro* and *in vivo* data have suggested that dual MEK/P13K inhibition has a synergistic antitumor effect in a non-small-cell lung cancer model [56]. Additionally, in a Phase II trial of selumetinib in which patients with *BRAF*-mutated melanoma were stratified based on pAKT expression, tumor regression was seen in three of the five patients in the low pAKT cohort [57]. No responses were seen in the high pAKT cohort. A number of early phase studies testing the hypothesis and the feasibility of this approach have preliminary results or are currently underway. As there is preclinical data to support MEK + PI3K inhibition, a number of combination trials have been undertaken with varying results. As an example the combination of GDC-0973 + the PI3K inhibitor GDC-0941 had been tested in different schedules, including 21 days on/7 days off, as well as 7 days on/7 days off, and was felt to be generally well tolerated. [58]. With 46 evaluable patients, there is also early evidence of some clinical activity, including PRs seen in three patients with *BRAF* mutant melanoma [58]. Another combination study with AS703026 and the dual PI3K/mTOR inhibitor SAR245409 is ongoing and has shown that continuous dosing of each agent is tolerated (NCT01390818). Preclinical data have also suggested that co-targeting IGF-1 may show synergistic activity with MEK inhibition, opening up new possibilities for combination strategies in the future [59].

Table 1. Selected studies of BRAF + MEK inhibitor combination therapy.

Study	Agents	Trial design	Patients	RR	PFS (mon)	Status	Ref.
Trials with reported results							
Flaherty *et al.*	Dabrafenib Trametinib	Three arm, randomized Phase II Dabrafenib 150 b.i.d. vs dabrafenib 150 mg b.i.d. + trametinib 1 mg daily or dabrafenib 150 mg b.i.d. + trametinib 2 mg daily	162 (total) 54 (150/2 arm)	76%	9.4	Completed accrual	[49]
McArthur *et al.*	Vemurafenib Cobimetinib	Single arm, multiple dosing cohorts (Expansion cohorts: Cobimetinib 60 mg daily 21/7 + vemurafenib 960 mg b.i.d. or 720 mg b.i.d.)	63	85% (BRAFi naïve) 15%	NR†	Completed accrual	[50]
			65	15% (prior BRAFi)	2.8		
Ongoing trials							
	Dabrafenib Trametinib	Randomized Phase III Dabrafenib 150 b.i.d. + placebo vs dabrafenib 150 mg + trametinib 2 mg daily	n/a	n/a	n/a	Completed accrual 2013	
	Dabrafenib Trametinib Vemurafenib	Randomized Phase III Vemurafenib 960 mg b.i.d. vs Dabrafenib 150 + trametinib 2 mg daily	n/a	n/a	n/a	Completed accrual 2013	
	Vemurafenib Cobimetinib	Randomized Phase III Vemurafenib 960 mg b.i.d. + placebo vs vemurafenib 960 mg b.i.d. + cobimetinib 60 mg daily	n/a	n/a	n/a	Completed accrual 2013	
	LGX818 MEK162 Vemurafenib	Three arm randomized Phase III LGX818 vs LGX818 + MEK162 vs vemurafenib	n/a	n/a	n/a	enrolling	

†With 10 months of followup, median PFS has not yet been reached for this cohort.
NR: Not reached; n/a: Not applicable; PFS: Progression-free survival; RR: Response rate.

MEK inhibition in uveal melanoma

Uveal melanoma is a rare disease, and accounts for approximately 5% of all melanomas [60]. It appears to be a genetically distinct subset from cutaneous melanoma, as *BRAF* and *NRAS* mutations are not generally found. However, it is clear that MAPK signaling still plays an important role, as nearly 80% of uveal melanomas harbor a mutation in the GTPases *GNAQ* or *GNA11*, resulting in constitutive MAPK pathway activation [61,62]. Preclinical data suggested that this activity may be susceptible to MEK inhibition [61,63], and a subset analysis of 20 uveal melanoma patients treated on a study comparing selumetinib versus temozolomide showed a trend in improved time to progression that favored the selumetinib arm [30,64]. Based on these observations, a Phase II study comparing selumetinib with temozolomide in patients with *GNAQ/11*-mutated melanoma was initiated [65]. In this study 98 patients with metastatic uveal melanoma were randomized to receive chemotherapy (temozolomide or dacarbazine) or selumetinib at 75 mg b.i.d. Patients who were initially randomized to the chemotherapy arm could cross over to receive selumetinib treatment at disease progression. Eighty-four percent of patients were found to have an exon 5 mutation in either *GNAQ* or *GNA11*. Approximately half of the patients in the selumetinib arm had evidence of tumor regression, with 15% meeting criteria for a clinical response by RECIST, as compared with only 11% with regression and no RECIST responses in the chemotherapy arm [65]. Overall, 76% of patients on the selumetinib arm achieved stable disease, with a median duration of response of 23 weeks [65]. Notably, there appeared to be less evidence of tumor regression in patients who received selumetinib in crossover, with 23% experiencing tumor regression and no confirmed responses. PFS was improved in both the overall and mutant population with selumetinib, but overall survival was not statistically different between the two arms [65]. This trial is the first study to demonstrate any improvement in clinical outcome with a systemic therapy for patients with metastatic uveal melanoma. Though the responses were not durable, it importantly highlights this as a viable therapeutic approach and provides a basis for subsequent work. Other MEK targeted approaches in patients with uveal melanoma are also underway, including a Phase Ib/II study of MEK162 in combination with the protein kinase C inhibitor AEB071 (NCT01801358).

MEK-targeted therapy in NRAS-mutated melanoma

NRAS mutations are reported in approximately 15–25% of melanomas, and currently there are no approved targeted therapies for this patient population [66]. While

MEK theoretically represents a common target in both RAS and BRAF driven tumorigenesis, differing levels of antitumor activity have been seen among specific MEK inhibitors. Analyses now suggest that distinct mechanisms of action may account for this, and inhibitors which prevent phosphorylation of MEK, thereby leading to a stable RAF–MEK complex, may have more activity in RAS driven tumors. This effectively blocks the feedback loop which leads to MEK phosphorylation via wild-type RAF in these cells. Conversely, agents that selectively inhibit activated MEK appear to be more active in BRAF driven cancers, where there is a high basal rate of MEK activity [67].

Preclinical analyses using the selective MEK inhibitor MEK162 demonstrated inhibition of *NRAS*-mutated melanomas *in vitro* and *in vivo* [41]. Early evidence of potential clinical activity in NRAS driven disease was seen in a Phase I study, with one patient with *NRAS* mutated cholangiocarcinoma achieving a PR and another nine patients with stable disease [42]. A Phase II study, which included patients with *NRAS*-mutated melanoma has also shown promising results [43]. In the cohort of 30 patients with *NRAS*-mutated disease treated at 45 mg b.i.d., six patients had a PR (three confirmed) and another 40% experienced stable disease [43]. The toxicity profile appeared manageable, with rash, dermatitis, peripheral edema and diarrhea being the most common events reported. This was one of the first studies to demonstrate evidence of clinical activity with a targeted agent in the *NRAS*-mutated melanoma population. Based on these results, a randomized Phase III study comparing MEK162 to dacarbazine is currently underway (NCT01763164). Other MEK inhibitors are currently being tested in this population as well, including a Phase II trial of pimasertib versus dacarbazine, which is currently enrolling (NCT01693068). While these results are promising, single agent MEK inhibition is unlikely to result in durable disease control and future strategies will likely center around rationally designed combination strategies. In the *NRAS* mutated population, preclinical evidence additionally suggests that specifically targeting the cell cycle may be clinically relevant. *In vitro* and *in vivo* data has demonstrated that co-targeting MEK and cyclin CDK4 results in both apoptosis and cell cycle arrest, with resultant increased tumor regression [68]. Based on these results, a Phase Ib/II trial of MEK162 in combination with the CDK4/6 inhibitor LEE011 is currently enrolling (NCT 01781572).

Current place in practice & future directions

The treatment landscape for melanoma continues to evolve rapidly, and there are more treatment options available for patients than ever before. While there are now a number of targeted therapeutic options that can provide meaningful clinical benefit, they seldom result in cure for patients diagnosed with metastatic melanoma. While the current data available for MEK inhibition in melanoma suggest promising clinical activity, it is unlikely that there will be a distinct patient subset in which MEK inhibitor monotherapy represents the best available option. While single agent trametinib was approved based on an improvement in overall survival when compared with chemotherapy, the response rates and duration of PFS for MEK inhibitors appear to be consistently lower than those reported for selective BRAF inhibitors in the *BRAF* mutant melanoma population, albeit these agents have never been compared in a prospective study. The recent approval of dabrafenib and trametinib in combination likely represents a new standard in the treatment of *BRAF*-mutated melanoma. Based on the currently available data, MEK inhibitors may be appropriate for patients with *BRAF*-mutated melanoma who are intolerant of BRAF inhibitors.

While single agent MEK inhibitors have limitations, the critical advance lies in the fact that the availability of this early clinical success provides a platform to continue to build upon existing knowledge. The availability of MEK inhibitors with more manageable toxicity profiles allows for the investigation of combination strategies, including with other targeted agents, cytotoxic chemotherapies, as well as immunologic approaches. Novel classes of immunomodulators, including CTLA-4 and PD-1 directed therapies hold particular promise. As single agents, these therapies have shown the potential to induce durable responses, albeit there are many patients who do not benefit from therapy [69–72]. Preclinical data have suggested the potential for synergy between targeted and immunotherapeutic approaches, as inhibition of $BRAF^{V600}$ may lead to increased expression of melanocyte differentiation antigens, thereby improving T-cell recognition [73]. Clinical trials using combination strategies are currently underway, including two trials assessing the safety of the anti-CTLA-4 antibody ipilimumab in combination with dabrafenib and trametinib (NCT01767454; NCT01940809). Additionally, a study of cobimetinib with the anti-PD-L1 antibody MPDL3280A is also ongoing (NCT01988896).

Future studies will likely yield more insight into mechanisms of resistance and will allow for more of an evidence-based approach when designing dual and even triple targeted strategies. Increased knowledge about the molecular and genetic alterations which drive melanomagenesis has the potential to provide new insights into other novel targets. Improvements

in current technology with molecular profiling and next-generation sequencing assays will likely provide more information in order to define new subsets of patients that could derive benefit from targeted therapies. Clinical trials incorporating these approaches will remain of critical importance as more options become available in order to best define the optimal treatment approach for patients.

Conclusion

The MAPK pathway has long been a target for therapeutic interventions in oncology, as it is well known to play an essential role in cell survival. Increased insight into MAPK pathway dysregulation as a major contributor to the pathogenesis of melanoma has yielded landmark advances in therapy, initially with the development of selective BRAF inhibitors and now with MEK targeted agents. MEK inhibition has shown clinical activity across multiple melanoma subtypes, including both *BRAF* and *NRAS* mutated disease, as well as uveal melanoma. It is likely that novel combination strategies will continue to emerge and build upon the promise seen in these initial studies, with the ultimate goal of benefiting many more patients.

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Executive summary

- The RAS-RAF-MEK pathway is a key mediator of oncogenesis in a large proportion of melanomas.
- Preclinical data have suggested that targeting this pathway via MEK inhibition could lead to potential therapeutic benefit.
- Selective MEK inhibitors are now at various phases of clinical development and have shown clinical activity in several melanoma subtypes.
- The selective MEK inhibitor trametinib was recently US FDA approved for the treatment of $BRAF^{V600}$ melanoma based on an improvement in overall survival seen in a randomized Phase III study when compared with chemotherapy.
- Trametinib is also approved for use in combination with dabrafenib based on an improvement in PFS when compared with dabrafenib monotherapy.
- Early studies involving patients with *NRAS*-mutated and uveal melanoma have also shown promising results, and larger studies are currently ongoing.
- Future studies will focus on novel combinations and further defining patient subsets who are most likely to derive benefit from these agents.

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