Vemurafenib and BRAF Inhibition: A New Class of Treatment for Metastatic Melanoma

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Abstract:

The United States Food and Drug Administration recently approved vemurafenib for the treatment of BRAF V600E mutant metastatic melanoma. Vemurafenib is a competitive small molecule serine-threonine kinase inhibitor that functions by binding to the ATP binding domain of mutant BRAF. Compared to dacarbazine chemotherapy, vemurafenib significantly improved the six month overall survival of patients from 64% to 84% and exhibited a response rate of approximately 50%. Median progression free survival was also significantly improved with vemurafenib as compared with dacarbazine (5.3 vs 1.6 months, respectively) and this was consistent among groups analyzed including age, sex, geography, ECOG status, disease stage, and serum LDH. The success of targeting melanoma genomics has created a paradigm shift for future drug development. Currently, the elucidation of resistant mechanisms to vemurafenib therapy remains an important area of active investigation that will shape rational drug treatments for melanoma. The development of vemurafenib, the role of BRAF targeting, and the changing landscape of treatment for melanoma provide a new foundation for clinical investigation.
Introduction

The recent approval of vemurafenib by the United States Food and Drug Administration (FDA) for the treatment of BRAF<sup>V600E</sup> mutant melanoma marks a paramount change in the clinical management of melanoma patients. Historically, treatment options for melanoma were limited. Chemotherapy has long been considered a standard of care; however it is associated with a modest response rate and no proven overall survival benefit(1). Immunotherapy has also been of interest in melanoma. While highly efficacious in a subset of patients, immunotherapy for melanoma currently lacks needed predictive biomarkers for efficacy and toxicities. The recent development of the CTLA-4 blocking monoclonal antibody ipilimumab (Yervoy®) has begun to change the previously limited enthusiasm for this type of treatment. Yet, even the benefit of ipilimumab in melanoma is still limited to a select number of patients(2). The identification of mutant BRAF as a therapeutic target and the emergence of vemurafenib open new avenues of research and bring promise for personalized medicine to the clinical care of melanoma patients.

BRAF as target in melanoma

Signaling through and downstream of the mitogen-activated protein kinase (MAPK) pathway has been shown to drive the growth of most cutaneous melanomas(3). More specifically, mutations in NRAS and BRAF have been characterized to constitute up to approximately 80% of the driver lesions in this pathway(4, 5). BRAF itself accounts for approximately 60% of these, with greater than 90% of BRAF mutations resulting from the substitution of glutamic acid for valine in exon 15, at codon 600 (V600E), and effecting the kinase domain of the protein. The pharmacologic difficulties of developing inhibitors of the RAS isoforms are well documented(6, 7). However, given the preponderance of BRAF mutations in melanoma, there has been continued interest in the development of inhibitors of BRAF for nearly a decade.

Previous attempts to target RAF for therapeutic purposes have been unsuccessful. The multi-targeted kinase inhibitor sorafenib was initially developed with this purpose in mind. Clinical trials eventually ruled out the utility of this agent as a single agent and in combination with chemotherapy(8, 9). The disappointing results with sorafenib brought into question whether BRAF could be adequately targeted in melanoma with therapeutic benefit. The successful development of vemurafenib has now overcome this uncertainty. Importantly, this was accomplished through a greater understanding of the RAF isoforms and a novel pharmacologic development strategy that allowed selective and potent (> 80%) inhibition of the V600E protein(10) while still maintaining a tolerable side effect profile and oral delivery route.

Upon growth signaling, such as activation of RAS, normal BRAF forms both homo- and heterodimers with the other RAF isoforms ARAF and CRAF (also known as RAF1). These dimers then cause activation of MEK and continued signaling down the MAPK pathway. Mutated BRAF, however, signals as a monomer, independent of upstream growth stimuli. Treatment with vemurafenib leads to inhibition of downstream signaling by mutant BRAF monomers. In addition, vemurafenib can also cause activation of downstream MEK by normal RAF homo- and heterodimers in non-BRAF mutated cells(11). This has been shown to be caused by transactivation of the nondrug-bound partner in BRAF to CRAF heterodimers and
CRAF to CRAF homodimers(12). This interaction of different isoforms, and the non-selectivity of the prior generation of RAF inhibitors for BRAF<sup>V600E</sup>, explains the failure of sorafenib. Sorafenib is at least as potent and likely more potent than vemurafenib against wild-type B- and CRAF as opposed to mutant BRAF.

The paradoxical activation of MEK by non-mutant RAFs not only outlines the specificity of vemurafenib for mutant BRAF but also provides an explanation for the vemurafenib treatment complication of squamous cell carcinoma (SCC) development. Via <i>in vitro</i> systems, it has been documented that MEK activation after BRAF<sup>V600E</sup> inhibition is accomplished by BRAF/CRAF dimerization and subsequent CRAF signaling(13). This has been confirmed by experiments inactivating (kinase-dead) BRAF, showing a similar effect to BRAF-inhibiting agents leading to downstream activation of MEK(14). A second model has proposed dose dependence for downstream MEK activation or inhibition based on lower or higher doses of BRAF inhibitors(12). Interestingly, both these experimental models require upstream activation, such as RAS or Epidermal Growth Factor Receptor (EGFR) activation. Such activation would not be unexpected in otherwise non-malignant skin tissue that was previously exposed to ultraviolet light exposure; however this has not been explicitly shown <i>in vivo</i> to date.

The development of a second malignancy during treatment, such as SCC with vemurafenib, is of concern however the clinical significance of these lesions needs to be considered closely. While the MEK/ERK activation seen in non-malignant tissue appears to be a mechanistic side effect of vemurafenib, the resultant lesions identified have not posed a significant clinical problem. These lesions have uniformly been removed without clinical sequelae(15). In fact, it has been proposed that the description of these lesions as SCC may be a pathologic misclassification, given that SCC implies a disease with an eventual possibility of metastatic spread. Instead, the lesions in question tend act in a manner more consistent with keratoacanthoma and thus are unlikely to do more than grow locally. Monitoring for the growth of these lesions will clearly be a part of clinical care of patients being treated with vemurafenib going forward however.

**Preclinical data**

Vemurafenib (Zelboraf®, PLX4032, RG7204, RO5185426, N-(3-{[5-(4-chlorophenyl)-1H-pyrrolo[2,3-b]pyridin-3-yl]carbonyl}-2,4- difluorophenyl)propane-1-sulfonamide) is an oral serine-threonine kinase inhibitor. Owing to the failure of prior BRAF inhibitors, a structure-guided discovery approach was pursued to identify molecules containing a pyridoimidazolone compatible with the ATP pocket-binding domain of BRAF. PLX4720, a 7-azaindole derivative, was the initial result of these investigations(16). This molecule established a new class of more specific kinase inhibitors with selectivity for mutant BRAF<sup>V600E</sup>. Subsequent studies demonstrated marked effects on apoptosis, proliferation, and blockade of downstream ERK phosphorylation. This translated into inhibition of growth in V600E mutant melanoma cell lines as well as tumor regression in xenograft models. Via this drug discovery approach, a panel of related small molecules was also discovered including PLX4032 (vemurafenib). PLX4720 and PLX4032 were evaluated simultaneously. Owing to a more advantageous pharmacokinetic
profile in dogs and cynomolgus monkeys, vemurafenib was chosen to take forward into further clinical development(10).

Vemurafenib was shown to be highly specific for BRAF<sup>V600E</sup> with an inhibitory concentration at 50% (IC<sub>50</sub>) of 31 nM. In melanoma cell lines as well as melanoma and colon cancer xenografts, vemurafenib was observed to inhibit tumor growth in a dose-dependent fashion(17). Animal studies in rats and beagle dogs showed no significant toxicity at a 1000 mg/kg/day on a 28 day dosing schedule or in longer studies with effective exposures tested up to 2600 µM in rats and 820 µM in beagle dogs(10). Notably, the effective exposure described in rats is higher than that administered to human patients.

**Clinical studies**

The initial phase I trial of vemurafenib included a 55 patient dose escalation followed by a dose expansion of 32 patients(18). All advanced solid tumor patients were eligible for participation in the dose escalation however tumors associated with a high incidence of BRAF V600E mutation made up the bulk of the accrual (49 melanoma, 3 thyroid, 1 rectal, 1 ovarian carcinoma). Eligibility requirements of note included Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 and the absence of brain metastases. Patients received daily drug dosing until the development of toxicity or progression of disease. Vemurafenib was initially developed in a crystalline formulation and administered at a starting dose of 200 mg daily. After 26 patients were treated, accrual was stopped due to poor pharmacokinetics. Serum sampling revealed that available drug levels were lower than required for efficacy based on pre-clinical modeling. The drug was then reformulated into a more highly bioavailable micro-precipitated powder, available as an oral capsule, and accrual was restarted. Twenty-nine patients were treated to a maximum dose administered of 1120 mg twice daily. After reformulation, the pharmacokinetics of vemurafenib demonstrated a linear increase in mean area under the curve (AUC) with increasing dose level. Additionally, a mean maximum concentration at steady state of approximately 86 µM and mean half-life of approximately 50 hours were documented.

Vemurafenib was generally tolerated well with no dose-limiting toxicity until the 720 mg twice daily dose level was initiated. Dose-limiting toxicities observed at the maximum administered dose included rash, fatigue and arthralgia. Squamous cell carcinoma (nearly always keratoacanthoma type) was an unexpected side effect seen during the dose escalation. The recommended phase II dose was deemed to be 960 mg twice daily. From doses of 240 mg twice daily and higher, 11 out of 16 melanoma patients were observed to obtain a response with 10 partial responses and one complete response. Additionally, three patients with papillary thyroid cancer had responses.

The phase I dose expansion accrued only melanoma patients documented to harbor BRAF V600E mutations and showed a striking response rate. Twenty-six of 32 patients (81%) exhibited a response and some experienced marked improvement in quality of life, as indicated by decreased narcotic use. The median progression-free survival (PFS) of responders was greater than 7 months and toxicities seen in the dose expansion were similar to those seen in
the dose escalation. Approximately 40% of patients required dose reduction and approximately one third of patients had development of keratoacanthoma type SCC.

Given these impressive results, vemurafenib was rapidly taken into phase II and phase III trials in melanoma. The results of the phase II study (BRIM-2) were presented at American Society of Clinical Oncology (ASCO) meeting in 2011(19). Notable eligibility criteria for the study included documentation of BRAF V600E mutation, exposure to a prior line of therapy, ECOG status of 0 or 1 and no evidence of brain metastases. The primary endpoint of the study was best overall response rate (BORR) and a pre-specified stratification by age, stage (M1a-c), performance status and LDH was pursued.

One hundred thirty-two patients were treated in BRIM-2 at 960 mg twice daily. The primary endpoint of the study was met with a 52% response rate. Benefit was seen across all dose levels however the benefit was less robust in patients who had elevated LDH at the start of treatment. The median duration of response was 6.8 months with a median PFS of 6.2 months. Toxicities were similar to the phase I study with arthralgia, rash, photosensitivity, fatigue, alopecia, pruritus, and skin papilloma making up the majority of grade 1-2 events. Grade 3 events were predominately rash, arthralgia and liver function abnormalities. Squamous cell carcinoma, majority keratoacanthoma type, was again seen at the frequency of 24%. Approximately 40% of patients required dose reductions during treatment on study.

The results of the phase III (BRIM-3) study of vemurafenib in BRAF V600E mutant melanoma were recently published(20) and these data have now led to FDA approval. BRIM-3 was a two arm randomized study comparing vemurafenib, 960 mg oral twice daily, to dacarbazine chemotherapy, 1000 mg/m² administered every 3 weeks. The initial primary endpoint was overall survival; PFS was later added as a co-primary endpoint after the results of the phase I and II studies were available. Eligibility requirements were similar to BRIM-2 requiring the patient’s tumor to harbor the mutation in BRAF^{V600E}, good performance status, and no history of central nervous system (CNS) metastases. Notably 20 patients treated on study were eventually found to have non-V600E mutations (19 V600K, 1 V600D).

Within one calendar year, 2107 patients were screened and 675 patients were randomized 1:1 for treatment on either study arm of BRIM-3. Patients were stratified by age, stage, performance status, geography, and LDH. The arms of the study well balanced. A planned interim analysis after 196 study deaths by an independent review board recommended cross-over of all patients being treated on the decarbazine arm as the study co-primary endpoints had been met. Overall survival at 6 months was found to be 84% in the vemurafenib arm and 64% in the decarbazine arm. Progression-free survival could be evaluated in 81% (549/675) of patients with a median value of 5.3 months for the vemurafenib arm versus 1.6 months for decarbazine arm. The survival benefit of vemurafenib was observed to be consistent in all subgroups, including LDH. The hazard ratio for tumor progression in the vemurafenib arm was 0.26 with a 95% confidence interval of 0.2-0.3. The response rate of each arm was 48% for vemurafenib (two complete responses) and 5% for decarbazine. Additionally, nearly all patients receiving vemurafenib obtained some tumor regression even if this did not meet Response
Evaluation Criteria in Solid Tumors (RECIST) 1.1 criteria for a response. Of the 20 non-V600E mutant patients treated, 10 patients (all V600K) were randomized to the vemurafenib arm. Of these, four patients had partial responses.

Vemurafenib was well tolerated in the BRIM-3 trial with the incidence of grade 1-2 and grade 3-4 adverse events similar to prior studies. Notably, 38% of patients required dose reduction in the vemurafenib arm. Sixty-one patients (18%) had development of SCC, keratoacanthoma type; however all were treated with local therapies. The length of response duration was not calculated as an insufficient number of patients had progressed at the time of publication.

Pharmacodynamics

BRAF is an intermediary in signal transduction through the MAPK pathway. Activation of BRAF is associated with downstream activation of ERK, subsequent elevations of cyclin D1, and an overall increase in cellular proliferation. Treatment with vemurafenib in clinical trials has been associated with reductions in all of these markers. In the vemurafenib phase I dose escalation, pre- and post-treatment (day 15) tumor biopsies were obtained from all patients and analyzed by immunohistochemistry (IHC) for pharmacodynamic effects(10). Nuclear and cytosolic ERK pathway activation was observed before and after treatment. While a plasma exposure of at least 300 µM was required to see tumor volume reduction, treatment at nearly all dose levels was associated with reduction in phosphorylated-ERK (p-ERK) and proliferation by Ki67. Decreases in cytoplasmic p-ERK, but not nuclear p-ERK, correlated with clinical benefit. Importantly, those patients obtaining a clinical response showed an at least 80% reduction in cytoplasmic p-ERK staining. Similarly in the phase I dose expansion, seven patients had pre- and post-treatment biopsies(18). All post-treatment specimens analyzed revealed marked reductions in p-ERK, cyclin D1, and Ki67 after two weeks of treatment with vemurafenib. Inhibition of mutant BRAF induced metabolic activity has also been documented. In nearly all patients (independent of confirmed clinical response) a marked decrease in the FDG-avidity of tumor lesions by positron emission tomography (PET) can be observed by two weeks of treatment. These results reinforce the exquisite sensitivity of BRAF V600E for vemurafenib and imply that near complete MAPK pathway inhibition is required to obtain clinical benefit in the treatment of melanoma driven by mutant BRAF.

Comparison with Other Agents

There have been major changes in the clinical management of melanoma over the past year. Ipilimumab was approved for treatment of metastatic melanoma independent of line of therapy and vemurafenib is now available. These drugs sit in stark contrast to one another by mechanism, clinical treatment course, and long-term outcome. The time course of response with ipilimumab is variable and inherent in the use of this agent is allowance for potential non-clinically significant progression of disease prior to clinical response(21). Further, while the response rate of ipilimumab was described as approximately 10-15%(2, 22), it has demonstrated the ability to induce long-term stable disease and complete remissions. By contrast, vemurafenib has a response rate of greater than 50% and is associated with rapid improvement in quality of life. It is not however associated with long-term complete remissions.
but rather has a median PFS on the order of six to seven months. Therefore, the use of these agents should be considered not necessarily as competing alternatives but rather cooperating possibilities available for use as dictated by patient circumstance. One area of clinical investigation of great interest is the potential that vemurafenib and ipilimumab may be synergistic. Whereas non-specific inhibitors of the MAPK pathway such as MEK inhibitors have been reported to reduce T-cell function(23), vemurafenib has no known effect on the immune system to date (presumably at least partly due to its V600E specificity). Furthermore, treatment with vemurafenib has been shown to increase melanoma differentiation antigen (gp100, MART-1, Tryp-1) expression and improve antigen-specific T-cell recognition(23). A much anticipated clinical trial combining these two agents will begin to accrue patients in the near future.

Patient Selection

Therapeutic treatment with vemurafenib is dependent on molecular selection of patients by BRAF mutational status. A commercial assay, known as cobas® 4800 BRAF V600 Mutation Test(24), was FDA approved in conjunction with vemurafenib and is now available for clinical use (Table 1). This test is a real-time polymerase chain reaction assay designed to detect the BRAF V600E (T1799A) mutation. The cobas® BRAF test is highly predictive for V600E however also detects other BRAF V600 mutations with less sensitivity. This may be important going forward given the variable incidence of other BRAF V600 mutations in sub-populations of melanoma patients such as older patients where the incidence of V600K mutations have been reported to be above 20%(25, 26). While vemurafenib has not been evaluated thoroughly in these patients, it does appear that the drug has clinically relevant activity.

Conclusion and Future Directions

Vemurafenib has established a new paradigm for targeted drug development and rapid clinical actualization. Vemurafenib demonstrates a high response rate in BRAF V600E mutant melanoma and survival advantage in comparison to chemotherapy. However, for most patients the clinical benefit is limited with a PFS just greater than six months. Already mechanisms of resistance to vemurafenib therapy have begun to be elucidated and clinical programs attempting to abrogate both this and the on-target toxicities of BRAF inhibition are being pursued.

There are at least four mechanisms of resistance to vemurafenib described to date. These include upstream of mutation of NRAS, activation of membrane bound receptor tyrosine kinases (platelet-derived growth factor receptor- β (PDGFR-β)) with subsequent signaling through other growth pathways (phosphatidylinositol 3-kinases (PI3K)/AKT)(27), overexpression of COT(28), and downstream mutation of MEK(29) (Figure 1). Novel combination regimens (BRAF plus MEK or BRAF plus PI3K/AKT inhibitors) are currently being evaluated clinical trials in hopes of circumventing resistance mechanisms. The combination of BRAF plus MEK inhibitors is also being evaluated in the treatment naive setting under the hypothesis that the addition of MEK inhibition will abrogate the on-target toxicity of SCC development.
Moreover, it is unclear at this time whether combination regimens of vemurafenib and other agents will be of increased benefit. It does seem very likely however that combining vemurafenib with other targeted agents, particularly those specific for resistant escape pathways, will prove fruitful. Furthermore, vemurafenib is likely to be useful in diseases beyond melanoma given that BRAF is the most commonly mutated oncogenic kinase in the human genome. Multiple other cancers including thyroid, colon, lung, and ovarian cancer harbor V600E mutations in varying incidence and are worthy of additional clinical testing.


Table 1.

**cobas® 4800 BRAF specifications and BRAF<sup>V600</sup> isoform sensitivity.**

<table>
<thead>
<tr>
<th>cobas® 4800 BRAF V600 Mutation Test</th>
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<tbody>
<tr>
<td>Clinical Specimen Required</td>
<td>FFPET* slide (≥5-µm section)</td>
</tr>
<tr>
<td>Limit of Detection of FFPET</td>
<td>5% mutant allele**</td>
</tr>
<tr>
<td>Minimal tumor content within specimen</td>
<td>15%</td>
</tr>
<tr>
<td>DNA requirement within specimen</td>
<td>125 ng</td>
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<tr>
<td><strong>Mutation Detection Sensitivity</strong></td>
<td></td>
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<tr>
<td>V600E</td>
<td>97.3%</td>
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<tr>
<td>V600K</td>
<td>65.8%</td>
</tr>
<tr>
<td>Other V600</td>
<td>Not Established***</td>
</tr>
</tbody>
</table>

* FFPET – Formalin-Fixed Paraffin-Embedded Tissue  
** Compared to 20% for Sanger Sequencing  
*** Insufficient Sample Size For Assessment of V600D, V600E<sub>2</sub>, V600R
Figure Legend

Figure 1. MAPK signaling in melanoma: Wild-type, with vemurafenib treatment and current resistance mechanisms to vemurafenib treatment. A preponderance of melanomas are driven by MAPK signaling. Under wild-type conditions (Panel A-left), RAF isoforms dimerize upon upstream stimulation of receptor tyrosine kinases and signaling through RAS to RAF. RAF dimers then signal through MEK and ERK eventually leading to downstream growth, proliferation and survival. In BRAFV600E mutant melanoma (Panel A-right) signaling between RAS and RAF is disconnected due to the constitutive, oncogenic signaling by mutant BRAF. Vemurafenib inhibits signaling by BRAFV600E leading to apoptosis of these cells. Multiple mechanisms of resistance to vemurafenib have been described to date (Panel B). Predominately these have included mechanisms for reactivation of MAPK signaling (upstream mutation of RASQ61K (Star 1), overexpression of other kinases that bypass BRAFV600E, such as CRAF or COT (Star 2) or downstream mutation of MEKC121S (Star 3)). Signaling through alternative growth pathways by activation of upstream receptor tyrosine kinases (Star 4) has also been described.
Growth, proliferation, and survival

Resistence mechanisms to BRAF\textsuperscript{V600E} signaling in melanoma

1. Resistance mechanism #1: Mutation in RAS
2. Resistance mechanism #2: Overexpression of other kinases (CRAF and COT)
3. Resistance mechanism #3: Mutation in MEK
4. Resistance mechanism #4: Activation of upstream RTKs (PDGFR-B or IGF-1R)
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