Unresectable or metastatic melanoma remains an aggressive disease with a high mortality rate. In the US, the incidence of advanced melanoma continues to rise, and an estimated 9,710 patients will die from the disease in 2014. Prior to 2011, systemic therapy for cutaneous melanoma was limited to cytotoxic chemotherapy, high-dose interleukin 2, or combination biotherapy. A minority of patients did respond, and some responses were quite durable. However, we could not predict who would respond, and no evidence existed that these treatments improved median progression-free survival (PFS) or overall survival (OS).

A New Direction in Immunotherapy

A greater understanding of immune activation has identified immune checkpoints that negatively regulate T-lymphocyte activation. Two such checkpoints, B7/CTLA-4 and PD-1/PD-L1, have been the target for monoclonal antibody therapy (Figure 1).

Iпilimumab

CTLA-4 is a T-lymphocyte surface protein that recognizes B7 on antigen-presenting cells and leads to down-regulation of T-lymphocyte activation. Iпilimumab is a humanized monoclonal antibody that blocks CTLA-4, thereby releasing T-cells from suppression, although recent data raise the possibility that ipilimumab may function by depleting regulatory T-cells from within tumors. The objective response rate to ipilimumab, using traditional RECIST criteria (Response Evaluation Criteria in Solid Tumors), is about 15 percent. Although these responses are sometimes delayed and may not be evident until several months after initiation of therapy, most responses are quite durable. An additional 25 percent of patients will experience stabilization of disease, which also can be extremely durable. Overall, this led to a near doubling of OS at 2 years (23.5 vs. 13.7 percent) in the first randomized trial, which compared ipilimumab to a peptide vaccine. A second randomized trial of ipilimumab + dacarbazine vs. dacarbazine alone also showed an OS advantage for ipilimumab — a 3-year OS of 20.8 percent in the ipilimumab plus dacarbazine group compared to 12.2 percent for dacarbazine alone.

This improvement in long-term OS is likely due to the durability of ipilimumab’s anti-melanoma effects. In compiling all survival data from 1,861 patients treated with ipilimumab, Schadendorf, Hodi, and Robert recently reported an estimated 3-year OS rate of 22 percent (95 percent CI 20-24 percent). Remarkably, approximately 84 percent of patients alive at 3 years were estimated to be alive at 5 and 10 years, confirming that clinical benefit from ipilimumab is generally durable. Indeed, complete responses are essentially cures. Among the 15 complete responders reported by the NCI, only one had disease recurrence with follow-up of 4.5-8 years.

Continued on page 2
Treatment of Metastatic Melanoma, from page 1

The T-cell activating effects of ipilimumab are relatively nonspecific, and autoimmune reactions are the most common adverse events, especially colitis, rash, pruritus, and skin depigmentation. Endocrinopathies (especially hypophysitis), hepatitis, and pancreatitis are also seen and require management.8,9 The incidence and complexity of these toxicities have discouraged some community oncologists from using ipilimumab.

**Anti-PD-1/-PDL1**

A second immune checkpoint, the protein programmed death 1 (PD-1) and one of its ligands, PD-L1, is another target for immunotherapy. PD-1 is an inhibitory receptor on T-lymphocytes, and after chronic T-lymphocyte activation, expression of its ligand PD-L1 on tumor cells and other cells in the tumor microenvironment can protect the tumor cells from immune destruction. Monoclonal antibodies that block PD-1 or PD-L1 are currently in late-stage clinical trials, although not yet FDA-approved. Initial results indicate that PD-1/PD-L1 blockade results in higher response rates and a more favorable side effect profile than seen with ipilimumab.10-13 We await the results of several randomized trials comparing ipilimumab with anti-PD-1 therapy.

**Targeting the MAPK Pathway**

Along with the advancements in immunotherapy, parallel advancements have been made in understanding the molecular biology of melanoma cells. It appears that these cells are absolutely reliant on the MAPK pathway (Figure 2), and almost all melanomas have mutually exclusive driver mutations that activate the MAPK pathway, most commonly mutations in BRAF (40-60 percent of cases), NRAS (about 20 percent of cases), or loss of NF1 function (about 4 percent of cases).14 In BRAF-activated cells, the MAPK pathway is hyperactivated, leading to feedback inhibition of upstream RAS. This maintains the RAF kinases in the monomeric state which, under normal circumstances, would fail to signal downstream MEK. But mutated BRAF can signal as a monomer. However, as a monomer, it is very susceptible to pan-RAF inhibitors such as vemurafenib and dabrafenib. These drugs immediately shut down the MAPK pathway in BRAF-mutated cells. Interestingly, in cells without a BRAF mutation, the RAF kinases are free to dimerize, and in these cells, RAF inhibitors inhibit one member of the dimer pair but trans-activate the other member. The result is that RAF inhibitors actually stimulate the MAPK pathway in cells with wild-type BRAF. This is the basis for the specificity of the anti-melanoma effect. The RAF inhibitors shut down the MAPK pathway in the BRAF-mutated melanoma cells but do not inhibit the pathway (in fact, slightly stimulate the pathway) in the patient’s normal tissues.

**RAF Inhibitors**

Vemurafenib was the first RAF inhibitor to receive FDA approval for advanced melanoma with a BRAFV600E mutation. Phase I and II data showed striking and rapid antitumor activity in patients with BRAFV600E-mutated melanoma.16,17 The randomized phase III trial (BRIM-3) comparing vemurafenib to dacarbazine showed both a PFS and OS advantage in favor of vemurafenib, with a median PFS of 5.3 months vs. 1.9 months, an RR of 48 percent vs. 5 percent, and an estimated median OS of 13.6 months vs. 9.7 months.18,19 This trial and subsequent studies showed that less common BRAF mutations — V600K and V600R — also are sensitive to vemurafenib treatment.19,20 Common toxicities associated with vemurafenib therapy include arthralgias, fatigue, rash, painful callus formation of the soles and/or palms, photosensitivity, hair thinning, and cutaneous squamous proliferations, most commonly keratoacanthomas but occasionally squamous cell carcinoma (SCC). These toxicities are reversible with discontinuation of the drug (although we do not know if SCCs regress), but can often be managed with drug holidays, antiinflammatory drugs (either NSAIDs

---

**Figure 1. CTLA-4 and PD-1**

A. In lymphatic tissue, antigen-presenting cells (APC) activate naïve T-cells via the T-cell receptor (TCR) and stimulatory receptor CD28. This leads to expression of CTLA-4 on the T-cell surface, which binds to B7 with higher avidity than does CD28. Interaction of CTLA-4 with B7 leads to T-cell inactivation. Ipilimumab binds to CTLA-4 and reverses this inactivation.

B. In peripheral tissue, tumor cells upregulate PD-1 ligands, which bind to PD-1 on activated T-cells, leading to T-cell inhibition or death. Monoclonal antibodies that bind to either PD-1 or PD-L1 interfere with this, allowing antitumor T-cells to survive and kill the tumor cells.
or low-dose prednisone), and topical keratolitics and steroids. Some investigators have detected new primary BRAF wild-type melanomas while patients are on the drug. These have been very superficial, and it is still unclear if the frequency is truly increased. Keratoacanthomas generally are seen within the first 8 weeks of therapy and often stop appearing afterwards.

Dabrafenib, the second RAF inhibitor to gain FDA approval, has performed on par with vemurafenib. The randomized phase III trial (BREAK-3) of dabrafenib vs. dacarbazine showed an RR of 50 percent vs. 6 percent and a PFS of 5.3 months vs. 2.7 months. A subsequent phase II trial in patients with V600E or V600K BRAF-mutated melanoma and at least one asymptomatic brain metastasis showed that 49/139 (35 percent) had an intracranial response with dabrafenib therapy. The adverse event profile of dabrafenib is similar to that of vemurafenib, except that dabrafenib does not induce photosensitivity. It is thought to induce cutaneous squamous proliferations less frequently than vemurafenib, although the two drugs have not been compared side to side. In our experience, dabrafenib is definitely associated with cutaneous toxicities. It is also occasionally associated with fever.

Despite the favorable response to RAF inhibitors, resistance develops at a median time of 6-7 months, although it is important to stress that resistance is not inevitable. Long-term follow-up shows that approximately 14 percent of patients remain on RAF inhibitors and free from relapse at 18 months of treatment. Several mechanisms of RAF inhibitor resistance have been described, but virtually all lead to reactivation of the MAPK pathway. The most common mechanisms are: a post-transcriptional splice variant of the mutated BRAF mRNA that enhances dimerization in the absence of RAS activation; overexpression of the mutated BRAF protein; and acquisition of an NRAS mutation (reviewed in Cancer Cell). Based on preclinical models, there is interest in exploring intermittent dosing schedules as a way to prevent or delay the development of resistance. [See “Oncogene Addiction and Overdose: Intermittent Treatment in Models of Drug-Resistant BRAF-Mutated Melanoma,” in this issue.]

**MEK Inhibitors**

MEK, being downstream of RAF is also a target of therapeutic interest. In a phase III trial of previously untreated BRAF-mutated melanoma, patients were randomized to receive either the MEK inhibitor trametinib or chemotherapy. Trametinib showed a response rate of 22 percent (compared with 8 percent in the chemotherapy arm), an improvement in median PFS (4.8 months for trametinib vs 1.5 months for chemotherapy), and a benefit in estimated OS at 6 months (61 percent versus 67 percent). Based on this study, trametinib was FDA-approved for use in BRAF-mutated melanoma that had not been previously treated with a RAF inhibitor. In reality, trametinib is rarely used for this indication, as the RAF inhibitors are more effective single agents.

Common adverse events from trametinib include rash (Figure 3), diarrhea, and fatigue. MEK inhibitors also commonly cause CPK (creatine phosphokinase) elevations, and while it is usually not symptomatic, significant muscle weakness is occasionally seen. In rare cases, MEK inhibitors have also been associated with central serous retinopathy.

Given the frequent cutaneous toxicities, namely acneiform rash, xerosis, alopecia, and paronychia for MEK inhibitors, and hand-foot skin reaction, xerosis, alopecia, skin neoplasms (keratoacanthomas and squamous cell carcinomas), and photosensitivity for RAF inhibitors, close collaboration with dermatologic colleagues is important. For the acneiform rash caused by MEK inhibitor, topical steroids and oral antibiotics are commonly used as a preventative or treatment method. For hyperkeratosis or thickening of the palms and soles associated with the use of RAF inhibitors, otherwise referred to as hand-foot skin reaction, keratolitics (creams containing ammonium lactate 12 percent, topical urea 20-40 percent, or salicylic acid 6 percent) are helpful for the callus formation on the palms and soles (Table 1). Vemurafenib is a highly photosensitive agent, so the use of a broad-spectrum sunscreen with an SPF of at least 30 when exposed to the sun, along with protective wear, is critical to prevent painful sunburns. In addition, for similar reasons, vemurafenib should be discontinued approximately one week prior to planned radiation therapy.

**Combination Therapies**

There is great interest in exploring combination therapies. Combining RAF and MEK inhibitors is a rational strategy to inhibit the MAPK pathway.

---

Figure 2. MAP kinase pathway in a melanoma cell

Continued on page 4
more completely and to delay or prevent emergence of resistance. In a randomized phase II trial, 162 patients with BRAF-mutated metastatic melanoma (Stage IIIC or IV) were randomized to dabrafenib treatment alone (150 mg twice daily), or dabrafenib plus trametinib at 1 mg/d or 2 mg/d. The combination of dabrafenib + trametinib at 2 mg/d was associated with a median PFS benefit of 9.4 months compared to 5.3 months with dabrafenib monotherapy. The response rate with this combination was 76 percent compared to 54 percent for dabrafenib monotherapy, and had a longer median PFS (10.5 months vs 5.6 months). Although the combination is associated with less cutaneous toxicity than monotherapy (less hyperkeratosis and fewer keratoacanthomas/SCCs), it is also associated with more frequent systemic toxicities, including fever, chills, and fatigue in more than half of patients. Based on the improved response rate and PFS data, however, the FDA recently granted this combination accelerated approval. A formal phase III trial has been completed, and we await the results.

Efforts are now under way to combine targeted therapy and immunotherapy. The rationale is based on the observation that RAF inhibition releases MITF (microphthalmia-associated transcription factor) inhibition, which leads to increased expression of melanoma-specific proteins, hopefully including relevant tumor rejection antigens. The initial effort, a phase I trial combining vemurafenib and ipilimumab, was closed early due to a high rate of significant hepatotoxicity. Currently, combinations of RAF inhibitors and immunotherapy agents should be used only in the context of a clinical trial.

A phase I immune checkpoint inhibitor trial combining ipilimumab with the anti-CD80 antibody, durvalumab, was shown to be safe and to have intriguing clinical activity. The objective response rate was 60 percent, far higher than what has been seen with ipilimumab alone. Multiple questions remain: is the combination better than nivolumab alone? Is sequential therapy equivalent to (or better than) combination therapy? What is the durability of these responses? Many of these questions will likely be answered by the phase III trial that just recently completed accrual, in which patients were randomized to ipilimumab, nivolumab, or combination therapy.

Choosing First-Line Therapy for BRAF-mutated Melanoma

In patients with BRAF-mutated melanoma, uncertainty remains as to first-line therapy — single agent RAF inhibitors, combination dabrafenib/trametinib, or immunotherapy. (Currently ipilimumab is the only FDA-approved option, but anti-PD-1 antibodies are expected to be FDA-approved in the near future.) Because of the high response rates and rapid onset of action, RAF inhibitors may be preferred first-line treatments in patients with BRAF-mutated melanomas with bulky disease or who are symptomatic. Also, in patients with brain metastases, RAF inhibitors appear to have a higher response rate than ipilimumab or even whole-brain radiotherapy and may again be the preferable first-line therapy. Alternatively, in patients with low burden of disease and no brain metastases, immunotherapy might be considered as a first-line treatment even in the setting of a BRAF mutation.

Given the high incidence of developing resistance to RAF inhibitors, some physicians switch patients to ipilimumab once a maximal tumor response has been achieved. This strategy has the potential advantage of providing immunotherapy in a setting of decreased tumor burden, and maintains the option of returning to RAF inhibitor therapy at a later date should immunotherapy not be effective. However, this strategy has not yet been tested in a prospective randomized trial.

Conclusions

Over the past 5 years, new treatment options for unresectable or metastatic melanoma have changed the field completely, resulting in improved OS for the first time. We are seeing long-term survivors in our clinics, which is a welcome sight. Still, many challenges lie ahead to improve outcomes further. In targeted therapy, resistance and lack of durable complete responses remain a problem. We also have to develop therapeutic options for patients whose tumors do not harbor a BRAF mutation. On the immunotherapy side, we need to understand the mechanisms of resistance and identify which checkpoint mechanisms are operative in which patients. Combination therapies hold promise but are likely to be associated with more toxicity. Continued innovative clinical trials will remain critical.
### Table 1: Treatment algorithms for common RAF and MEK inhibitor cutaneous toxicities

<table>
<thead>
<tr>
<th>Severity (CTCAE v.4)</th>
<th>Xerosis and Hand/Foot Skin Reaction to RAF Inhibitors</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0</td>
<td>Gentle skin care instructions with use of fragrance-free soaps and detergents</td>
<td>Avoid friction to the hands and feet, use thick gloves and socks during activity</td>
</tr>
<tr>
<td>Grade 1</td>
<td>Continue drug at current dose and monitor for change in severity</td>
<td>Moisturizing cream to the body and topical keratolytic (salicylic acid 6%, urea 20-40%, ammonium lactate 12%) to hands and feet bid</td>
</tr>
<tr>
<td>Grade 2</td>
<td>Continue drug at current dose and monitor for change in severity</td>
<td>Topical high-potency steroid (i.e., clobetasol) bid AND pain control with NSAIDs/GABA agonists/narcotics or topical lidocaine cream or patches</td>
</tr>
<tr>
<td>Grade ≥ 3</td>
<td>Interrupt treatment until severity decreases to grade 0-1, and continue treatment of skin reaction with the following:</td>
<td>Topical high potency steroid bid (i.e., clobetasol) AND pain control with NSAIDs/GABA agonists/narcotics or topical lidocaine cream or patches</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Severity (CTCAE v.4)</th>
<th>Acneiform (papulopustular) Rash to MEK Inhibitors</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0</td>
<td>Prophylactic treatment during weeks 1-6 and 8</td>
<td>Oral antibiotics for 6 weeks at start of therapy (doxycycline 100 mg bid OR minocycline 100 mg bid) AND alcohol-free over-the-counter moisturizing creams or ointment bid</td>
</tr>
<tr>
<td>Grade 1</td>
<td>Continue drug at current dose and monitor for change in severity</td>
<td>Topical low/moderate strength steroid daily AND topical antibiotic bid OR combined topical steroid and antibiotic</td>
</tr>
<tr>
<td>Grade ≥ 3</td>
<td>Dose-modify; obtain bacterial/viral/fungal cultures if infection is suspected; continue treatment of skin reaction with the following:</td>
<td>Oral antibiotic for 6 weeks (doxycycline 100 mg bid OR minocycline 100 mg bid OR oxytetracycline 500 mg bid) AND topical low/moderate potency steroid</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Severity (CTCAE v.4)</th>
<th>Maculopapular Rash to RAF Inhibitor</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0</td>
<td>Gentle skin care instructions given; over-the-counter moisturizing creams</td>
<td>Continue drug at current dose and monitor for change in severity</td>
</tr>
<tr>
<td>Grade 1</td>
<td>Continue drug at current dose and monitor for change in severity</td>
<td>Topical steroid bid AND oral antihistamines</td>
</tr>
<tr>
<td>Grade 2</td>
<td>Continue drug at current dose and monitor for change in severity</td>
<td>Topical steroid bid AND oral antihistamines OR oral steroids (0.5 mg/kg or equivalent)</td>
</tr>
<tr>
<td>Grade ≥ 3 Or Intolerable grade 2</td>
<td>Dose-modify; obtain bacterial/viral/fungal cultures if infection is suspected; continue treatment of skin reaction with the following:</td>
<td>Oral antihistamines AND oral steroids (0.5 mg/kg or equivalent)</td>
</tr>
</tbody>
</table>

Treatment of Metastatic Melanoma, from page 5

### Treatment of Metastatic Melanoma

#### References


### Table 2: FDA-approved systemic agents for advanced melanoma

<table>
<thead>
<tr>
<th>Drug (Brand)</th>
<th>Category</th>
<th>Dosage/Frequency</th>
<th>Performance</th>
<th>Common Side Effects</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vemurafenib (Zelboraf®)</td>
<td>RAF Inhibitor</td>
<td>960 mg po bid, which can be reduced to 720 mg po bid or 480 mg po bid if intolerable</td>
<td>RR 50 percent Median PFS 5–7 m Median OS 13.6 m</td>
<td>Arthralgia Rash Photosensitivity Cutaneous SCC Keratoacanthoma Transaminitis</td>
<td>Rapid management of symptomatic metastases; activity in brain metastases</td>
</tr>
<tr>
<td>Dabrafenib (Tafinlar®)</td>
<td>RAF Inhibitor</td>
<td>150 mg po bid</td>
<td>RR 50 percent Median PFS 5.3 m</td>
<td></td>
<td>Rapid management of symptomatic metastases; consider in combination with MEK inhibitor; benefit demonstrated in patients with brain metastases</td>
</tr>
<tr>
<td>Trametinib (Mekinist®)</td>
<td>MEK Inhibitor</td>
<td>2 mg po daily</td>
<td>RR 22 percent Median PFS 4.8 m</td>
<td>Rash Peripheral edema Diarrhea In rare cases, central serous retinopathy CPK elevation</td>
<td>Used in combination with RAF Inhibitor</td>
</tr>
<tr>
<td>Combination Dabrafenib/Trametinib</td>
<td>RAF Inhibitor plus MEK Inhibitor</td>
<td>Dabrafenib: 150 mg po bid Trametinib: 2 mg po daily</td>
<td>RR 76 percent Median PFS 9.4 m</td>
<td>Pyrexia Rash Fatigue Nausea/Vomiting Arthralgia Peripheral edema</td>
<td>Accelerated approval based on Phase II data Higher CR rate Longer duration of response</td>
</tr>
<tr>
<td>Ipilimumab (Yervoy®)</td>
<td>Anti-CTLA-4 Antibody</td>
<td>3mg/kg IV q3 weeks for a total of 4 doses; can consider re-challenge</td>
<td>RR 15 percent OS at 3 yrs 22 percent</td>
<td>Diarrhea Colitis Dermatitis Rarely hypophysitis</td>
<td>Delayed onset of action Durable response</td>
</tr>
</tbody>
</table>

*RR= response rate, PFS= progression-free survival, OS= overall survival; SCC= squamous cell carcinoma, CR=complete response*


---

**From the Editors, continued from back page**

BRAF molecule, mutated in about 50 percent of melanomas, would result in cell cycle arrest and cure. However, despite some durable responses, the development of drug resistance has proven to be the norm rather than the exception. We have come to realize that the MAP-kinase pathway is in fact extremely intricate and will take much more manipulation before we solve the problem of drug resistance.

Some researchers speculate that BRAF-mutated melanomas harbor a subpopulation of malignant cells that grow more efficiently with BRAF inhibition, essentially becoming addicted to the drug itself and being nourished by it. In other words, when BRAF inhibition stops the growth of BRAF-sensitive malignant cells, it may selectively enhance the growth of resistant cells, ultimately defeating the therapy. Researchers are now seeking to create a balance between the BRAF-sensitive and resistant cell populations so that neither grows out of control, creating a homeostasis that might prove lifesaving.

In our fascinating second story, Dr. Martin McMahon and Marian Deuker discuss their research into intermittent dosing with BRAF inhibitors as one means of creating such a homeostasis.

Recent advances in melanoma therapy after decades of disappointment have prompted optimism, if not impatience, for a cure. Unfortunately, many hurdles remain and the road to further breakthroughs may be long. But with researchers working out the intricacies of how best to shut down defective pathways and harness the immune system, there is indeed much reason for hope that each new discovery will help more patients to live longer and better.
Oncogene Addiction and Overdose: Intermittent Treatment in Models of Drug-Resistant BRAF-Mutated Melanoma

Marian M. Deuker
Martin McMahon, PhD
Helen Diller Family Comprehensive Cancer Center and Department of Cell and Molecular Pharmacology
University of California, San Francisco

Over the past 12 years, BRAF (v-raf murine sarcoma oncogene viral homolog B) has risen in stature: no longer an insufficiently studied regulator of cell signaling, BRAF has emerged as an important oncogenic driver of numerous cancers and a prominent target for successful pathway-targeted melanoma therapy.1,2 Indeed, the development of BRAF inhibitors (e.g., vemurafenib and dabrafenib), which selectively target the most common mutationally activated form of the protein in melanoma, has both improved treatment options for patients with BRAF-mutated melanoma and revealed unique features of RAS-regulated intracellular signaling.2,3 Unfortunately, dramatic melanoma regressions promoted by BRAFV600E-targeted therapies are often transient, with almost inevitable onset of recurrent disease driven by strikingly diverse mechanisms of drug resistance.4,5

These observations have fueled the development of preclinical systems to explore the causes and consequences of drug resistance and test novel therapeutic strategies to prevent the onset of drug-resistant melanoma.6

The Preclinical HMEX 1906 PDMX Model

The use of patient-derived melanoma xenografts (PDMX), serially passaged through immunocompromised mice, provides a complementary strategy to the use of either cell culture or genetically engineered mouse (GEM) models to study drug-resistant melanoma.7 While PDMX models are undoubtedly subject to selective pressure during establishment in mice, they avoid the detrimental stresses of cell culture, including: growth in plastic dishes, an oxygen-rich environment, and the use of growth supplements such as serum. Moreover, when used to model drug resistance, PDMX tumors are exposed to the drug via oral administration — a delivery method that more accurately reflects drug exposures in patients.

To that end, small fragments of a vemurafenib-naive, lymph node-derived BRAF-mutated melanoma metastasis were subcutaneously implanted into immunocompromised mice. Some of these fragments grew into tumors that, when resected, could be serially propagated through larger numbers of mice, thereby generating the HMEX1906 PDMX model.8 At that point, a cohort of tumor-bearing mice was orally administered vemurafenib and, as expected, HMEX1906 melanomas displayed striking regression. However, when such mice were dosed continuously for greater than 50 days, stable drug-resistant tumors emerged that grew to end-stage. As expected, vemurafenib treatment of the original sensitive HMEX1906 tumors elicited profound inhibition of phosphorylation of the ERK1/2 (pERK1/2) MAP kinases, downstream effectors of oncogenic BRAFV600E. By contrast, drug-resistant HMEX1906 tumors displayed a higher basal level of pERK1/2 that was incompletely inhibited following exposure to vemurafenib. Hence, the HMEX1906 PDMX model proved useful for preclinical analysis of mechanisms of drug sensitivity and resistance in melanoma.

Extensive analysis was then performed on vemurafenib-sensitive or -resistant HMEX1906 melanomas to identify the mechanism(s) of resistance. Exome sequencing failed to detect any new mutations that might explain the properties of the resistant tumors. However, further analysis revealed that drug-resistant melanomas expressed elevated levels of mutated BRAF mRNA and BRAFV600E protein. Indeed, in one resistant tumor (45V-RT5), elevated BRAFV600E expression resulted from further amplification of the mutated BRAF gene. However, while all of the other
resistant tumors displayed elevated BRAF<sup>V600E</sup> expression, none of them showed increased BRAF gene copy number. Hence, two different mechanisms of vemurafenib resistance — both of which resulted in increased BRAF<sup>V600E</sup> expression — arose from a small fragment of a PDMX. The continued dependence of resistant tumors upon BRAF<sup>V600E</sup> signaling was confirmed using either genetic or pharmacological inhibitors of the pathway. Hence, as first demonstrated by Robert Schimke at Stanford University in 1977, these data emphasize the importance of increased expression of the drug target as a relevant mechanism of cancer drug resistance.<sup>8,9</sup>

**Drug-Resistant, Drug-Dependent Tumors**

At this point, the study might have been concluded were it not for two provocative findings. First, it was noted that the transplantation efficiency of vemurafenib-resistant tumors was significantly higher if the mice were receiving vemurafenib at the time of the transplant, suggesting that the tumors were not simply drug-resistant, but also drug-dependent for optimal tumorigenesis. More telling was the observation that, while the original sensitive tumors readily gave rise to cell lines in vitro in the absence of vemurafenib, it was difficult to generate a cell line from the resistant melanomas unless cells were cultured in the presence of a moderate vemurafenib concentration (50 nM). These data confirmed that the drug-resistant tumor cells were also drug-dependent for growth and that, for these cells, culture either in too much or too little vemurafenib suppressed proliferation of the vemurafenib-resistant cells.

Thus, the tumor fitness benefit conferred by elevated expression of BRAF<sup>V600E</sup> in the presence of vemurafenib becomes a fitness deficit in the absence of vemurafenib. These observations are in accord with previous studies that showed how cells respond to both the quality (i.e., which pathways are on/off) and quantity (i.e., the integrated magnitude) of signal pathway activation.

**Figure 2. Intermittent BRAF inhibitor therapy to forestall the onset of drug-resistant melanoma**

Graph A reflects pERK level. Optimal tumor growth occurs when the cells have an optimal pERK level (green zone). Tumor cells with excess or insufficient pERK (red zones) will not grow optimally. The tumor volume (of both drug-sensitive and drug-resistant tumor cells) corresponding to the different pERK levels is depicted in graph B.

Prior to initiation of BRAF inhibitor therapy for BRAF-mutated melanomas, the bulk of the cells have optimal BRAF<sup>V600E</sup>→MEK→ERK (pERK) activity for tumor growth (graph A: the tumor cells are in the green zone with optimal pERK levels). In this state, tumor cells that will be sensitive to BRAF inhibitor (drug-sensitive) are growing at an optimal rate (graph A: blue line is in the green zone) and the rare variant cells possessing intrinsic drug resistance are not growing optimally (Graph A: black line is in the red zone). The corresponding tumor volume (Graph B) shows a greater volume of drug-sensitive blue cells and few drug-resistant black cells. Addition of BRAF inhibitor leads to insufficient BRAF<sup>V600E</sup>→MEK→ERK activity in the bulk of the drug-sensitive tumor, leading to tumor regression (blue line heads into the red zone in Graph A). However, BRAF inhibitor therapy immediately begins to select for expansion of melanoma cells with elevated BRAF<sup>V600E</sup> expression, corresponding to drug-resistant tumor cells, and these cells now get pushed into the optimal green zone in graph A; they begin to grow because of tumor fitness benefit in the presence of drug. Upon cessation of drug administration, drug-resistant melanoma cells slow their growth, and the drug-sensitive tumor cells reinitiate proliferation. In short, as the drug is alternately administered or withdrawn, the drug-sensitive tumor cells go from optimal to insufficient levels of pERK and back again, while the drug-resistant tumor cells go from optimal to excessive levels of PERK and back again. Thus, the volume of drug-sensitive and drug-resistant tumor cells keeps rising and falling in opposite directions, creating a homeostasis that keeps the cancer from growing out of control, which may translate to increased patient survival.
Oncogene Addiction and Overdose, from page 9

Specifically, it had previously been demonstrated that low levels of RAF pathway activation could promote the growth of cultured cells, whereas higher-level activation of the same pathway could elicit cell cycle arrest that, in primary cells, was irreversible and displayed features of cellular senescence.1,10 Hence, even though the HMEX1906 melanoma cells are addicted to oncogenic BRAFV600E signaling, they remain sensitive to the antiproliferative effects of high-level BRAFV600E→MEK→ERK pathway activation (Figure 1).

To test the relevance of this phenomenon in vivo, drug-resistant melanomas were implanted into mice that were dosed daily with vemurafenib. Whereas these continuously dosed mice showed sustained tumor growth, cessation of drug administration led to a highly reproducible decrease in melanoma cell proliferation accompanied by tumor regression, which correlated with a spike in the level of pERK1/2 (Figure 2).

However, eventually, a selective or adaptive response occurs, leading to reduced BRAFV600E→MEK→ERK activity, which allows for tumor regrowth.

Intermittent Dosing

The observation that vemurafenib-resistant tumors displayed a fitness deficit in the absence of drug, with a concomitant decrease in pERK1/2. These studies indicated that, although elevated BRAFV600E expression confers vemurafenib resistance on HMEX1906 cells, upon drug removal the elevated BRAFV600E expression is now unopposed, thereby promoting a level of BRAFV600E→MEK→ERK signaling that elicits antiproliferative activity leading to tumor regression (Figure 2). However, eventually, a selective or adaptive response occurs, leading to reduced BRAFV600E→MEK→ERK activity, which allows for tumor regrowth.

An intermittent drug-dosing schedule might forestall the onset of drug resistance by alternating the selective pressures on the drug-sensitive versus drug-resistant cell populations.

Next Frontiers

The major challenge ahead is to translate studies conducted in the well-controlled laboratory environment into the more complex world of clinical trials. This is indeed daunting due to the remarkable heterogeneity observed within BRAF inhibitor-resistant melanomas.5,15 Although drug resistance due to overexpression of BRAFV600E or the presence of truncated BRAFV600E splice variants may conform to the model described above, it seems unlikely that all mechanisms of BRAF inhibitor resistance will confer a fitness deficit in the absence of drug.6

Furthermore, even within a single melanoma, different resistance mechanisms may coexist — not all of which will benefit from intermittent dosing.9,16 Even the design of intermittent dosing clinical trials is complicated by the inability to predict the mechanisms of resistance that will emerge in any one lesion in any one patient. Hence, in the absence of a well-defined patient stratification strategy, it may be challenging to design a clinical trial that is sufficiently powered to detect a benefit in a subgroup of individuals.

An additional challenge is that the therapeutic landscape for patients with BRAF-mutated melanoma is rapidly evolving with the advent of new agents (dabrafenib, LGX818, trametinib, cobimetinib, etc.), some of which will be used in combination. Finally, when cancer cells are exposed to conventional or pathway-targeted chemotherapy, there is a selection for preexisting cell variants that have a fitness benefit in the presence of drug. Given the remarkable heterogeneity of melanoma identified by genome sequencing efforts, it is possible that intermittent dosing will simply select for those cells with mechanisms of
resistance that are oblivious to intermit-
tent dosing.

Despite the substantial challenges, a clinical trial is currently under way
to test the utility of intermittent dos-
ing with a BRAF inhibitor (LGX818, NCT01894672) in patients with stage IV
or unresectable stage III BRAF-mutated melanoma. Planning is also under way
to compare the ability of intermittent versus continuous dabrafenib plus
trametinib to promote progression-free
survival in patients (S1320).

**Intermittent Combination Therapy: Sequential vs. Concurrent Administration**

Assuming that such studies yield promising outcomes, what might be
the best way to translate intermittent dosing of BRAF mutant
pathway-targeted inhibitors into clinical practice? In the past few years, the response of mel-
oma patients to immunomodulators such as anti-CTLA-4, anti-PD1, or
anti-PDL1 has generated considerable
excitement.17 Since concurrent admin-
istration of both pathway-targeted and immunomodulatory therapies may be
contraindicated either because of drug
interference or unacceptable toxicity, an
intermittent dosing regimen may allow
such combinations to be employed in a
rational sequential manner. For ex-
ample, a patient with a BRAF-mutated
melanoma might receive BRAF mutant
pathway-targeted therapy for an initial
period, until the deepest possible tumor
regression has been achieved. At that
time, the patient could be switched to
an immunomodulatory agent to provoke
a durable immune response against the
residual disease. The patient might then
be switched back and forth between the
pathway-targeted and the immuno-
modulatory therapy, with the intent of
eliciting the deepest and most durable
remission. Clearly, the development
of such a regimen would benefit from
preclinical data supporting the concept.
However, preclinical studies to test the
utility of alternating pathway-targeted
and immunomodulatory therapy are
only feasible using either GEM models
or PDMXs transplanted into immuno-
compromised mice reconstituted with a
“humanized” immune system.18,19

**Conclusions**

The recent clinical successes of path-
way-targeted and immunomodula-
tory therapies have converted advanced
melanoma from a disease without ef-
ficacious treatment options into one
with treatments affording remarkable
disease control. This revolution in the
revolution of BRAF-mutated melanoma
also offers hope to patients with other
recalcitrant cancers that have limited
treatment options. However, attendant
to each success are new challenges. Ini-
tial experience with pathway-targeted
melanoma therapy has revealed the depth of heterogeneity that allows
cancer cells to evade even the newest
and most innovative treatments. Fortu-
nately, the rapid rate of cancer evolu-
tion is paralleled by a rapid evolution in
our ability to employ multiplex genetic and biochemical analyses to probe the
inner workings of the cancer cell. Such
detailed maps of the cancer cell prom-
ise to transform cancer from a disease
treated with toxic chemotherapies to
one treated with rational drug combi-
nations designed to selectively ablate
malignant cells and prevent lethal drug
resistance.

The authors acknowledge our UCSF
colleague, Dr. Adil Daud, for his com-
ments on this manuscript.

**References**

1. Davies H, Bignell GR, Cox C, et al. Mutations of
417(6892): 949-954.

2. Chapman PB, Hauschil A, Robert C, et al. Im-
proved survival with vemurafenib in melanoma
with BRAF(V600E) mutation. New Engl J Med

and ERK signalling in cells with wild-type BRAF.

4. Wagle N, Emery C, Berger MF. Dissecting therape-
utic resistance to RAF inhibition in melanoma by
27(22):3085-3096.

5. Lito F, Rosen N, Solit DB. Tumor adaptation and
resistance to RAF inhibitors. Nat Med 2013;

Modelling vemurafenib resistance in melanoma re-
sults reveals a strategy to forestall drug resistance.

7. Siolas D, Hannon G. Patient-derived tumor xenog-
rafts: transforming clinical samples into mouse

8. Schimke RT, Alt FW, Kellemes RH, Kaufman RJ, Ber-
tino JR. Amplification of dihydrofolate reductase
genes in methotrexate-resistant cultured mouse
cells. Cold Spring Harb Symp Quant Biol 1978; 42
Pt 2:649-657.

whole-exome sequencing identifies (V600E) B-RAF
amplification-mediated acquired B-RAF inhibitor

10. Woods D, Parry D, Cherwinski H, Bosch E, Lees
E, McMahon M. RAF-induced proliferation or cell
cycle arrest is determined by the level of RAF ac-
tivity with arrest mediated by p21Cip1. Mol Cell Biol
1997; 17(9):598-5611.

11. Zhu J, Woods D, McMahon M, Bishop JM. Senes-
cence of human fibroblasts induced by oncogenic

sion of RAS-mutant leukemia during RAF inhibitor

13. McMahon M, Woods D. Regulation of the p53 path-
way by Ras, the plot thickens. Biochim Biophys

14. Krizhanovsky V, Yon M, Dickens RA, et al. Senes-
cence of activated stellate cells limits liver fibrosis.

and clonal evolution in melanoma during BRAF
Nov 21.

RAF inhibitor resistance is mediated by dimeriza-
tion of aberrantly spliced BRAF(V600E). Nature

17. Callahan MK, Wolchok JD. At the bedside: CTLA-4-
and PD-1-blocking antibodies in cancer immuno-

cooperates with PTEN loss to induce metastatic melanoma. Nat Genet 2009; 41(5):544-
552.

19. Shultz LD, Brehm MA, García-Martínez JV, Greiner
DL. Humanized mice for immune system investiga-
tion: progress, promise and challenges. Nat Rev


21. Weinstein IB, Joe AE. Mechanisms of disease:
Oncogene addiction — a rationale for molecular
targeting in cancer therapy. Nat Clin Pract Oncol
“Targeted therapy” has been the springboard for this revolution, often replacing chemotherapy’s scattershot attack on both diseased and healthy cells or relegating it to a complementary role. By gaining insights into the molecular drivers of cell division, senescence, and apoptosis, and unraveling many of the immune system’s complex interactions with cancer cells, scientists have been able to develop strategies specifically inhibiting not only defective genes that switch on melanomas, but also certain brakes on the immune system that stop T-cells from attacking. These new therapies offer hope to patients who once had little. Still, only 16 percent of patients with distant metastases attain 5-year survival, and much remains to be learned about how best to employ these new therapies: when and how to use them, how to choose among them, and whether to use them alone, in combination, or sequentially to minimize toxicities and circumvent drug resistance.

In our lead story, Drs. Momtaz, Lacouture, and Chapman explore in detail just how far we’ve come in this targeted revolution, and how much further we have to go.

The BRAF inhibitor vemurafenib is an object lesson in how complex the process of discovery will be. The first drug approved to block signaling along the mitogen-activated protein kinase (MAPK) pathway driving melanoma cells, it has provided at once some of the greatest thrills and greatest disappointments of the targeted era. Initially, based on these breathtaking results, many were naïvely optimistic that the MAP-kinase pathway would prove easy to manipulate and that simply blocking the

Continued on page 7