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

Jason J. Luke\(^1\) and F. Stephen Hodi\(^2\)

Abstract
The U.S. Food and Drug Administration recently approved vemurafenib for the treatment of BRAF valine in exon 15, at codon 600 (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 with dacarbazine chemotherapy, vemurafenib significantly improved the 6-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 versus 1.6 months, respectively), and this was consistent among groups analyzed, including age, sex, geography, Eastern Cooperative Oncology Group status, disease stage, and serum lactate dehydrogenase. 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 U.S. Food and Drug Administration (FDA) for the treatment of BRAF valine in exon 15, at codon 600 mutant (BRAF\(^{V600E}\)) 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. Although 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; Bristol-Myers Squibb) 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 V600E and affecting 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, interest in the development of inhibitors of BRAF has been sustained for nearly a decade.

Previous attempts to target RAF for therapeutic purposes have been unsuccessful. The multitargeted kinase inhibitor sorafenib was initially developed with this purpose in mind. Clinical trials eventually ruled out the utility of sorafenib 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 process was accomplished through a greater understanding of the RAF isoforms and a novel pharmacologic development strategy that allowed selective
BRAFV600E inhibition is accomplished by BRAF/CRAF carcinoma (SCC) development. Via vemurafenib treatment complication of squamous cell mutant BRAF, but also provides an explanation for the not only outlines the specificity of vemurafenib for wild-type B– and CRAF as opposed to mutant BRAF.

The paradoxical activation of MEK by nonmutant 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 in vitro systems, it has been documented that MEK activation after BRAFV600E inhibition is accomplished by BRAF/CRAF dimerization and subsequent CRAF signaling. This sequence has been confirmed by experiments inactivating (kinase-dead) BRAF, showing a similar effect to BRAF-inhibiting agents leading to downstream activation of MEK. A second model has proposed dose dependence for downstream MEK activation or inhibition on the basis of lower or higher doses of BRAF inhibitors. Interestingly, both of these experimental models require upstream activation, such as Ras or epidermal growth factor receptor activation. Such activation would not be unexpected in otherwise nonmalignant skin tissue that was previously exposed to ultraviolet light exposure; however, this has not been explicitly shown in vivo 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. Although the MEK/ERK activation seen in nonmalignant tissue seems 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. 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 to 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; Genentech; PLX4032, RG7204, RO5185426, N-(3-{[1H-pyrrolo[2,3-b]pyridin-3-yl]-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 pyridomdiazolone compatible with the ATP pocket-binding domain of BRAF. PLX4720, a 7-azaindole derivative, was the initial result of these investigations. This molecule established a new class of more specific kinase inhibitors with selectivity for mutant BRAFV600E. Subsequent studies showed marked effects on apoptosis, proliferation, and blockade of downstream ERK phosphorylation. These findings 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.

Vemurafenib was shown to be highly specific for BRAFV600E with an inhibitory concentration at 50% (IC50) of 31 nmol/L. In melanoma cell lines as well as melanoma and colon cancer xenografts, vemurafenib was observed to inhibit tumor growth in a dose-dependent fashion. Animal studies in rats and beagle dogs showed no significant toxicity at a 1,000 mg/kg/day on a 28-day dosing schedule or in longer studies with effective exposures tested up to 2,600 μmol/L in rats and 820 μmol/L in beagle dogs. 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. All advanced solid tumor patients were eligible for participation in the dose escalation; however, tumors associated with a high incidence of BRAFV600E 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 because of poor pharmacokinetics. Serum sampling revealed that available drug levels were lower than required for efficacy based on preclinical 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 1,120 mg twice daily.
After reformulation, the pharmacokinetics of vemurafe-
nib showed a linear increase in mean area under the curve
with increasing dose level. Additionally, a mean maxi-
num concentration at steady state of approximately 86
μmol/L and mean half-life of approximately 50 hours
were documented.

Vemurafenib was well tolerated 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.
SCC (nearly always keratoacanthoma type) was an unex-
pected 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 1 complete
response. Additionally, 3 patients with papillary thyroid
cancer had responses.

The phase I dose expansion accrued only melanoma patients
documented to harbor BRAFV600E 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 the
American Society of Clinical Oncology meeting in 2011
(19). Notable eligibility criteria for the study included
documentation of BRAFV600E 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, and a prespecified stratification
by age, stage, performance status, geography, and LDH. The
arms of the study were well balanced. A planned interim
analysis after 196 study deaths by an independent review
board recommended cross-over of all patients being trea-
ted on the decarbazine arm, as the study coprimary
endpoints had been met. Overall survival at 6 months
was found to be 84% in the vemurafenib arm and 64% in
the decarbazine arm. PFS could be evaluated in 81% (549
out of 675) of patients, with a median value of 5.3
months for the vemurafenib arm versus 1.6 months for
the 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 inter-
val of 0.2 to 0.3. The response rate of each arm was 48%
for vemurafenib (2 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 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, 4
patients had partial responses.

Vemurafenib was well tolerated in the BRIM-3 trial, with
the incidence of grade 1 to 2 and grade 3 to 4 adverse
events similar to those from prior studies. Notably, 38% of
patients required dose reduction in the vemurafenib arm.
Sixty-one patients (18%) had development of SCC, kera-
toaanthoma type; however, all were treated with local
therapies. The 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

The results of the phase III (BRIM-3) study of vemur-
afenib in BRAFV600E mutant melanoma were recently
published (20), and these data have now led to FDA
approval. BRIM-3 was a 2-arm randomized study com-
paring vemurafenib, 960 mg orally twice daily, to dacar-
bazine chemotherapy, 1,000 mg/m2 administered every 3
weeks. The initial primary endpoint was overall survival;
PFS was later added as a coprimary endpoint after the
results of the phase I and II studies were available.
Eligibility requirements were similar to those for BRIM-
2, necessitating the patient’s tumor to harbor the muta-
tion in BRAFV600E, good performance status, and no
history of central nervous system metastases. Notably,
20 patients treated on study were eventually found to
have non-V600E mutations (19 V600K, 1 V600D).

Within 1 calendar year, 2,107 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 were well balanced. A planned interim
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MAPK pathway. Activation of BRAF is associated with
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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 posttreatment (day 15) tumor biopsies were obtained from all patients and analyzed by immunohistochemistry for pharmacodynamic effects (10). Nuclear and cytosolic ERK pathway activation was observed before and after treatment. Although a plasma exposure of at least 300 μmol/L 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 Ki-67. 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, 7 patients had pre- and posttreatment biopsies (18). All posttreatment specimens analyzed revealed marked reductions in p-ERK, cyclin D1, and Ki-67 after 2 weeks of treatment with vemurafenib. Inhibition of mutant BRAF-induced metabolic activity has also been documented. In nearly all patients (independent of confirmed clinical BRAF-induced metabolic activity has also been documented. In nearly all patients (independent of confirmed clinical response, a marked decrease in the 2[18F]fluoro-2-deoxy-D-glucose avidity of tumor lesions by positron emission tomography can be observed by 2 weeks of treatment. These results reinforce the exquisite sensitivity of BRAFV600E 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

Major changes have taken place 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 nonclinically significant progression of disease prior to clinical response (21). Further, although the response rate of ipilimumab was described as approximately 10% to 15% (2, 22), ipilimumab has shown 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 6 to 7 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 nonspecific 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 metabolic activity has also been documented. In nearly all patients (independent of confirmed clinical response, a marked decrease in the 2[18F]fluoro-2-deoxy-D-glucose avidity of tumor lesions by positron emission tomography can be observed by 2 weeks of treatment. These results reinforce the exquisite sensitivity of BRAFV600E 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.

Table 1. cobas 4800 BRAF specifications and BRAFV600 isof orm detection

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Requirements</th>
</tr>
</thead>
<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&lt;sup&gt;a&lt;/sup&gt;</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>
</tr>
</tbody>
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<sup>a</sup>Compared with 20% for Sanger sequencing.

Vemurafenib has established a new paradigm for targeted drug development and rapid clinical actualization.
Vemurafenib shows a high response rate in BRAF\textsuperscript{V600E} mutant melanoma and survival advantage in comparison with chemotherapy. However, for most patients, the clinical benefit is limited, with a PFS just greater than 6 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.

At least 4 mechanisms of resistance to vemurafenib have been described to date. These mechanisms include upstream of mutation of NRAS, activation of membrane-bound receptor tyrosine kinases \textit{[platelet-derived growth factor receptor-β (PDGFR-β)]}, with subsequent signaling through other growth pathways \textit{[phosphoinositide 3-kinases (PI3K)/AKT (27), overexpression of COT (28), and downstream mutation of MEK (Fig. 1; ref. 29)]}. Novel combination regimens \textit{(BRAF plus MEK or BRAF plus PI3K/AKT inhibitors)} are currently being evaluated in clinical trials in hopes of circumventing resistance mechanisms. The combination of BRAF plus MEK inhibitors is also being
evaluated in the treatment-naïve 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.

Disclosure of Potential Conflicts of Interest

F. S. Hodi, clinical trial research support, Genentech/Roche, Bristol-Myers Squibb, Novartis, Pfizer, and Synta; nonpaid consultant, Genentech, Roche, Novartis, and Bristol-Myers Squibb. J. J. Luke disclosed no potential conflicts of interest.

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