Metastatic melanoma is well known for its aggressive clinical behavior and therapeutic resistance. The standard systemic therapy has shown limited clinical activity with no significant survival benefit. Melanoma is a molecularly heterogeneous malignancy. Several key genetic lesions governing melanoma initiation and progression have been identified, the most common being a point mutation in the \textit{BRAF} proto-oncogene, which is detected in 50–60\% of metastatic melanoma. The prevalence of \textit{RAF} alteration in metastatic melanoma and other human cancer has prompted significant efforts in the development of BRAF targeted therapy. Several BRAF inhibitors have entered clinical trials, and have shown significant responses even in patients with late-stage melanoma. In this article, we review the rationale, clinical activity and safety of BRAF targeted therapy for metastatic melanoma.

Keywords: BRAF inhibitors • drug resistance • metastatic melanoma • targeted therapy

Melanoma is the sixth most common cancer in the USA and its incidence is rising [1]. The development of distant metastatic disease is associated with poor outcome with a median survival of approximately 6–10 months and a 5-year survival rate of less than 10\% [2,3].

Until recently, systemic therapies have offered limited benefit. Single agent dacarbazine does not offer a survival benefit when compared with best supportive care [4] and high-dose bolus IL-2 is a highly toxic therapy with less than 15\% response rate. Moreover, combination of various therapeutic agents have failed to alter the natural history of the disease. Most recently, based on the improved overall survival by 4 months, anti-cytotoxic T-lymphocyte antigen 4 antibody, ipilimumab (Yervoy\textsuperscript{TM}, Bristol-Myers Squibb) became the first therapeutic agent approved by the US FDA for refractory metastatic melanoma since 1998 [5].

Over the past decade, important advances have been made that have yielded exciting results and are changing the paradigm of treatment of metastatic melanoma. These advances include the identification of specific somatic mutations and the development of novel agents that target various components of signaling pathways. The most promising of these therapies is targeting \textit{BRAF} proto-oncogene in the MAPK pathway. In this report, we review the rationale, clinical activity and safety of BRAF targeted therapy.

Rationale

In the last decade, considerable excitement has been generated by the identification of genetic mutations in various components of signal pathways involving melanoma initiation and progression, particularly those involved in the MAPK and PI3K/AKT pathways. Identification of the molecular alterations/mutations has prompted significant efforts in the development of drugs targeting various components of the MAPK pathway.
Intracellular signaling pathways

The MAPK and PI3K/AKT intracellular signaling pathways mediate extracellular growth signals from the cell membrane to the nucleus via a cascade of phosphorylation events (Figure 1). Several aberrations in various components of the MAPK and PI3K/AKT signal transduction pathways involving melanoma proliferation and survival have been identified.

In the MAPK pathway, the stimulation of membrane-bound receptor tyrosine kinase leads to the activation of the RAS protein. This in turn activates the RAF kinase family – ARAF, BRAF and CRAF (currently known as RAF1). The RAF kinase activates the MEK protein, which subsequently activates ERK by phosphorylation. Activated ERK translocates into the nucleus where it targets various transcription factors that regulate cell proliferation and differentiation-related genes [6,7]. Phosphorylated ERK (pERK) is often used as a biomarker of MAPK pathway activation in vitro and in pharmacodynamic studies. Activation of RAS protein also activates the PI3K/AKT pathway that leads to downstream activation of mTOR. This is an important signaling pathway for tumor cell survival.

Figure 1. Intracellular signaling pathways in melanoma with sites of inhibition of targeted therapies.
BRAF-targeted therapy for metastatic melanoma

■ BRAF mutation

In 2002, the Sanger Institute reported the results of a landmark study that evaluated the mutational status of various cancers from over 500 cell lines and clinical specimens [8]. Although present in only 7% of human cancers, they found that over 59% of melanoma cell lines and 67% of the melanoma clinical specimen harbored a mutation in the gene encoding BRAF protein. This has also been seen in clinical studies, which have shown the presence of BRAF mutation in 50% of metastatic melanomas [9].

Preclinical data from animal models using immunocompromised mice and human melanoma cell xenograft provided further evidence that implicated BRAF mutation as a significant driver for cell proliferation [10–12]. Coupled with the high prevalence of point mutation, the BRAF protein thus became an important focus for the development of targeted therapy. BRAF is a serine-threonine kinase. The most common mutation (present in over 90%) is a point mutation in exon 15 (T1799A) which leads to the substitution of glutamic acid for valine at position 600 (BRAFV600E), and results in the protein folding into its active conformation [13]. The constitutively active protein serine kinase activity of the mutated BRAF is 10.7 times higher and leads to a sustained activation of the MAPK pathway. Other point mutations in BRAF proto-oncogene have been identified some of which also activate the MAPK pathway (e.g., BRAFV600K, BRAFV600D and BRAFV600R).

■ Other mutations

NRAS is an oncogene and is the upstream activator in the MAPK pathway. Mutations are reported to occur in 10% to 25% of melanomas. Interestingly, mutations in NRAS and BRAF are mutually exclusive [14,15]. This is important since it implies that the vast majority of melanomas are driven by an activated MAPK pathway via either BRAF or NRAS mutation.

PTEN is a tumor suppressor gene and amongst its various functions is the inactivation of the PI3K/AKT pathway. Loss of PTEN function is rarely present with an NRAS mutation. However, the combination of mutated BRAF and silencing of PTEN expression is common in human melanoma (~20%) [16]. Dankort and colleagues have demonstrated in a preclinical model that, “BRAFV600E cooperates with PTEN loss to induce metastatic melanoma” [17].

Clinical activity

The prevalence of RAF alterations in human cancer has prompted the efforts in the development of drugs targeting the MAPK pathway. Many of these are in clinical trials in patients with metastatic melanoma. These inhibitors are grouped in two categories: non-selective RAF inhibitors and selective RAF inhibitors (Figure 1 & Table 1). With few exceptions, in general the RAF inhibitors have the advantages of ease of administration (oral agents) and acceptable safety profiles.

■ Nonselective RAF inhibitors

Sorafenib

Sorafenib (BAY43–9006, Nexavar® Bayer Healthcare Pharmaceutical Inc.) was one of the first agents to be evaluated as a targeted therapy in metastatic melanoma. It has inhibitory activity against multiple kinases, including RAF kinases, VEGFR-2/3, PDGFR-2 and c-KIT [18,19].

In Phase I pharmacokinetic and pharmacodynamic studies, the maximum tolerated dose (MTD) of sorafenib resulted in near complete suppression of pERK [20,21]. However, this did not translate into significant clinical efficacy in patients with solid tumors. A Phase II clinical study of sorafenib as a single agent did not show clinical response in advanced melanoma, although seven patients (19%) achieved stabilization of disease [22]. The combination of chemotherapy with sorafenib yielded mixed results. In a Phase I/II study of sorafenib in combination with carboplatin and paclitaxel, 11 patients (31%) achieved partial response and another 19 patients (54%) had stable disease in the expansion cohort of patients with metastatic melanoma [23]. In a randomized Phase II study of dacarbazine with or without sorafenib in patients with advanced melanoma, the combination arm achieved an overall response rate of 24% with a progression-free survival of 41% at 6 months [24]. In another multi-arm, randomized Phase II study comparing two schedules of temozolomide in combination with sorafenib in patients with advanced melanoma, 47 patients with no brain metastases achieved a disease control rate of 72% (30% partial response, 6% minor response and 36% stable disease) [25].

Tumor tissue, when available, was analyzed for specific mutations in these studies but none of them showed a correlation of response with the presence of BRAF mutation. It was thought that sorafenib may play a role in potentiating the effects of chemotherapy. However, Phase III randomized studies did not support this notion. In a Phase III study, carboplatin and paclitaxel in combination with sorafenib or placebo as a second-line treatment in patients with unresectable stage III or stage IV disease did not show any significant difference in median progression-free survival or the response rate [26]. An Eastern Cooperative Oncology Group double-blind, randomized, Phase III trial (E2603) comparing carboplatin/paclitaxel with
or without sorafenib in metastatic melanoma showed no significant difference in overall survival during the third interim analysis [27].

The most common toxicities associated with sorafenib are hand-foot syndrome, skin rash and diarrhea that are often mild-to-moderate in severity and easily manageable. Sorafenib is currently approved for metastatic renal cell cancer which is uniquely VEGF driven. However, the multikinase inhibitory activity of sorafenib may still have a role to play in metastatic melanoma, especially when drug resistance develops and/or in the treatment of mucosal melanoma. Further evaluation of sorafenib in combination with various chemotherapies, immunotherapies and other targeted therapies is currently ongoing [101].

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Targets</th>
<th>Manufacturer</th>
<th>Current status</th>
<th>Common toxicities</th>
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<tr>
<td>Sorafenib</td>
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<td>Nexavar®</td>
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</tr>
<tr>
<td>RAF265 (CHIR-265)</td>
<td>RAF, VEGFR-2, KIT and PDGFR-2</td>
<td>Novartis</td>
<td>Phase I</td>
<td>Dose-limiting toxicities: pulmonary embolism, visual changes, hyperlipasemia, diarrhea, ataxia and thrombocytopenia</td>
</tr>
<tr>
<td>XL281 (BMS-908662)</td>
<td>CRAF, BRAF and BRAFV600E</td>
<td>Exelixis/Bristol-Myers Squibb</td>
<td>Phase I</td>
<td>Fatigue, nausea, vomiting, diarrhea and anorexia</td>
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**Selective RAF inhibitors**

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<tr>
<th>Compounds</th>
<th>Targets</th>
<th>Manufacturer</th>
<th>Current status</th>
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<tr>
<td>Vemurafenib (PLX4032/ RG7204)</td>
<td>BRAFV600E</td>
<td>Plexxikon Hoffmann-La Roche</td>
<td>Awaiting US FDA approval</td>
<td>Fatigue, rash, arthralgia and photosensitivity</td>
</tr>
<tr>
<td>GSK2118436 (SB-590885)</td>
<td>BRAFV600E</td>
<td>GlaxoSmithKline</td>
<td>Phase II Phase III</td>
<td>Fatigue and rash</td>
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**Other inhibitors**

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<tr>
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<th>Targets</th>
<th>Manufacturer</th>
<th>Current status</th>
<th>Common toxicities</th>
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<tr>
<td>Selumetinib (AZD6244/ ARRY-142886)</td>
<td>MEK</td>
<td>Array Biopharma AstraZeneca</td>
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</tr>
<tr>
<td>PD0325901</td>
<td>MEK</td>
<td>Pfizer</td>
<td>Phase II terminated</td>
<td>Reason for termination: retinal vein thrombosis and neurological side effects</td>
</tr>
<tr>
<td>GSK1120212</td>
<td>MEK</td>
<td>GlaxoSmithKline</td>
<td>Phase III</td>
<td>Diarrhea and rash</td>
</tr>
<tr>
<td>AS703026</td>
<td>MEK</td>
<td>EMD Serono/Merck Serono</td>
<td>Phase I/II</td>
<td>Diarrhea, rash and nausea/vomiting</td>
</tr>
<tr>
<td>E6201</td>
<td>MEK</td>
<td>Eisai Ltd.</td>
<td>Phase I</td>
<td>Nausea, vomiting, dizziness and peripheral edema</td>
</tr>
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XL281/BMS-908662

XL281 (developed by Exelixis Inc. and out-licensed as BMS-908662 to Bristol-Myers Squibb) is a second-generation, broad-spectrum, kinase inhibitor with a high oral bioavailability and improved potency for RAF inhibition including CRAF, BRAF and activated BRAFV600E [28]. A Phase I study evaluated escalating doses of XL281 in 30 patients with solid tumors that included 5 with metastatic melanoma [29]. The MTD was established at 150 mg daily. The MTD was further expanded to 40 patients that included 11 patients with metastatic melanoma. Tumor pharmacodynamic studies revealed a robust pathway inhibition with decrease in ERK and MEK phosphorylation that was independent of the RAS/RAF genotype. Clinical benefit was noted including one patient with ocular melanoma (harboring KITM541L mutation) achieved a partial response. Stable disease for greater than 3 months was also reported that included two patients with papillary thyroid cancer and two patients with colorectal cancer.

A Phase I, dose-escalation study to evaluate the safety and pharmacokinetics of XL281 as once- or twice-daily dosing in patients with solid tumors, including melanoma (NCT00451880) and a Phase I/II study of BMS-908662 alone or in combination with cetuximab in patients with KRAF or BRAF mutation in metastatic colorectal cancer (NCT01086267) are currently recruiting patients.
RAF265

RAF265 (CHIR-265, Novartis International AG) is another second-generation, oral, small molecule RAF inhibitor. Preclinical data has shown potent inhibition of mutant BRAF in addition to inhibition of VEGFR-2, c-KIT and PDGFR-2 [30–32]. The preliminary results of the first-in-human Phase I study of RAF265 in patients with advanced melanoma were reported recently [33]. The MTD of oral RAF265 on a continuous daily schedule was defined at 48 mg. Clinical activity was observed at multiple dose cohorts in patients with BRAF mutated (16%) and wild-type (13%) melanoma. Since RAF265 daily at 67 mg consistently caused delayed dose limiting hematologic toxicity, an intermittent schedule is being explored.

■ Selective RAF inhibitors

Vemurafenib (PLX4032/RG7204)

Vemurafenib (PLX4032, Plexxikon Inc., and currently co-developed as RG7204 by F. Hoffman-La Roche Ltd.) is a highly selective BRAF inhibitor that to date has shown great efficacy in the treatment of metastatic melanoma. This agent was developed using Plexxikon’s proprietary Scaffold-Based Drug Discovery program. The molecular structure of the mutated BRAF protein was used as a model to synthesize a panel of inhibitors that could interact with key kinase domains of the protein in its active conformation.

Preclinical data showed that many of the inhibitors had a high potency and selectivity for mutated BRAF over wild type. PLX4032 was highly potent against A375 melanoma cell line and was chosen for further development. Xenograft models revealed a significant delay in tumor growth with tumor regression that was associated with inhibition of MEK phosphorylation and decreased expression of cyclin D1 [34–36]. Although it is highly selective for BRAFV600E, in vitro data has demonstrated that it may also inhibit other BRAF mutations including BRAFV600D, BRAFV600K and BRAFV600R at different inhibitory concentrations (IC50) [37].

The preliminary results of the Phase I study were presented in 2009 [38] and updated results of the extension cohort were recently published [39]. This multicenter, dose-escalation trial was conducted to evaluate the safety and pharmacokinetic characteristics of treatment with continuous, twice-daily dosing of PLX4032. In the dose-escalation phase of the study, 49 (89%) of the 55 patients enrolled had metastatic melanoma. More than 70% of the patients with metastatic melanoma had visceral disease and nearly half had previously received three or more systemic therapies.

Patients initially received a crystalline formulation of the drug and no antitumor activity was seen. Pharmacokinetic studies revealed that dose levels of 200–1600 mg twice daily failed to achieve the required serum levels for efficacy with this formulation. The drug was then reformulated as a micro-precipitated bulk-powder with a higher bioavailability, and all patients, including those previously enrolled, were treated with the new drug formulation.

To better define the response rate, 32 additional patients with melanoma harboring BRAFV600E mutation were enrolled in the extension cohort and treated with the recommended Phase II dose. This yielded highly encouraging results with two patients achieving complete response and 24 patients achieving partial response (an overall response rate of 81%). Tumor response was seen at all sites (including visceral disease) and was seen even in patients who were heavily treated with multiple systemic therapies. The duration of response ranged 2–18 months. Although some of the patients were still being followed at the time of reporting of the results, the median progression free survival was estimated at 7 months.

A multicenter, open-label, Phase II study evaluated the efficacy of vemurafenib in melanoma (BRIM-2) [40]. In this study, primary end point was best overall response rate (BRR) and 132 previously treated patients with metastatic melanoma harboring BRAFV600E mutation were enrolled. At median follow-up of 7 months, BRR was 52.3% and median duration of response was 6.8 months. Stable disease was observed in 29.5% of the patients and only 13.6% had progression of disease.

A large, multicenter, open-label, randomized Phase III study (BRIM3) was conducted to determine if vemurafenib improved overall survival (OS) and progression-free survival (PFS) in melanoma patients with BRAFV600E mutation. The preliminary results of this randomized study of vemurafenib versus dacarbazine were recently presented [41]. A total of 675 treatment-naive patients were enrolled at 103 centers worldwide between January and December 2011. At the preplanned interim analysis, the hazard ratio for OS and PFS were 0.37 (95% CI: 0.26–0.55; p < 0.0001) and 0.26 (95% CI: 0.20–0.33; p < 0.0001), respectively, both in favor of vemurafenib when compared with dacarbazine in patients with previously untreated metastatic melanoma. In general, tumor response to therapy with vemurafenib is very rapid. Evaluation with 18-fluorodeoxyglucose (FDG)-PET revealed a dramatic reduction of metabolic uptake as early as day 15 following initiation of therapy [39,42]. This rapid response was also seen in day 15 tumor biopsy specimens which showed a marked reduction of pERK, cyclin D1 and Ki-67 as compared with baseline [43]. Clinically, the rapidity of response was associated with alleviation of symptoms within 1–2 weeks after beginning treatment, as was evidenced by a decreased need for narcotic analgesia in some patients [39].
Based on the results of the Phase III study, the developers have initiated an Expanded Access Program (NCT01248936) that is currently available at three centers within the USA. In addition, the company is working with the FDA to obtain approval for treatment of BRAFV600E mutated metastatic melanoma.

The most common side effects reported with vemurafenib are arthralgia, rash, nausea, photosensitiveness, fatigue, pruritus and palmer-plantar dysesthesia. The majority of these side effects are mild-to-moderate and are proportional to the dose and exposure to the drug. An important adverse event of vemurafenib is the development of cutaneous squamous cell cancer (SCC) that was reported in eight patients (15%) in Phase I study and ten patients (31%) in the extension cohort.

GSK2118436/SB590885
SB590885 (GlaxoSmithKline) is a highly potent, selective BRAF inhibitor [44] and has been developed for in-human clinical trials as GSK2118436. In a Phase I/II study, 61 patients were enrolled that included 52 patients with metastatic melanoma harboring BRAF mutation [45]. This agent was well tolerated and at the time the results were reported, MTD had not been reached. Inhibition of pERK was dose-dependent. Plasma levels were proportional to the dose received and exceeded the therapeutic target at doses as low as 35 mg daily. Greater than 20% decrease in tumor size (PR) was seen by 8–9 weeks of initiating therapy in 18 (60%) out of the 30 evaluable patients with BRAF-mutated, metastatic melanoma without brain metastases. When available, a decrease in FDG-PET uptake was seen in 79% of the patients.

Cerebral involvement is very common in melanoma, affecting 40–50% of patients with advanced disease. Prognosis of these patients is grim, with median survival of approximately 4 months from the time of diagnosis of brain metastases. Therapeutic development for the management of melanoma metastatic to the CNS is severely restricted because brain involvement is a common exclusion criterion to clinical trial participation.

Preliminary results from part 2 of the Phase I/II dose escalation study evaluating GSK2118436 in a cohort of treatment-naïve BRAFV600E positive melanoma patients with asymptomatic brain metastases strongly indicate that this BRAF inhibitor is active against both extra- and intracerebral melanoma lesions [46]. All of the seven evaluable patients had tumor response in the brain, with three complete resolutions of brain metastases.

With these highly encouraging results, GSK2118436 has proceeded into further clinical evaluation [101] including Phase II (NCT01153763) and a Phase III study comparing GSK2118436 with dacarbazine (NCT01227889) in treatment-naïve metastatic melanoma. This agent is also being evaluated in a multicenter, Phase II trial in both previously treated and treatment-naïve patients with BRAF-mutated melanoma that has metastasized to the brain (NCT01266967).

Downstream inhibitors of MAPK pathway: MEK inhibitors

The direct substrate of BRAF activation in the MAPK pathway is the MEK protein. Targeting MEK inhibits the downstream propagation of an activated BRAF or RAS protein. The first agent to be clinically developed in this class of MEK inhibitors was CI-1040 (PD184352, Pfizer Inc.). However, the initial Phase I and II studies showed poor clinical efficacy of this agent [47,48].

Selumetinib (AZD6244/ARRY-142886)

Selumetinib (ARRY142886, Array Biopharma Inc., out-licensed and developed in collaboration as AZD6244, AstraZeneca PLC) is another second-generation, oral, highly selective, allosteric MEK inhibitor.

A Phase I, dose-escalation study evaluated the safety of selumetinib (formulated as an oral powder for reconstitution) in 57 patients with advanced cancers [51]. More than two-thirds of the patients had previously received at least two systemic therapies. Evaluation of tumor biopsies showed a strong inhibition of ERK phosphorylation. One patient with metastatic melanoma had 70% tumor shrinkage after 3 months of selumetinib but developed brain metastases. One patient with metastatic uveal melanoma had prolonged disease stabilization for greater than 22 months.

In an open-label, multicenter Phase II study, 200 chemo-naïve patients with metastatic melanoma were randomized to receive either selumetinib 100 mg twice daily or temozolomide 200 mg/m² daily for 5 days every 28 days [9]. Six patients had an objective response in the selumetinib arm of which five had melanoma harboring BRAF mutation while nine patients had an objective response (including three patients with BRAF mutation) in the temozolomide arm. However, there was no significant difference in progression-free survival between the two arms.
In a subsequent study, a new formulation of selumetinib with a hydrogen sulfate salt (Hyd-Sulfate) was used to improve patient compliance and convenience of dosing. In a Phase I, multicenter, dose-escalation study, 75 mg twice daily was determined to be the MTD, which was well tolerated with minimal need for treatment interruption or dose reduction [52]. Pharmacokinetic studies showed that the oral bioavailability of the 75 mg Hyd-Sulfate capsule was higher than that of the 100 mg free base suspension.

The most common side effects of this agent include skin rash (commonly on the torso), diarrhea, nausea, vomiting, edema and fatigue. Blurred vision was noted as ocular toxicity, most of which occurred at the higher doses, although no structural abnormalities were identified. The adverse events associated with the Hyd-Sulfate capsule are either early toxicities including dermatitis aceneiform, rash, erythema, skin exfoliation, diarrhea, or late toxicities including dry skin, pruritus, skin fissures, paronychia, nausea, vomiting and peripheral edema.

Selumetinib is currently undergoing further evaluation in clinical trials at various stages [101].

GSK1120212

GSK1120212 (GlaxoSmithKline) is a potent, highly selective, oral MEK inhibitor. In a Phase I, dose-escalation study, 84 patients with solid tumors or lymphoma were enrolled that included 29 patients with metastatic melanoma [53]. The MTD was established at 3 mg daily and the recommended dose for future studies was 2 mg daily. Tumor mutation analysis was available for 20 melanoma patients, five of whom achieved partial response and eight had stable disease. A total of 11 melanoma patients had mutated BRAF and three of these patients had achieved partial response. Another five patients had stable disease, which included a patient who had previously been treated with PLX4720. Three patients had progression of disease on treatment of which two developed new brain metastases. Evaluation of tumor biopsies showed greater than 90% reduction of ERK phosphorylation and Ki-67 at the recommended dose.

GSK1120212 also showed rapid onset of antitumor activity. In four melanoma patients for whom FDG-PET was available for evaluation, metabolic uptake was reduced by 23–48% within 2 weeks, even at doses as low as 0.5–1 mg daily. Dose-limiting toxicities included rash, diarrhea and central serous retinopathy, all of which were reversible.

Based on the promising results of the above study, G1120212 is undergoing further clinical investigation. A Phase II study is evaluating the role of GSK1120212 in patients who have previously been treated with or without a BRAF inhibitor (NCT01037127). An open-label, randomized, Phase III study is currently ongoing to compare the survival benefit of GSK1120212 versus dacarbazine or paclitaxel (NCT01245062).

Based on the finding that the MEK protein is frequently reactivated at the time of development of tumor resistance to BRAF inhibitors, the combination of this MEK inhibitor with the BRAF inhibitor GSK2118436 is being evaluated in a Phase I/II study for safety, pharmacokinetics, and efficacy [54,55]. Preliminary results of Phase I portion were reported at the 2011 ASCO annual meeting [56]. BRAF inhibitor GSK2118436 at 150 mg twice a day was combined safely with MEK 1/2 inhibitor GSK1120212 2 mg daily. No SCC have been observed thus far and skin rash occurred with less frequency as compared with previous trials of single agent GSK2118436 and GSK1120212, respectively. Of 16 evaluable patients, 13 patients had partial response and three had stable disease for an overall response rate of 81%. The preliminary antitumor activity warrants further investigation and the randomized Phase II portion is currently accruing patients.

AS703026

AS703026 (EMD Serono/Merck Serono) is a highly selective, potent, noncompetitive MEK inhibitor. In a Phase I study, 68 patients with metastatic solid tumors including nine patients with metastatic melanoma were enrolled to evaluate two intermittent dosing schedules [57]. Partial response was observed in two patients with previously treated melanoma. Most common treatment related toxicity included asthenia, diarrhea, acne-like reaction, nausea, constipation and vomiting. This drug is currently being evaluated in Phase I and II studies in a variety of malignancies (NCT01016483 and NCT00957580).

E6201

E6201 (Eisai Inc.) is a potent, MEK inhibitor. In an open-label, Phase I dose escalation study, 25 patients with advanced solid tumors received weekly intravenous infusion of E6201 for 3 out of 4 weeks [58]. The MTD was established at 320 mg/m2. One patient with metastatic ocular melanoma harboring wild-type BRAF mutation had stable disease for more than 10 months. This drug was well tolerated and the most common toxicities were nausea, vomiting, constipation, dizziness and peripheral edema. Patients are currently being accrued to the expansion cohort (NCT00794781).

Special considerations

- Keratoacanthoma-like squamous cell carcinoma of the skin & RAF inhibitors
  A unique feature of RAF inhibitors is their ability to induce squamous cell carcinoma (SCC) of the skin. The spectrum of these lesions ranges from actinic keratoses...
to well-differentiated keratoacanthoma-like SCC to classic invasive SCC. These cutaneous neoplasms were first reported in patients treated with sorafenib [59–61]. Published retrospective reviews indicated that sorafenib-induced SCCs occurred in approximately 6–7% of patients at a median onset of 6.7 months. Interestingly, the incidence of SCCs increased significantly with the selective BRAF inhibitors, 31% with vemurafenib and 12% with GSK2118436 [38]. Of note, with selective BRAF inhibitors, these skin lesions appeared much earlier when compared with sorafenib.

At the present time, it is recommended that patients with advanced melanoma being treated with RAF inhibitors undergo thorough dermatologic examinations at baseline and then every 2 months. Once identified, SCCs should be managed with complete surgical resection without drug interruption. Fortunately, SCC of tissues other than the skin has not been observed with RAF inhibitors. However, clinicians should be vigilant to monitor for the development of lesions suggestive of a primary carcinoma [38,62].

The mechanism by which RAF inhibitors induce SCCs has not been fully understood. Current working hypothesis is built on the observation that the same RAF inhibitor can be inhibitory in one cell line but stimulatory in another. In tumor cells that harbor BRAFV600E, RAF inhibitors suppress pathway activation and induce cell death. However, in the cell lines carrying oncogenic RAS and wild-type RAF, RAF inhibitors unexpectedly stimulate drug-bound BRAF monomer to heterodimerize with CRAF and hyper-activate CRAF, causing activation of MAPK pathway [62–67]. Since mutant RAS has been identified in 22% of SCCs it is highly probable that RAF inhibitors behave as paradoxical activators in cutaneous squamous cells and stimulate MAPK pathway to induce keratoacanthoma-like SCCs.

**Mechanism of drug resistance to RAF inhibitors**

Despite the unprecedentedly high response rate seen with RAF inhibitors in patients with advanced melanoma, duration of response is disappointingly short, implicating rapid emergence of drug resistance [59]. To date, the mechanisms of resistance to RAF inhibitors have not been fully elucidated. It is also unclear whether disease progression is caused by acquisition of novel drug-resistant mechanisms, by selection and expansion of primary resistant tumor clones, or both [68].

**Tumor heterogeneity as a medium for drug resistance**

Melanoma tumors are not homogenous. Heterogenous clones with diverse genetic make-up react differently to RAF inhibitors. It has been established that RAF inhibitors suppress MAPK pathway activation and induce apoptosis in the tumor cells harboring BRAFV600E. Unexpectedly, RAF inhibitors paradoxically activate MAPK signaling via transactivation of RAF dimers to promote growth in the clones carrying mutant RAS and wild-type BRAF [63–65]. Recently, pre-existing BRAF amplification has been identified in a small population of colorectal tumor cells [69]. If also present in melanoma tumors, these minor subsets, with exaggerated MAPK signaling, are able to evade BRAF inhibition and maintain growth. More common in melanomas, especially in the acral lentiginous subtypes and those arising from skin area with chronic sun damage, is amplification of CCND1, a downstream component of the MAPK pathway [70]. Melanoma cell lines with increased CCND1 copies are less dependent on MAPK signaling for proliferation and therefore are less susceptible to BRAF inhibition. It has been recently shown that melanoma cell lines with PTEN loss, when exposed to RAF inhibitors, underwent significantly less apoptosis than the PTEN-expressing cell lines [71]. Indeed, PTEN aberrations have been correlated with suboptimal tumor response in a small group of patients with metastatic melanoma being treated with GSK2118436 [72].

Clinical evidence begins to emerge as proof of this concept. When FDG-PET was used to assess clinical response after 2-week treatment with GSK2118436, heterogeneous FDG uptake in preselected target lesions was demonstrated in 26% of patients [73]. Interestingly, heterogeneity in tumor response appeared to signify a shorter time to disease progression.

Altogether, these observations suggest that disease progression in the patients who had initially responded to BRAF inhibitors could partly be due to drug-induced selection and expansion of melanoma clones with unfavorable genotypes. These observations also rationalize the need for pretreatment tumor genotyping to maximize the therapeutic index and to strategize against drug resistance.

**Mechanisms of secondary resistance**

Unlike other tyrosine kinase inhibitors, secondary mutations to the drug-binding domain of BRAFV600E kinase have not been identified to date [55,74]. Below is the list of proposed models of drug resistance to this class of agents.

Resistance occurring upstream of RAF

Located upstream of RAF is RAS. Acquisition of activating NRAS mutations can manipulate RAF inhibitors to exhibit opposing effect on MAPK signaling, conferring drug resistance [55]. Further upstream from RAS are a number of growth factor receptors, of which up-regulation has also been recognized as mediator of...
resistance to RAF inhibitors. For instance, increased expression of PDGFRβ or IGF1R has been identified not only in vemurafenib-resistant melanoma cell lines but also in patient-derived tumor biopsies. Both of these RTKs, via cellular pathways that are unclear at this time, are able to reactivate MAPK signaling despite RAF inhibition [55,74].

Resistance occurring at RAF level
Amplification of BRAF is one potential method colorectal cancer cells use to escape drug-induced apoptosis after chronic exposure to RAF inhibitors [69]. Although gains in BRAF copies have not yet been demonstrated in melanoma cell lines, it is highly possible that BRAF amplification may also mediate resistance to RAF inhibitors in melanoma tumors. At RAF level, other ways for cancer cells to survive drug effect include increasing CRAF expression or rerouting among the three RAF isoforms to bypass pathway blockade at BRAF [74,75]. Indeed, after weeks of treatment with selective inhibitors of BRAFV600E, rebound in pERK, which appeared to correlate with elevated level of CRAF, was found in a subset of drug-resistant melanoma cells [75]. More recently, it has been demonstrated that BRAFV600E positive melanoma cells, under the selective pressure of RAF-inhibiting drugs, could flexibly switch among the three RAF isoforms, ARAF, BRAF and CRAF, to reactivate MAPK signaling [74]. At the present, the exact mechanisms underlying the substantial increase in CRAF expression or the seamless switch to ARAF or CRAF remain unknown.

Resistance occurring downstream of RAF
Immediately downstream of RAF is MEK. Development of MEK mutations has been identified in both melanoma cell lines and patient-derived tumor samples after treatment with selumetinib or vemurafenib [54,76]. MEK mutations lead to the recovery of pERK, conferring secondary resistance to MEK inhibition and cross-resistance to RAF inhibitors [54]. Most recently, researchers at Dana-Farber Cancer Institute have discovered a novel cancer gene named COT, also known as MAP3K8, with the ability to activate MAPK signaling through both MEK-dependent and MEK-independent mechanisms [77]. Expression of COT has been shown to mediate both de novo and secondary resistance in BRAFV600E melanoma cells to both BRAF and MEK inhibitors.

Resistance involving other signaling pathways
Growth signaling networks in melanomas are highly complex. There is increasing evidence to suggest that communication between MAPK and PI3K/AKT pathways exist, providing further protection to melanoma cells when one pathway is not functioning appropriately. Rebound in pERK, in spite of preserved MEK inhibition, was found in a PLX4720-resistant BRAFV600E melanoma cell line [78]. Here, ERK reactivation appeared to correlate with increased PI3K/AKT signaling. This association was strengthened when inhibition of PI3K/AKT pathway was shown to reinstate sensitivity to PLX4720-induced apoptosis in the resistant clone. Constitutive AKT3 activation could also promote survival of melanoma cells exposed to RAF inhibitors through ERK-independent mechanisms. In fact, increased AKT3 phosphorylation suppressed the expression of pro-apoptotic proteins such as Bim-EL and Bmf in melanoma cells, rendering them resistant to BRAF inhibition [79].

Future perspective
We have witnessed that the genetic make-up of melanoma tumors dictates response to signaling pathway targeted therapy. Therefore, genotype-guided treatment planning is important to ensure therapeutic response to this class of agents.

Combining RAF inhibitors with other targeted agents to circumvent drug resistance and/or enhance response has been suggested. Dual RAF and MEK inhibition has demonstrated the promise of preventing drug resistance in melanoma cell lines [54]. This strategy has been tested in a Phase I trial combining GSK2118436 with GSK1120212 in patients with advanced melanoma (NCT01072175). Based on the safety data and promising antitumor activity, the randomized Phase II study is ongoing. Concurrent inhibition of MAPK and PI3K/AKT signaling has shown synergistic antitumor activity in melanoma cell lines [80]. Combination therapy of a RAF or MEK inhibitor with a PI3K or mTOR inhibitor has just entered Phase I clinical trials in patients with advanced solid tumors [101].

Acquisition of chemoresistance in intrahepatic cholangiocarcinoma cells by activation of AKT and ERK 1/2 has prompted examination of signaling pathway inhibitors with chemotherapy [81]. For instance, docetaxel with or without selumetinib is currently being compared in a randomized Phase II trial in patients with advanced melanoma harboring wild-type BRAF (NCT01256359).

Another strategy for which clinical trials are being planned is to combine BRAF inhibitors with immunotherapy. Theoretically, rapid tumor antigens released after drug-induced apoptosis can effectively stimulate antigen specific cytotoxic T-lymphocytes responses, of which activation and proliferation are augmented by concurrent immunotherapy. Additional rationale for this approach stems from preclinical data that increased MAPK signaling can decrease melanoma antigen expression in tumor cells. This may be a mechanism...
whereby tumors evade recognition by immune system [82,83]. Indeed, BRAF inhibitors such as PLX4720 were shown to upregulate Melan-A/MART-1 expression in BRAF-mutated cell lines without affecting cytotoxic T-lymphocytes proliferation and function. To test the hypothesis of synergistic antitumor activity, the clinical trial of vemurafenib in combination with ipilimumab will be started soon.

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Executive summary

- Melanoma is a molecularly heterogeneous malignancy and point mutations in the BRAF proto-oncogene are detected in 50–60% of metastatic melanoma.
- The prevalence of mutant BRAF and the constitutively activated MAPK signaling pathway in metastatic melanoma have prompted the development of BRAF targeted therapy.
- Several BRAF inhibitors have entered clinical trials and demonstrated significant clinical responses even in patients with late-stage melanoma.
- BRAF inhibitors are generally well tolerated, with common side effects being fatigue, skin rash, nausea, vomiting, and diarrhea.
- A unique feature of RAF inhibitors is their ability to induce squamous cell carcinoma of the skin; therefore, it is essential that patients treated with RAF inhibitors undergo thorough dermatologic examinations at baseline then every 2 months.
- Despite generating rapid responses in patients with advanced melanoma, duration of response to BRAF inhibitors is often less than 7 months, implicating rapid emergence of drug resistance.
- To date, the mechanisms of resistance to RAF inhibitors have not been fully elucidated.
- Combining RAF inhibitors with chemotherapy, immunotherapy, and/or other targeted agents will be the future direction to circumvent drug resistance and/or enhance response.

Bibliography

Papers of special note have been highlighted as:

- of interest
- of considerable interest

BRAF-targeted therapy for metastatic melanoma

Review: Clinical Trial Outcomes


- Compared the BRAF inhibitor vemurafenib with the current standard of care. Vemurafenib was found to improve overall survival and progression-free survival.


- The rapid onset of response with vemurafenib correlates with decrease in fluoro-deoxyglucose-PET uptake.


Review: Clinical Trial Outcomes

Noor, Trinh, Kim & Hwu

Assesses a combination of inhibitors to multiple targets in the signaling pathway. This is an important strategy to overcome drug resistance.

- Discussion of the importance of tumor heterogeneity in melanoma and the development of resistance to targeted therapy.


BRAF-targeted therapy for metastatic melanoma


Website

ClinicalTrials.gov
www.clinicaltrials.gov