How Zelboraf™ (Vemurafenib), a New FDA-Approved Therapy, Extends Life For Patients with Metastatic Melanoma

The overall median survival for patients with stage IV melanoma is less than a year, with approximately 10 percent long-term survivors. Although patients can respond to chemotherapy, it has not been possible to show that it improves median overall survival. High-dose interleukin-2 (IL2), an FDA-approved treatment for stage IV, can induce long-term responses in a small percentage of patients (<10 percent) but is highly toxic and difficult to administer. The discovery in 2002 that about half of melanomas harbor an activating mutation in the BRAF gene\(^1\) provided a novel target for therapy which has led, in a relatively short period of time, to a new treatment paradigm for metastatic melanoma.

The MAP Kinase Pathway

One of the primary evolutionary problems a eukaryotic cell had to overcome was how to transmit extra-
cellular signals from the cell surface to the nucleus. One of the primary pathways that evolved was the MAP kinase (MAPK) pathway (Figure 1a). When a ligand binds to the appropriate receptor tyrosine kinase, a conformational change is induced that leads to the phosphorylation and activation of Ras (H, N, and K). Activated Ras induces the dimerization of the Raf kinases (A, B, and C) as well as their phosphorylation. These activated dimers phosphorylate MEK, which phosphorylates ERK. Activated ERK enters the nucleus and acts as a transcription factor, turning on the transcription of several genes that lead to cellular proliferation and survival. There is also a complicated negative feedback mechanism that keeps this system under control.

Approximately 50 percent of melanomas harbor an activating mutation in the BRAF gene that leads to an activating mutation of the valine at position 600, usually to glutamic acid (V600E) but sometimes to arginine or lysine. In these cells, the MAPK pathway is constitutively activated, being driven by this mutated BRAF kinase. These cells do not have activated Ras, so the Raf kinases are not dimerized. This is an important mechanism for the specificity of BRAF inhibitors.

Vemurafenib (also known as PLX4032), binds to the ATP-binding site of mutated BRAF and locks it into an active conformation, but without ATP, the mutated BRAF cannot phosphorylate downstream MEK and the MAPK pathway is turned off. However, vemurafenib can also bind to CRAF and to a lesser degree, wild-type BRAF. In the wild-type cell, the Raf kinases are dimerized, and when vemurafenib binds to wild-type CRAF or BRAF, this conformational change induces a conformational change in the other member of the dimer pair resulting in transactivation and increased MAPK pathway activity. Thus, the specificity of vemurafenib comes from the fact that in cells with BRAF mutations, the cell is driven by activated BRAF, which exists primarily as monomers and is inhibited by the drug (Figure 1b). This model is supported by data from several in vitro studies.

**Early Clinical Trials – Phase I and Phase II**

The first human phase I trial with vemurafenib (then designated PLX4032) began in 2006. Early on in the study, at the lower dose levels, it became clear that the drug could induce dramatic responses in melanoma tumors harboring a V600E mutation (Figure 2). The maximum tolerated dose (and the recommended phase II dose) was determined to be 960 mg po bid. An extension cohort of 32 melanoma patients with V600E mutations treated at this recommended phase II dose showed that most patients experienced tumor shrinkage and that 56 percent qualified as having a partial or complete response by RECIST [Response Evaluation Criteria in Solid Tumors] criteria. At 2 years, 7 of 22 patients were still on the study drug.

Recently, the results of a phase II trial (termed BRIM2) were reported. In this multicenter trial, 132 melanoma patients harboring a BRAFV600E mutation who had disease progression after at least one prior therapy were treated with vemurafenib at 960 mg po bid. In this trial, in which the tumor responses were reviewed by a central, independent panel, the response rate by RECIST criteria was 52 percent, consistent with the phase I experience. The median overall survival had not been reached at 12 months, indicating that responses can be durable.

![FIGURE 1a. The Mitogen-Activated Protein Kinase (MAPK) Pathway](image-url)

When bound by their ligand, various receptor tyrosine kinases lead to ERK activation, which triggers cell proliferation and anti-apoptotic pathways. ERK also activates DUSP (dual-specificity phosphatases) and Sprouty, which negatively feed back on ERK and Ras, respectively. In melanomas with BRAFV600E mutations, the MAPK pathway is activated from the level of RAF.

Adapted from Pratilas and Solit, *Clin Cancer Res* 2010; 16:3329, with permission.
In the two studies, the toxicities seen were similar. Arthralgias and fatigue were the most common dose-limiting toxicities. Other common toxicities were alopecia, rash, palmar-plantar dysesthesia, and photosensitivity. Although these were generally grade I or II in severity, occasionally they were grade III.

Drugs that inhibit Raf kinases are known to induce non-melanoma skin tumors. Consistent with this, approximately 25 percent of patients on vemurafenib developed skin tumors characterized as verrucae, keratoacanthoma, or squamous cell carcinoma. These tumors were simply excised. There were no instances of metastatic cancers (other than melanoma) or squamous cell carcinoma in other anatomical sites beyond the skin.

Phase III trial

This high response rate justified conducting a phase III trial in which stage IV (or unresectable stage III) previously untreated melanoma patients whose tumor harbored a BRAFV600E mutation were randomized 1:1 to either vemurafenib at 960 mg po bid or standard chemotherapy (dacarbazine 800 mg/m2 iv every 3 weeks).6 The primary endpoints were overall survival and progression-free survival. Between January and December, 2010, 675 patients were accrued to this trial among 104 participating centers worldwide. In January 2011, the planned interim analysis was performed, at which time the independent data safety monitoring committee announced that the primary endpoints of the trial had been met and recommended that patients randomized to dacarbazine be allowed to cross over to vemurafenib.

Despite the very short follow-up time (median 3 months), the vemurafenib group had a 63 percent lower hazard of death and a 74 percent lower hazard of progression compared to the dacarbazine group (Figure 3). Although median overall survival could not be reliably estimated at this first analysis due to the very short median follow-up, 6-month overall survival rates were 84 percent for the vemurafenib group compared to 64 percent for the
The clinical trials with vemurafenib consistently show a median time to progression of 5-7 months. This indicates that melanomas can develop resistance to vemurafenib relatively quickly. Therefore, it is critical to understand the mechanism of resistance.

The experience with inhibiting mutated KIT in GIST (gastrointestinal stromal tumors) using imatinib predicted that melanomas developing resistance to vemurafenib would develop a second mutation in BRAF that would prevent binding of the drug. This has not been the case. In some cases of resistance, the tumor has developed an activating mutation in upstream NRAS which reactivates the MAPK pathway. Other investigators have described potential resistance mechanisms that can reactivate the MAPK pathway through downstream events, or mechanisms that activate the parallel PI3K [phosphatidylinositol 3-kinases] pathway. It remains to be determined how frequently these mechanisms play a role in patients developing resistance to vemurafenib. It is likely that other mechanisms reactivating the MAPK pathway will be described in these patients.

**Future studies**

Vemurafenib was FDA-approved for use in metastatic melanoma just 9 years after the first report that melanomas often harbor BRAFV600E mutations. This represents a remarkable amount of collaborative work among those in the melanoma community and the pharmaceutical industry. Other BRAF inhibitors are also in development, most notably GSK2118436, which has recently completed a phase III trial. In this study, 200 patients were randomized 3:1 to GSK2118436 vs. dacarbazine, the primary endpoint being progression-free survival. Results are expected in 2012.

**Response in other BRAF mutations**

Although 90 percent of BRAF mutations are V600E, about 5 percent are V600K; other mutations make up the remaining 5 percent. In the phase II and phase III vemurafenib trials, a small number of patients were found retrospectively to have had a V600K mutation. Forty percent of these patients responded,
Combining Forces: Vemurafenib and Ipilimumab To Be Tried Together

Vemurafenib (Zelboraf™) was the second drug approved for metastatic melanoma this year. In March, the FDA approved ipilimumab (Yervoy™), a monoclonal antibody that binds to CTLA-4, which normally blocks lymphocyte activation against melanoma. Ipilimumab led to greater overall survival compared to a peptide vaccine, and a subsequent phase III trial comparing ipilimumab + dacarbazine vs. dacarbazine alone found that the combination therapy led to greater overall survival compared to dacarbazine alone.

With two new approved treatments for metastatic melanoma, each of which improves overall survival, physicians now have unique options.

The two drugs are quite different, as shown in the table. Ipilimumab is an immune-boosting therapy with a slow onset of effect and a low rate of objective responses, though the complete responses seen are generally durable. It can result in durable stable disease even without objective tumor shrinkage. Vemurafenib blocks mutated BRAF and has a much higher rate of objective responses, which generally occur rapidly.

Because these two drugs each have been shown to improve overall survival but have very different and complementary actions, there has been pressure on both drug companies involved (Bristol-Myers Squibb and Roche/Genentech) to combine them. There is reason to think that the two drugs could act synergistically. Evidence exists that inhibiting the MAPK pathway in BRAF-driven tumor cells can decrease production of immune-suppressive factors such as IL10 and enhance expression of differentiation antigens that could be recognized by the immune system.

In this way, vemurafenib could render melanoma cells more susceptible to immune attack.

As attractive as this combination is, it could prove less effective than either drug alone. For example, vemurafenib might adversely affect T cell function, undermining ipilimumab’s effects. Also, some of the toxicities seen with these drugs are overlapping (e.g., rash, elevated liver enzymes), which could limit the ability to administer the drugs together. Even if the combination is tolerable and not antagonistic, the combination may be no better than using the drugs in sequence. This will need to be tested.

To the credit of both drug companies, they overcame several difficulties to bring the combination trial about, and the phase I trial is about to start. This multicenter Phase I/II trial, headed by Drs. Jedd Wolchok, Stephen Hodi, and Antonio Ribas (from Memorial Sloan-Kettering, Dana-Farber Cancer Institute, and UCLA, respectively), will treat advanced melanoma patients who have a BRAFV600E mutation with both vemurafenib and ipilimumab at the FDA-approved doses (960 mg bid for vemurafenib; 3 mg/kg for ipilimumab). Patients will start vemurafenib either 2 or 4 weeks prior to starting ipilimumab. The dose of ipilimumab will be escalated to 10 mg/kg, if tolerated. A phase II cohort will be treated at the maximum tolerated doses. Ultimately, a three-arm trial comparing overall survival in patients randomized to either drug alone or the combination therapy should be considered. Patients who are randomized to either drug alone will, upon progression, certainly receive the other drug, so the study will test de facto whether the combination is superior to the two drugs used sequentially in either order.


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<tr>
<th>Mechanism of action</th>
<th>Vemurafenib</th>
<th>Ipilimumab</th>
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<tr>
<td>Blocks mutated BRAFV600E</td>
<td></td>
<td>Activates T cells against melanoma</td>
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| Specific indication         | Melanoma must have BRAFV600E mutation | Any melanoma, including uveal melanoma |

| Contraindications          | Wild-type BRAF                       | Autoimmune diseases                  |

| Objective response rate (RECIST criteria) | 50% | 12%, but long-term stable disease also seen commonly |

| Onset of response           | Rapid | Typically takes 12-20 weeks |

| Common toxicities           | Rash, arthralgias, fatigue, non-melanoma skin tumors including occasional squamous cell carcinoma, 1 LFTs | Autoimmune colitis, dermatitis, hepatitis, hypophysitis, pancreatitis, thyroiditis |
indicating that melanomas with V600K mutations are also sensitive to vemurafenib. In the future, it will be necessary to test the other less frequent, non-V600E mutations.

**Brain metastases**

The efficacy of BRAF inhibitors on brain metastases is currently unclear. Despite the fact that neither GSK2118436 nor vemurafenib were predicted to cross the blood-brain barrier, the phase I trial of GSK2118436 gave encouraging results. Of 10 patients treated with brain metastases, 8 showed shrinkage of >30 percent and several had complete responses in the brain. A formal phase II trial in patients with brain metastases has recently completed accrual with GSK2118436 and another is under way with vemurafenib.

**Combination studies**

The infrequency of complete responses with vemurafenib and the frequency with which melanomas develop resistance suggest that combination therapies will be needed. There are currently ongoing trials adding MEK inhibitors, ipilimumab (Yervoy™), or bevacizumab to BRAF inhibitors.

Other rational combinations are also under consideration. [See “Combining Forces: Vemurafenib and Ipilimumab To Be Tried Together,” on p. 5.]

**References**