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The discovery of vemurafenib for the treatment of BRAF-mutated metastatic melanoma

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Abstract

Introduction—In the era of precision medicine and sophisticated modern genetics, the discovery of the BRAF^{V600} inhibitor, vemurafenib, quickly became the model for targeted therapy in melanomas. As early as 2002, the majority of metastatic melanomas were described to harbor the BRAF^{V600} mutation, setting the stage for an explosion of interest for targeting this protein as a novel therapeutic strategy. The highly selective BRAF^{V600} inhibitor, vemurafenib, was identified initially through a large-scale drug screen.

Areas Covered—Here we examine vemurafenib's journey from discovery to clinical use in metastatic melanoma. Topics covered include preclinical data, single agent Phase 1,2 and 3 clinical trials, resistance issues and mechanisms, adverse effects including the development of squamous cell cancers, and combination trials.

Expert Opinion—Due to its tolerance, low toxicity profile, rapid tumor response, and improved outcomes in melanoma patients with BRAF^{V600} mutations, vemurafenib was advanced rapidly through clinical trials to receive FDA approval in 2011. While its efficacy is well documented, durability has become an issue for most patients who experience therapeutic resistance in approximately 6-8 months. In addition, a concerning toxicity observed in patients taking the drug include development of localized cutaneous squamous cell carcinomas (SCCs). It is hypothesized that drug resistance and SCC development result from a similar paradoxical activation of protein signaling pathways, specifically MAPK. Identification of these mechanisms has led to additional treatment strategies involving new combination therapies.

1. Introduction

Melanoma is the 6th most common malignancy with an estimated incidence of 76,380 cases in the United States and a death toll of 10,130 in 2016¹. Recently through an improved understanding of the underlying molecular tumorigenesis of melanoma, the novel treatment options including immunotherapy (i.e. CTLA4 and PD1 antagonists) and targeted therapies have made a significant impact on the disease. Clinical trials using these novel therapeutics have demonstrated an impact on overall and disease free survival². However, prognosis remains poor in AJCC stage IV disease with 1-year overall survival at 62% for M1a, 53% for

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M1b, and 33% for M1c melanomas³. Similarly, from a meta-analysis of phase II cooperative group trials, a benchmark for patients with stage IV melanoma revealed a 1-year overall survival of 25.5%⁴. In the past, treatment options for advanced melanoma were limited to non-specific chemotherapeutics, such as dacarbazine, resulting in relatively low patient response rates and intolerance due to toxicity. Improved modern genetics and molecular biology techniques identified the *BRAF*^{V600} mutation in ~60% of melanomas⁵. The *BRAF*^{V600} mutated gene product is translated to a constitutively activated BRAF protein that dysregulates the downstream mitogen-activated protein kinase (MAPK) signaling transduction⁵⁻⁷. This pathway activation is required in melanoma proliferation, apoptosis inhibition, and progression⁵⁻⁷.

Growing interest in precision medicine has led to high-throughput screens of chemical compounds with the goal of identifying a selective inhibitor of BRAF^{V600}. Vemurafenib was discovered as a highly specific BRAF^{V600} kinase inhibitor with selectivity against melanoma cells⁸. Shortly after its discovery, vemurafenib was tested in clinical trials, where it displayed a low toxicity, and more importantly, a rapid clinical response in advanced melanoma patients harboring the *BRAF*^{V600} mutation⁹⁻¹³. Despite a low number of adverse events, a significant amount of patients developed localized cutaneous squamous cell carcinomas (SCC). Another limiting factor for this drug is that treated patients eventually succumb to therapy resistance with progression of disease within 6-8 months of treatment initiation. Hypothesized mechanisms of both SCC development and vemurafenib resistance were believed to be a result of reactivation of the MAPK pathway as well as activation of other signaling pathways. This has led to a growing interest in combination therapy with vemurafenib and downstream MAPK inhibitors including MEK inhibitors^{14,15}. The goal of this review provide an overview highlighting the key events in the discovery and development of vemurafenib, from its beginnings as a cell-based screen for cells harboring the *BRAF*^{V600} mutation, to its clinical development and assessment as a promising novel therapeutic for advanced melanoma.

2. MAPK Signal Transduction

The MAPK signaling cascade is a highly conserved, protein-protein communication network responsible for mediating various cellular processes including proliferation, differentiation, cell-survival, apoptosis, and gene expression. Under normal physiologic conditions, the signal transduction is initiated through the complexing of a mitogen to its respective receptors and an activation of Rat Sarcoma (RAS)-GTPase via exchange of GDP to GTP^{16,17}. Subsequent constructions of scaffolding complex consisting of Son-of-Sevenless (SOS) and Growth-factor-receptor-bound protein 2 (GRB2) result in high affinity binding and activation of the Rapidly-accelerated fibrosarcoma (RAF) protein^{16,17}. RAF, a serine/threonine kinase, phosphorylates and catalyzes the activation of MAP/Extracellular signal-regulated kinase (ERK) 1 and 2 (MEK1/2)^{6,16-20}. MEK1/2, in turn, phosphorylate their sole substrates ERK1/2. While ERK1/2, a serine/threonine kinase, are responsible for activation and inhibition of more than 50 substrates resulting in an appropriate regulation of the cellular homeostasis²¹.

Dysregulation of the MAPK signaling pathway through mutations in effector proteins play a vital role in neoplastic transformation^{6,16-19}. In the recent era of precision medicine, targeting of mutated pathway members has drawn increasing interest.

3. B-RAF

RAF isoforms (A, B, and C) represent a class of serine/threonine kinases that operate as a hetero- or homo-dimers to activate downstream MEK and ERK²². The first *Raf* gene, *v-Raf*, was cloned from the murine sarcoma virus 3611 and characterized for its role in murine fibrosarcoma development in 1983^{18,23}. The human homolog to *v-Raf* was cloned and named *CRAF* (initially *RAF1*), and comes from a 80,626 bp gene on chromosome 3p25 encoding a 648 aa (72-74kDa) peptide^{18,19}. The discovery of *CRAF* resulted in subsequent identification of additional RAF isoforms, *ARAF* on chromosome Xp11.4-11.2 encoding 606 amino acid (68kDa) peptide; and *BRAF* on chromosome 7q34 encoding a 766 amino acid (94kDa) peptide²⁴⁻²⁷.

Structurally, the RAF proteins share three conserved regions (CR): two at the N-terminus (CR1 and CR2) and one at the C-terminus (CR3)^{6,19,20,28}. CR1 is composed of two domains, RAS-binding domain (RBD) and a cysteine-rich domain (CRD)⁶. CR2 includes a serine/threonine-enriched domain that functions as a binding site for scaffolding proteins, such as 14-3-3⁶. CR3 contains the kinase domain with a small N-terminal lobe and larger C-terminal lobe, typical of protein kinases⁶. Together, the two lobes contain the catalytic site responsible for RAF activation and MEK1/2 phosphorylation^{6,17,28}. The smaller lobe is composed of glycine-rich, anti-parallel β -sheets that bind to ATP⁶. The larger lobe is predominantly composed of α -helices that anchor MEK1/2⁶. In addition, this lobe contains the important aspartate-phenylalanine-glycine (DFG) motif, which is responsible for the regulation of the ATP-binding pocket. Mutations, such as V600, can destabilize and disrupt the inactive conformation of the DFG motif, resulting in a constitutively activated protein state²⁹. As such, the DFG motif is an attractive target of kinase inhibitors.

Due to its role as an activating mediator of the MAPK pathway, the RAF protein is highly regulated under normal physiologic conditions. In the absence of extracellular mitogenic signals, RAF is predominantly cytoplasmic and is part of a multi-protein complex, including HSP90 and p50^{6,20,22,28}. In this state, BRAF is phosphorylated in the CR2 domain (Ser365 and Ser729), resulting in formation of inhibitory complex with 14-3-3^{6,20,22,28}. With the induction of MAPK signal transduction, the RAS-GTP interacts with RAF-RBD and phosphorylates the Thr599 and Ser602, activating B-RAF²⁸. Upon activation, either a hetero- or homo-dimerization of the RAF (A, B, or C) protein occurs⁶. This dimerization is crucial to RAF protein kinase activity and contributes to the complex activation of downstream RAF signaling.

3.1. BRAF mutation

Constitutively activating *BRAF* mutations are well documented. These have been described to promote tumorigenesis through downstream tumor suppressor inactivation and inhibition of apoptosis. These mutations are also believed to increase the metastatic potential of a tumor⁷. Various human cancers including hairy cell leukemia, papillary thyroid carcinomas,

melanoma, colorectal cancer, gastric cancer, and hepatocellular carcinoma were found to contain activating *BRAF* gene mutations¹⁰. Particularly, *BRAF*^{V600E} (previously V599E due to sequencing error that left out three extra nucleotides in exon 1) was identified in approximately 50% of melanomas. This mutation accounts for 90% of all known *BRAF* mutations^{5,6}. In addition, studies have revealed a high percentage of *BRAF*^{V600} mutations in their melanoma progression models consisting of melanocytic nevi (82% of samples with V600E mutations) progressing to a primary melanoma (80% of samples) and ultimately to metastatic melanoma (68% of samples)³⁰. The mutation occurs within the activation site in the CR3 resulting in an introduction of negative charges to the DFG motif that promote active conformation⁶. Activated *BRAF*^{V600} turns on the downstream MAPK pathway, independent of upstream RAS activation, through direct phosphorylation of MEK³¹. Although *BRAF*^{V600} is critical in melanoma progression, it was found to be insufficient for melanoma tumorigenesis^{30,32}. This was further validated with the generation of *BRAF*^{V600} transgenic mice that developed hyperplastic nevi but no development of melanoma. This particular study revealed an important biologic paradigm, namely that additional mutations are required for melanoma progression³³. Nonetheless, *BRAF*^{V600} targeting gained increasing interest in the treatment of advanced melanoma.

4. BRAF Inhibitor – Preclinical

With identification of the *BRAF*^{V600} mutation, interest mounted to discover a kinase inhibitor of this protein with potential therapeutic benefits. Researchers sought for an ideal kinase inhibitor that would function as a potent and a highly selective enzymatic antagonist. Utilizing a high-throughput kinase screening method with a library of 20,000 compounds ranging in sizes of 150-350 daltons molecules that inhibited BRAF enzymatic activity were identified³⁴. The initial screening process identified 238 compounds, which were further characterized through protein-inhibitor co-crystallography analysis to ensure for highly selective binding. It was found that an optimal chemical structure with high affinity binding to the active kinase site of BRAF required a 7-azaindole group³⁴. Kinase-inhibitor bound protomer adopted the DFG-in conformation to enable the formation of a unique hydrogen bond between the backbone of NH of Asp594 and the sulfonamide nitrogen of the inhibitor. The inhibitor bound to α C-helix and displayed the DFG-in conformation with the activation loop locked away from the ATP-binding site by a salt-bridge between Glu600 and Lys507 causing inhibition of *BRAF*^{V600}³⁵. Further characterizations identified the first candidate compound: propane-1-sulfonic acid [3-(5-chloro-1H-pyrrolo[2,3-b]pyridine-3-carbonyl)-2,4-difluoro-phenyl]-amide, also known as PLX4720³⁴.

PLX4720 was highly selective for the activated forms of both wild type BRAF and BRAF with a V600 mutation with similar affinity³⁴. However, PLX4720 selectively inhibited *BRAF*^{V600} at a lower concentration *in vitro* by 10-fold compared to wild type and *in vivo* by 100× due to cellular selectivity for *BRAF*^{V600} expressing melanoma cell lines³⁴. Kinase inhibition resulted in potent decrease in downstream MAPK pathway activation, resulting in cell cycle arrest and increased apoptosis specifically in *BRAF*^{V600} melanoma cells³⁴. Further pharmacokinetic analysis of PLX4720 characterized the compound's potential as an orally administered agent, which showed excellent oral bioavailability. Xenograph studies performed on multiple, *BRAF*^{V600}-containing melanoma cell-lines in SCID mice treated

with 14-day course of orally-dosed PLX4720 revealed potent anti-tumor, dose-dependent (5-1000 mg/kg) effects with clear tumor regression without any *in vivo* adverse reactions³⁴.

PLX4032, later renamed vemurafenib, was also being concurrently investigated for its efficacy in inhibition of melanoma. Like PLX4720, vemurafenib exhibited dose-dependent anti-proliferative and apoptotic effects in melanoma cells via downstream inactivation of the MAPK signaling cascade^{8,36}. The anti-tumor effects were specific to the *BRAF*^{V600} vs. *BRAF*^{WT} resulting in G1 arrest in treated cells followed by apoptosis³⁷. Interestingly, this anticancer drug effect of vemurafenib was tested in thyroid cancer cells harboring the *BRAF*^{V600} mutation, but did not demonstrate similar anticancer success, establishing the drug's cellular specificity³⁶. Dose-dependent pharmacokinetic assessment in both rats (up to 2600μM) and dogs (up to 820 μM) for durations up to 26 and 13 weeks, respectively, was performed without any observed impairing toxicity³⁵. This exhibition of favorable pharmacokinetics in higher mammals (beagle dogs and cynomolgus monkeys) was the reason for vemurafenib being chosen over PLX4720 for further drug development³⁵. With promising pre-clinical data, vemurafenib was soon approved by the FDA for use in a phase I clinical trial.

4.1. Vemurafenib phase I

The BRAF Inhibitor in Melanoma phase I trial (BRIM1) was initiated to assess the role of vemurafenib in advanced stage (metastatic) melanoma in patients with the *BRAF*^{V600} mutation under the sponsorships of Roche Pharmaceuticals and Plexxikon¹³. Goals of the study were to evaluate drug pharmacokinetics and safety as well as characterize early clinical drug efficacy through assessment of tumor response rate, duration, and failure¹³. Adverse events were reported using the NCI Common Terminology Criteria for Adverse Events (CTCAE) guidelines. CT studies were performed at 8-week intervals (4 weeks for some patients) with grading of the findings per Response Evaluation Criteria in Solid Tumors (RECIST).

Following informed consent, the trial enrolled patients age 18 and over with advanced melanoma refractory to standard of therapy or without any available standard/curative therapy and the absence of known progressing or unstable brain metastasis. Patients also had to have an ECOG performance status score of 0 or 1, a life expectancy of 3 months or longer, and adequate organ function (hepatic, renal, and hematologic)¹³.

BRIM1 enrolled a total of 55 patients¹³. The trial was designed with two phases in mind: (1) Dose-Escalation (patients with any tumor genotype) and (2) Extension (patients with *BRAF*^{V600} tumor genotype). During the dose escalation phase, vemurafenib required re-formulation due to poor bioavailability with oral dosing and was converted from a crystalline to a microprecipitated bulk powder formulation. Vemurafenib dosing was initiated at 160 mg (capsule) using a BID regimen with subsequent dose escalation to 240, 320, 360, 720, and 1120 mg twice daily. Dose-limiting toxicity was reported at the 1120 mg dose with patients reporting a grade 3 rash, arthralgia, and fatigue. Therefore, 960 mg twice daily dosing was established as the maximal dose for the extension phase. Despite this dosing adjustment, 13 patients in the trial required revision of their dosing regimen during the extension phase. The trial identified most commonly grade 2 or 3 side effects including arthralgias, rash, nausea,

photosensitivity, fatigue, cutaneous squamous cell carcinoma, pruritus, and palmar-plantar dysesthesia. Interestingly, photosensitivity is more common with vemurafenib (38.9%) in comparison to other BRAF inhibitors such as dabrafenib (0.8%)³⁸. A total of 8 patients in the dose-escalation phase and 10 patients in the extension cohort experienced the development of cutaneous squamous cell carcinoma (SCC). In most patients, the median time of treatment to appearance of SCC was 8 weeks with some patients undergoing operative resections. There were no systemic SCC metastases observed¹³.

The tumor response from this phase I trial was exceptional. Overall response rate in the dose-escalation phase was 69% with 11/16 patients experiencing partial responses while 1 patient of the 16 experienced a complete response¹³. The duration of responses ranged from 2 months to greater than 18 months. In contrast, patients in the extension phase experienced significantly higher response rates, providing further validity to the specificity of the drug for melanomas harboring the *BRAF*^{V600} mutation. In this group, 81% of patients experienced an overall response with median progression-free survival of greater than 7 months¹³.

4.2. Vemurafenib Phase II

A multi-center phase II trial (BRIM2) was initiated to determine the clinical response rate of vemurafenib in *BRAF*^{V600} patients with previously treated advanced staged melanoma¹¹. The study utilized a vemurafenib dose of 960 mg BID that was previously established from the BRIM1 Phase 1 study. Patients included in the study were again over the age of 18 with histologically proven stage IV melanoma and progressive disease following prior systemic treatment. They also had to have an ECOG status 0 or 1, no other invasive cancers, and no underlying organ dysfunction (hematologic, hepatic, and renal). Different from BRIM1, however, this study included patients with controlled metastatic disease to the brain, which allowed for assessment for real-life clinical efficacy. Tumor assessment according to RECIST was performed at 6-week interval and at the final visit. Again toxic effects of the drug were graded according to CTCAE.

The trial enrolled a total of 132 patients. The results of the study revealed a complete response (CR) in 6% of patients and a partial response (PR) in 47%, resulting in overall response of 53%¹¹. The median duration of the response was 6.7 month and the median progression-free survival was 6.8 months. Progression of disease was identified in 14% of patients¹¹. During the trial, 45% of the patients required dose adjustments due to reported adverse events. As previously reported in Phase I trial, the common reported adverse events included arthralgia, rash, fatigue, and cutaneous squamous-cell carcinoma (occurred in 26% of patients with no evidence of progressive disease and a median time to development of 8 weeks following initiation of treatment)¹¹. One of the poor prognostic indicators identified during the trial was an elevated serum LDH level ($>1.5\times$ the normal serum)¹¹. In patients with this elevated LDH, the overall response was only 33%, which although lower than those without LDH elevation, was still a significant improvement in comparison to current standard of care therapy¹¹. The trial revealed enhanced anti-tumor effects with significant clinical response in patients harboring *BRAF*^{V600}.

4.3. Vemurafenib Phase III

Next a multi-center international Phase III trial (BRIM3) was designed to compare the clinical efficacy of vemurafenib versus dacarbazine¹². Previous studies have revealed response rates of 7-12% with dacarbazine and a median survival time of 5.6-7.8 months following therapy initiation³⁹. BRIM3 aimed to compare the drugs in their clinical efficacy including overall and progression-free survival for both vemurafenib and dacarbazine¹². Trial eligibility was similar to BRIM 1&2 but also included previously untreated stage IIIC or stage IV melanoma with *BRAF*^{V600}.¹²

The trial enrolled and randomized 680 patients to either vemurafenib or dacarbazine treatment arms¹². Overall survival at 6-months was evaluated for 672 of the patients treated and revealed an increased survival in vemurafenib (84%) in comparison to dacarbazine (64%)¹². Progression-free survival was evaluated in 549 patients and demonstrated a hazard ratio for tumor progression in vemurafenib of 0.26.¹² The estimated median progression-free survival was 5.3 months in the vemurafenib group and 1.6 months in the dacarbazine group¹². During the trial, patients in the dacarbazine group were allowed to crossover to the vemurafenib arm due to the significant clinical efficacy. Regarding tumor response, a superior confirmed objective response was seen in the vemurafenib group (48%) compared to 5% in the dacarbazine group¹². As expected, similar adverse events were observed in the vemurafenib group as previously reported in the BRIM2 trial. These adverse events led to dose modification or treatment interruption in 38% of patients in the vemurafenib group vs. 16% in dacarbazine group¹². Overall, the investigative drug resulted in a relative risk reduction in mortality of 63% and a reduction of 74% in the risk of tumor progression in patients with previously untreated, unresectable stage IIIC or IV metastatic *BRAF*^{V600} melanoma¹². The study solidified the role of vemurafenib as an efficacious therapeutic agent in the treatment of metastatic *BRAF*^{V600} melanoma.

4.4. Open Label Trials

With the clinical efficacy of vemurafenib demonstrated for advanced stage melanoma in a large multicenter Phase III trial, the FDA approved vemurafenib on August 17, 2011.⁴⁰ An open, multi-center, international study was then established to examine the effectiveness of the drug in a real-world clinical practice setting. Unlike the BRIM trials, this open label study specifically sought to include patients with poor prognostic indicators such as high ECOG performance scores, brain metastasis, and elevated LDH levels.⁴¹

The study was performed in 44 countries, excluding the USA. The study enrolled 3226 patients diagnosed with advanced metastatic *BRAF*^{V600} melanoma (including brain metastases) irrespective of ECOG status and serum LDH levels⁴¹. The goal of the trial was to examine the safety and objective tumor response of vemurafenib per RECIST criteria in this more diverse population.

Overall, the patients tolerated the treatment well but 95% experienced at least one adverse event with a majority (93%) being grade 1 and 2 by RECIST⁴¹. Like previous studies, most of these low-grade adverse events consisted of rash (48%), arthralgia (38%), fatigue (32%), photosensitivity (30%), alopecia (26%), nausea (19%), and hyperkeratosis (19%)⁴¹. These

side effects resulted in dose reduction in 14% patients. Several patients also experienced grade 3 adverse events (46%), consisting of cutaneous SCC (12%), rash (5%), arthralgia (3%), fatigue (3%), and photosensitivity (2%)⁴¹. The median time to development of SCC in this cohort was 2.6 months, which was comparable to the 8 weeks noted in the BRIM trials⁴¹. Interestingly, this study identified several unique adverse events related to treatment. These included the development of a new primary melanoma (1%), cutaneous T-cell lymphomas (3 cases) and one patient who developed progression of their chronic lymphocytic leukemia⁴¹. One important finding of the study was that presence of brain metastasis or increased LDH levels were not associated with an increase in adverse events⁴¹. This data suggests that the medication is well tolerated in the general population with expected adverse events.

In an analysis of patient outcomes, 42% of patients died during the study period however the cause of death in this group was unrelated to vemurafenib treatment in 98% of cases⁴¹. Adverse events leading to death were reported in 20 patients and included general physical health deterioration (5 patients), cerebral hemorrhage (5 patients), cerebrovascular accident (4 patients), and pulmonary embolism (4 patients)⁴¹. A complete response (CR) to treatment was observed in only 3% of the subjects while a partial response (PR) was demonstrated in 31% and stable disease was observed in 55%⁴¹. Disease progression was observed in 11% of patients with 16% of these having brain metastases, 14% with elevations of LDH, and 18% with an ECOG>2 at the beginning of the trial⁴¹. Median progression free survival was 5.6 months with an overall survival of 75% of patients at 6 months, 52% at 12 months, and 36% at 18 months following initiation of treatment⁴¹. Although these were slightly lower than previously reported in the BRIM studies, this study also included patients with elevated serum LDH levels, which was previously identified as a poor prognostic marker and correlated with reduced response rates. Nonetheless, the study demonstrated the safety and effectiveness (improvement of 1 year survival in comparison to previous study demonstrating 4-34%) of vemurafenib in the study population.

4.5. Resistance

Recent examination of the long-term survivors (n=9, defined as survival greater than 3 years) from the BRIM1 study identified a correlation of lower baseline tumor load with non-CNS metastasis and an ECOG performance score of 0 with that of overall survival⁴². The remainder of the patients in this group experienced disease progression. Similar to other kinase inhibitor studies, vemurafenib treatment revealed an initial dramatic clinical response followed by a rapid acquisition of drug resistance. The resistance to vemurafenib is not, however, due to an accumulation of additional *BRAF* mutations⁴³. A hypothesized mechanism of resistance requires both intrinsic and extrinsic pathway activations. As previously stated, BRAF is a member of the MAPK pathway. We define the mechanism of an intrinsic resistance as activation of the MAPK pathway. Conversely, we define the extrinsic resistance as activation of alternative signaling pathways.

While preclinical studies revealed the inhibiting effects of the vemurafenib-BRAF^{V600} protomer, recent studies reveal a paradoxical activating role of the drug. In BRAF^{V600}, the MAPK pathway is more sensitive to the ATP-competitive RAF inhibition due to increased

ATP-based, BRAF^{V600} activity⁴⁴. However, treatment of BRAF^{WT} cells resulted in an increased activation of the MAPK pathway, and this coincided with an increased expression and activation of CRAF^{44,45}. It is believed that the BRAF^{WT}–vemurafenib protomer both enhanced and stabilized the heterodimerization with CRAF, resulting in subsequent MAPK pathway reactivation⁴⁴⁻⁴⁶. The formation of the BRAF^{WT}–vemurafenib protomer can occur even in BRAF^{V600} melanomas owing to heterogeneity of cell types within the tumor. A recent study examining the melanoma micro-tumor environment utilized laser microdissection and genotyping to confirm this hypothesis of intra-melanoma cellular and genetic heterogeneity⁴⁷. As such, treatment with BRAF inhibitors can paradoxically select for non- BRAF^{V600} cells to survive and cause both drug resistance and possible development of cutaneous SCC.

Moreover, mutations in genes upstream of RAF, such as the activating *N-RAS*^{Q61K} mutation, allow for BRAF^{V600} melanomas to escape molecular targeting^{43,48}. In these cells, NRAS is thought to signal preferentially through CRAF to activate the downstream MAPK pathway⁴⁹. As such, inhibition of MEK downstream of BRAF is becoming a more attractive therapeutic strategy to avoid some of this resistance.

Examination of patient-derived, therapy-resistant melanomas revealed extrinsic signaling activation of other pathways such as the PI3K/AKT pathway^{43,46,48}. Molecular and pathologic analysis of the resistant samples revealed increased *PDGFRβ* expression as well as an increase in the activation of the PI3K/AKT pathway^{43,48}. Moreover, cell-lines derived from the resistant samples exhibited increased proliferation and growth, which was decreased following siRNA suppression of *PDGFRβ*.

As a result of these observations, various new preclinical studies have proposed methods of curtailing therapy resistance, such as the use of HSP90 inhibition with geldanamycin or downstream MEK inhibition^{43,45,50}. In another experiment, cells expressing increased *PDGFRβ* expression when treated with an investigational compound, XL888, resulting in activation of FOXO3-mediated BCL-2 interacting mediator of cell death (BIM) leading to induction of apoptosis.⁵¹

4.6. Cutaneous SCC

One of the more interesting adverse toxicities experienced with vemurafenib treatment, like other kinase inhibitors, is the development of cutaneous squamous cell carcinomas⁵². These lesions frequently appear approximately 8-12 weeks from start of treatment¹¹⁻¹³. The mechanism of cutaneous SCC development is also hypothesized to be secondary to a paradoxical activation of the MAPK pathway. Examination of SCC samples from vemurafenib-treated patients frequently demonstrates activating *HRAS*^{Q61L} mutations^{53,54}. An *in vitro* model containing melanoma cells with *HRAS*^{Q61L} mutations treated with vemurafenib revealed proliferation stimulation through increased MAPK-pathway transcriptional effects⁵³. In addition, treatment of *HRAS*^{Q61L}-SCC mouse models with MEK inhibitors resulted in decreased activation of pERK1, suggesting a possible treatment options for SCC in vemurafenib treated patients⁵³.

Another paradoxical activation of the MAPK pathway is through the increased expression of *CRAF*⁴⁵. CRAF knockdown studies with shRNA revealed decreased downstream MAPK activation in the setting of vemurafenib treatment⁴⁴. As described in previous sections, RAF activation requires either homo- or heterodimerization between different RAF kinases. In the setting of vemurafenib treatment, increased CRAF expression results in an increased rate of BRAF/CRAF heterodimerization as well as CRAF homodimerization resulting in MAPK activation⁴⁴. Vemurafenib stabilizes this CRAF/BRAF heterodimerization through stabilization of the ATP binding domain⁴⁶. Furthermore, this heterodimerization is also mediated by RAS (either wild type or mutant)⁷. Following cessation of vemurafenib treatment, paradoxical MAPK activation decreases. This supports the role of vemurafenib treatment in MAPK signaling activation⁴⁴. Therefore, it is suggested that vemurafenib treatment results in CRAF- dependent activation of MAPK in normal cutaneous cells resulting in a transformation to squamous cell carcinomas.

4.7. Vemurafenib – Combination Trial

With prior studies revealing activation of the MAPK pathway as a mechanism of both drug resistance and development of cutaneous SCC, combination therapy of vemurafenib and cobimetinib (MEK inhibitor) has gained increasing interest.⁵⁵ The efficacy of this combination therapy was demonstrated first in an *in vitro* study with concurrent administration of vemurafenib and cobimetinib to the BRAF^{V600} melanoma cell-lines with acquired resistance.⁵⁶

As a result, a phase I trial was initiated examining the safety of cobimetinib and vemurafenib as a dual therapy.⁵⁷ The study enrolled 129 patients (66 previously treated patients who progressed and 63 previously untreated patients) over the age of 18 with unresectable stage IIIC or IV melanoma harboring a BRAF^{V600} mutation. The study revealed dose-dependent toxic effects that were more frequent in patients naïve to vemurafenib. The adverse events included non-acneiform rash, diarrhea, fatigue, photosensitivity, and liver enzyme abnormalities. Cutaneous SCC was observed in 13% of patients previously naïve to vemurafenib and 9% in patients previously treated with vemurafenib.⁵² Toxic effects typical of MEK inhibitor treatment were also observed including: chorioretinopathy, retinal detachment, macular edema, and cardiomyopathy (one patient). Complete responses were observed in 10% patients treated with dual therapy who never received a previous BRAF inhibitor. Conversely, there was no complete response observed in patients who had previously progressed on vemurafenib. Higher partial response rates were seen in patients initially naïve to vemurafenib when compared to those who had received it prior to the study (78% vs. 15% respectively). Moreover, stable disease was reported in 36% of patients with disease progression prior to enrollment in the study. However, progression of disease was observed more in the patients who did not respond to previous therapy compared to drug-naïve patients (36% vs. 3%). The combination therapy was well tolerated and revealed a potential therapeutic response to an unresectable melanoma.

Building on the previous trial, an international, multi-center phase 3 trial evaluated the clinical efficacy of cobimetinib in combination with vemurafenib in previously untreated advanced stage BRAF^{V600} melanoma.⁵⁵ The study enrolled 495 patients who were

randomized to either study drug combination (cobimetib and vemurafenib) or control group (placebo and vemurafenib).⁵⁵ The combination arm clearly demonstrated significant progression-free survival with a median survival of 9.9 months vs. 6.2 months in the control arm.⁵⁵ The calculated hazard ratio for disease progression or death was 0.51 (95% CI, 0.39 to 0.68; $p < 0.001$)⁵⁵. In addition, investigators measured response rates and demonstrated a clear advantage of the combination therapy over vemurafenib alone. An objective overall response of 68% was observed in the combination arm vs. 45% in the control (vemurafenib only) arm ($p < 0.001$)⁵⁵. The combination study arm also exhibited increased complete response (CR) rates (10% vs. 4% in controls), while the rate of stable disease decreased (20% combo vs. 42% control) as did disease progression (8% in the combination arm vs. 10% in the control arm)⁵⁵. Such clinical improvement was seen in a setting of equivalent grade 3 adverse events (49% combo vs. 49% controls) and fewer grade 4 events (9% in the combination arm vs. 13% in the control arm)⁵⁵. In this evolving therapeutic landscape of targeted melanoma treatment, combinatorial MAPK pathway inhibition with cobimetinib and vemurafenib revealed improved clinical efficacy in advanced stage *BRAF*^{V600} melanoma. Due to the favorable outcomes observed in the combination trial, the FDA approved cobimetinib and vemurafenib as a combination therapy for melanoma in late 2015.

Lastly, a combination trial examining vemurafenib and the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) blocking antibody ipilimumab in patients with metastatic melanoma, previously not treated with either agent was performed⁵⁸. However, the trial was abandoned due to increased liver toxicity observed within 3 weeks of initiation of dual therapy⁵⁸. These results demonstrated new toxicity caused by combination therapy, even if both drugs were previously approved in independent uses.

5. Conclusion

In conclusion, the discovery and success of vemurafenib therapy on advanced stage *BRAF*^{V600} melanoma clearly represents an evolution in modern cancer targeted therapy. The framework behind the discovery of the *BRAF*^{V600} mutation and the rapid development of vemurafenib leading up to its FDA approval will continue to serve as a platform for future cancer drug discovery. Although there are still many hurdles to overcome including drug resistance, ongoing trials and thoughtful preclinical research continues to address these challenges through novel drug combination approaches and expansion of melanoma clinical stage treatment applications.

6. Expert Opinion

The characterization of the *BRAF*^{V600} gene mutation and the discovery of vemurafenib as a successful inhibitor of melanomas with this mutation clearly exemplify the innovation of modern cancer care in the era of precision medicine. Vemurafenib is a clinically efficacious, well tolerated, orally dosed chemotherapeutic with a manageable dosing schedule and mild to moderate adverse events. Together, these benefits support its use as a preferential treatment for advanced melanoma patients harboring the V600 mutation of BRAF. Prior to the discovery of vemurafenib, patients with advanced, metastatic melanoma were left with limited chemotherapy treatment options (i.e., dacarbazine) carrying a low response and

survival expectation. For patients treated with dacarbazine, that overall response rate was only 7-12% with a median survival following therapy initiation of only 6 months¹². The BRIM trials revealed a clear clinical superiority of vemurafenib in treating advanced, metastatic melanoma patients with *BRAF*^{V600} mutations, demonstrating overall response rates of approximately 50% and an increase of progression-free survival by 5 months, in comparison to dacarbazine¹². It has been reported that the expected 5-year relative survival of a patient with advanced metastatic melanoma is only 16%¹. With the advent of vemurafenib, this relative survival in melanoma patients positive for the *BRAF*^{V600} mutation is expected to increase. As the mutation is demonstrated in approximately 50% of melanomas, this targeted therapy is now able to benefit a larger portion of advanced melanoma patients who in the past were left with limited treatment options⁵. Longer duration therapeutic studies with vemurafenib are currently underway and are likely to reflect this opinion.

The efficacy of vemurafenib in the treatment of *BRAF*^{V600} is not limited to melanoma, as demonstrated in the recent clinical phase 2 “basket” trial for non-melanoma cancers harboring the mutation⁵⁹. In these cancers, the V600 mutation was identified with an incidence less than 5%⁵⁹. Particularly in colorectal cancer and non-small cell lung cancers, the mutation was suggested to cause a more aggressive tumor biology, resulting in decreased overall and disease free survival⁵⁹. The “basket” trial sought to further examine the therapeutic potential of vemurafenib in these rare, non-melanoma, *BRAF* V600 mutated cancers. The study included patients with NSCLC, ovarian cancer, colorectal cancer, cholangiocarcinoma, breast cancer, multiple myeloma, and “all-other” cancer harboring the mutation⁵⁹. The study results revealed response rates of 42% in NSCLC and 43% in Langerhans'-cell histiocytosis⁵⁹. Unfortunately, colorectal cancer and cholangiocarcinoma did not demonstrate therapeutic response⁵⁹. The study concluded that different tumor types with the same molecular biomarkers differ in their sensitivity to targeted therapies against that biomarker. Nonetheless, the trial demonstrated the treatment potential of vemurafenib in a subset of non-melanoma cancers with V600 mutations.

Although this review focuses primarily on the development and efficacy of vemurafenib, there exists additional FDA approved *BRAF*^{V600} therapeutics such as dabrafenib. The BREAK trials demonstrated tumor regression and improved progression free survival in patients treated with dabrafenib⁶⁰. The efficacy of different compounds targeting *BRAF*^{V600} demonstrated the importance of this underlying mutation in tumorigenesis.

Unfortunately, cancer is a disease of evolution that frequently develops resistance mechanisms to chemical treatment. Patients treated with vemurafenib often will develop a therapeutic resistance within 6-8 month of treatment initiation, resulting in disease progression¹¹⁻¹³. Some hypothesized resistance mechanisms are either dependent (or independent) on MAPK pathway reactivation. Preclinical resistance studies demonstrate stabilization of CRAF and upstream RAS mutations as proposed mechanisms of resistance^{44,49}. To address this resistance pathway, combination therapy using vemurafenib with the MEK inhibitor, cobimetinib, was established⁵⁵. In clinical combination studies, adding the MEK inhibitor to vemurafenib treatment increased overall response rates to 68% and increased progression free survival by 3 months⁵⁵. Regarding extrinsic resistance

mechanisms, preclinical studies identified both activation of the AKT/PI3K pathway or upregulation of *PDGFR β* as potential mechanisms^{43,48}. Further trials examining this resistance are certainly needed. Another area being focused on in the setting of targeted therapy for metastatic melanoma is related to the extent of disease required to benefit from treatment. Current indications for vemurafenib are for advanced metastatic disease, however, earlier stage III (A and B) patients with *BRAF*^{V600} mutations may also benefit from therapy and carry lower disease burdens to initiate resistance mechanisms.

Another area of ongoing and future investigation with vemurafenib is its use in combination with immunotherapy. Recent trials with ipilimumab (anti-CTLA4) and pembrolizumab (anti-PD1) demonstrated prolonged survival in patients with metastatic disease. Interestingly, the combination trial of vemurafenib and ipilimumab, however, was abandoned early due to hepatic toxicity⁵⁸. Combination studies with vemurafenib and pembrolizumab remain to be conducted, and future clinical studies will be needed to better evaluate the therapeutic role of BRAF inhibitors in combination studies with emerging, novel therapeutics.

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Article Highlights

- Shortly after its discovery, vemurafenib was tested in clinical trials with display of low toxicity, excellent tolerance, and most importantly, rapid clinical response in advanced melanoma patients harboring the BRAF^{V600E} mutation.
- Despite however a low number of adverse events, a significant amount of patients treated with vemurafenib developed localized cutaneous squamous cell carcinomas (SCC).
- Another limiting factor of vemurafenib is that treated patients eventually succumb to therapy resistance with progression of disease within 6-8 months of treatment initiation.
- The BRIM2 Phase II study with vemurafenib revealed enhanced anti-tumor effects with significant clinical response in patients harboring BRAF^{V600E} and identified elevated LDH levels as a poor prognostic indicator to response rates.
- The multicenter international BRIM3 Phase III study compared vemurafenib with dacarbazine and showed a significant improvement in overall and progression-free survival with vemurafenib but also demonstrated an increased number of adverse events and dose modifications and interruptions due to these toxicities.
- It is suggested that vemurafenib treatment results in CRAF dependent activation of MAPK in normal cutaneous cells resulting in a transformation to squamous cell carcinomas.
- A large multi-center phase 3 trial evaluated the clinical efficacy of cobimetinib in combination with vemurafenib in previously untreated advanced stage BRAF^{V600E} melanoma showing improved survival over vemurafenib alone in this population.
- This box summarizes key points contained in the article.

Figure 1.

Chemistry structure. PLX4720 and vemurafenib are isoforms containing 7-azaindole core, which is responsible for binding and inhibition of the BRAF^{V600E} activation core. Addition of 4-cholorphenyl group in vemurafenib is responsible for increased bioavailability and pharmacokinetics in higher mammals.

Figure 2.

BRAF structure. The protein consists of three conservative regions CR1, CR2, and CR3. CR1 contains the RAS binding domain (RBD) and cystein rich domain (CRD). CR2 contains the phosphorylation domain (PD) enriched in serine and threonine residues. CR3 contains the kinase domain (KD) and contains the valine 600 residue often mutated in melanoma. Red star: V600E mutation.

Figure 3.

Physiologic and BRAF^{V600E} MAPK pathway activation. Growth factor activation of receptor tyrosine kinase results in activation of downstream BRAF through hetero-/homodimerization resulting in ERK activation for increased transcription, differentiation, and proliferation. BRAF^{V600E} mutation results in RTK-independent, constitutive activation of downstream MAPK activation for melanoma tumorigenesis. Vemurafenib inhibits BRAF^{V600E} to shut down this aberrant pathway activation.

Figure 4.

Mechanism of resistance and SCC development. BRAF^{V600E} inhibition via vemurafenib induces paradoxical activation of MAPK through increased CRAF activity and acquired NRAS mutation. In addition, increased PDGFRb expression with subsequent activation of PI3K/AKT pathway for increased resistance. It is hypothesized that these reported mechanisms are shared in drug resistance as well as cutaneous squamous cell carcinoma development.

Table 1
Summarization of the clinical trials with Vemurafenib. DE: Dose-escalation, E: Escalation, V: Vemurafenib, D: Dacarbazine, C: Control, E:
Experimental/ Treatment

Adverse Reaction										
Study	Year	Number of Patients	Grade 1&2	Grade 3	Cutaneous SCC	Overall Response	Complete Response	Partial Response	Progression	
BRIM1	2010	87 (DE: 55; E: 32)	89%	11%	DE: 15%; E: 31%	DE: 69%; E: 81%	DE: 6%; E: 6%	DE: 63%; E: 75%	DE: 31%; E: 19%	
BRIM2	2012	132	98%	60%	26%	53%	6%	47%	14%	
BRIM3	2011	675 (V: 337; D: 338)	92%	V: 38%; D: 14%	12%	V: 48%; D: 12%	V: 1%; D: 0%	V: 47%; D: 12%	V: 52%; D: 95%	
BRIM7	2014	129			C: 9%; E: 13%	C: 57%; E: 92%	C: 0%; E: 10%	C: 15%; E: 78%	C: 36%; E: 3%	
coBRIM	2014	495		C: 59%; E: 65%	C: 11%; E: 2%	C: 45%; E: 68%	C: 4%; E: 10%	C: 40%; E: 57%	C: 10%; E: 8%	