

Translating BRAF Mutations into Effective Therapy for Patients with Melanoma

By Jeffrey A. Sosman, MD

Overview: Melanoma expresses mutations in critical cancer-causing genes, including BRAF, NRAS, CKIT, and GNAQ or GNA11. One of these mutations occurs in nearly 70% of melanomas and for the most part they are mutually exclusive. The anatomic site of the primary melanoma is strongly associated to the gene that is mutated. These mutations lead to unregulated activation of the MAP kinase pathway, which promotes cell proliferation, prevents apoptosis, and promotes angiogenesis. New drugs such as vemurafinib (PLX4032) and GSK2118436 are potent BRAF inhibitors that are selective for the V600E BRAF in cancer cells. Both drugs have demonstrated rapid clinical responses and their overall response rate is very high (> 50%). Vemurafinib has completed phase I to III testing and phase III data released has demonstrated for the first time that both overall survival (OS) and progression-free survival (PFS) are improved compared with chemotherapy

HISTORICALLY, MELANOMA has been classified according to clinical and pathologic characteristics such as histology (depth, level, ulceration, and lymphocyte infiltration) and anatomic site of origin and was thought of as a single disease in terms of treatment. In general, treatment of advanced melanoma has been ineffective. Although a few patients demonstrate durable and sometimes complete responses to immunotherapy, the overwhelming majority of patients have disease that has been refractory to all systemic therapy. Over the past decade, it has become evident that subsets of melanoma can be further defined at the molecular level by recurrent driver mutations that occur in multiple oncogenes, including BRAF, GNA11, GNAQ, KIT, and NRAS (Fig. 1). Such driver mutations lead to constitutive activation of mutant-signaling proteins that induce and sustain tumorigenesis, a process known as “oncogene addiction.” Mutations in BRAF, CKIT, and NRAS can be found in approximately 70% of all melanomas. These mutations are seldom found concurrently in the same tumor. The distribution of mutation varies by site of origin and also by the absence or presence of chronic solar damage (Fig. 1).

RAS has been implicated in the pathogenesis of several cancers. Activating mutations within the RAS gene results in constitutive activation of the RAS GTPase, even in the absence of growth factor signaling. The result is a sustained proliferation signal within the cell.

Somatic mutations in NRAS have been found in 10% to 25% of all malignant melanomas¹⁻³ and were described as far back as the 1980s. In the majority of cases, these mutations are missense mutations, which introduce an amino acid substitution at positions 12, 13, or 61. The result of these mutations is constitutive activation of NRAS signaling pathways. NRAS mutations are found in all melanoma subtypes, but may be slightly more common in melanomas derived from chronic sun-damaged skin.^{1,3}

Up until 2002, NRAS mutations, which were and still remain nondruggable, were the only recurrent activating oncogene mutation found in melanoma. However, the landscape of melanoma research both from a preclinical and clinical perspective changed profoundly because of the discovery of mutations in the BRAF gene in solid human

with dacarbazine in the first-line setting. Squamous cell cancer of the skin develops in a large number of patients (30%). These cases are of the keratoacanthoma type, and are easily excised. Resistance to the BRAF inhibitors occurs uniformly after a period of effective treatment. Several early studies looking at patient's tumor tissues suggest several mechanisms for resistance. These include mutation of an upstream gene (NRAS) to activate the MAP kinase pathway; activation/overexpression of an alternate receptor tyrosine kinase signaling pathway (e.g., insulin-like growth factor receptor 1 [IGFR1], platelet-derived growth factor receptor (PDGFR) beta); and activation/overexpression of an activating kinase capable of activating the MEK protein and bypass the blockade. After 30 years with little progress, a paradigm shift in our approach to melanoma in the clinic has taken place. The future is much brighter for patients with melanoma.

tumors.⁴ The most commonly identified mutation in the BRAF gene arises in the kinase domain at nucleotide 1799, leading to a change in the V600 amino acid valine to glutamic acid, resulting in constitutive activation of BRAF kinase. The mutated BRAF kinase is able to activate downstream components of the pathway even in the absence of an upstream (external) signal. This results in dysregulated downstream signaling, gene expression, and, ultimately, excessive cell proliferation and survival.⁵⁻⁸ Oncogenic BRAF signaling is implicated in approximately 50% of melanomas, 30% to 70% of thyroid tumors, 30% of serous low-grade ovarian tumors, and 10% of colorectal cancers (CRCs) as well as infrequently (less than 5%) in a number of other cancers.⁴ The pervasive nature of oncogenic BRAF signaling across human cancers has focused attention on the development of targeted anticancer agents able to attenuate the aberrant signaling generated by the mutant BRAF kinase. During this same period small-molecule inhibitors of signaling kinases that are activating cancers became an effective therapy in some cases. Their success was first demonstrated by the pioneering work of Druker and Sawyer in chronic myeloid leukemia where imatinib had an essential role in the treatment of this disease. Imatinib has changed the entire disease course for many patients through its inhibitory effects on bcr-abl. Imatinib, a multitargeted kinase, also has inhibitory effects on CKIT and PDGFR. These effects have translated into very significant clinical benefits for patients with gastrointestinal stromal tumors (KIT and PDGFR).

The foundation for targeted therapy had been established in melanoma in 2002. However, the path was not as simple or straightforward as had been hoped. Initially, the avail-

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Melanoma is Comprised of Clinically Relevant Molecular Subsets

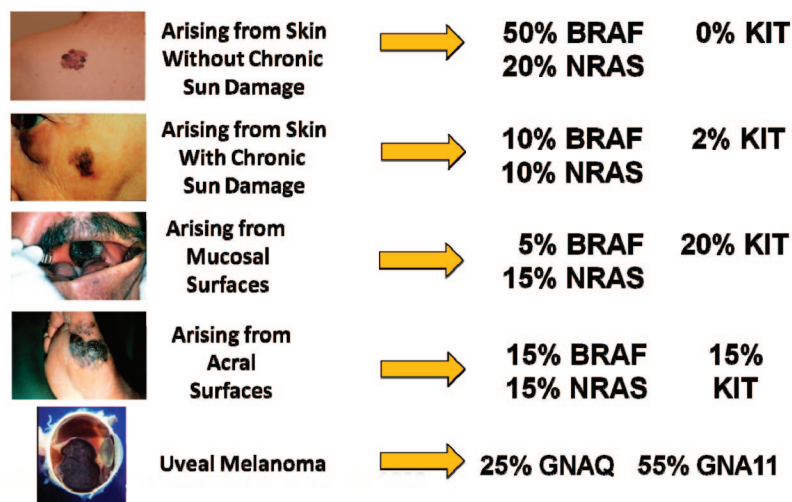


Fig. 1. Anatomic sites and the frequency of genetic mutations.

able RAF inhibitor sorafenib entered clinical trials before 2002 when BRAF mutations in melanoma were first de-

scribed. This agent eventually was determined to be multi-targeted kinase inhibitor (MTKI) with activity against vascular endothelial growth factor receptors and PDGFR. Its RAF activity as a single agent was minimal. This was less surprising as investigators demonstrated the intracellular and in vivo activity of sorafenib against V600E BRAF was extremely poor. Initial reports in combination with chemotherapy were promising. However, no association between BRAF mutations and response was found. Finally, a randomized phase III trial failed to demonstrate significant improvement in response, PFS, or OS, putting an end to the studies of sorafenib in melanoma as a single agent or combination with chemotherapy.

PLX4032/RG7204/Vemurafinib

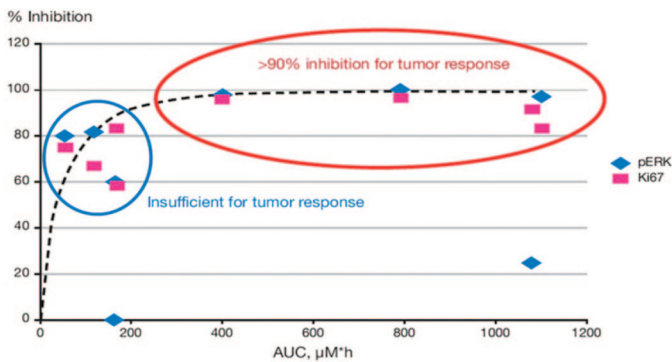
In 2007, clinical trials began with a new small molecule developed through a crystallographic approach by investigators at Plexikon, PLX4032, that demonstrated much greater potency as well as selectivity for V600E BRAF kinase. In vitro biochemical assays have shown that PLX4032 exhibits selectivity within a broad range of kinases. In panel testing of over 200 kinases, PLX4032 showed a similar potency for BRAFV600 (31 nmol/L; the most common mutation seen, encompassing 90% of BRAF-mutant melanoma tumors) and CRAF (48 nmol/L), and selectivity compared with other kinases.⁹ Selectivity for the mutated protein was apparent most impressively in vivo (within cell) where a more physiologic interaction between the wild-type RAS and wild-type RAF kinases and the mutated V600E BRAF kinase exists.

The in vivo selectivity of PLX4032 has translated into cellular selectivity in a series of experiments designed to evaluate the effect of PLX4032 on RAF-MEK-ERK pathway inhibition and proliferation suppression in a panel of cancer cell lines.¹⁰ Cell lines tested for inhibition of MEK and ERK phosphorylation included melanoma cell lines expressing BRAFV600E, BRAFV600D, BRAFV600R, or BRAFWT. PLX4032 was reported to inhibit both phosphorylation of MEK and ERK, and cellular proliferation in all BRAFV600E-expressing melanoma cell lines tested, includ-

KEY POINTS

- BRAF mutations are found in 50% of melanomas, and 90% of these have a mutation at amino acid V600E. This mutation leads to unregulated activation of the MAP kinase pathway, which promotes cell proliferation, prevents apoptosis, and promotes angiogenesis.
- New drugs, such as vemurafinib (PLX4032) and GSK2118436, are potent BRAF inhibitors that are selective for the V600E BRAF in cells. Both drugs have demonstrated rapid clinical responses and their overall response rate is very high (50%). Vemurafinib has completed phase I to III testing, and a 132-patient phase II trial showed a response rate over 50% with a median duration of nearly 7 months. Phase III trials have demonstrated for the first time that both OS and PFS are improved compared with chemotherapy in first-line setting.
- Squamous cell cancer of skin develops in a large number of patients (30%) and are easily treated with surgical excisions. These are of the keratoacanthoma type.
- Resistance to the BRAF inhibitors occurs uniformly after a period of effective treatment. Several early studies suggest several mechanisms for resistance. These include mutation of an upstream gene (NRAS) to activate the MAP kinase pathway; activation/overexpression of an alternate receptor tyrosine kinase signaling pathway (e.g., insulin-like growth factor receptor 1, platelet-derived growth factor receptor beta); and activation/overexpression of an activating kinase capable of activating the MEK protein and bypass the blockade.

To what extent should the target be inhibited?



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Fig. 2. Pharmacodynamic effects correlate with antitumor response for PLX4032.

ing Colo829 and LOX. PLX4032 also exhibited potent inhibitory effects on MEK and ERK phosphorylation and cellular proliferation in melanoma cell lines that expressed other mutations at the V600 position, such as BRAFV600D, BRAFV600R, and BRAFV600K, but not in cells with wild-type BRAF.¹⁰⁻¹³ Thus, PLX4032 displays a high degree of selectivity against BRAFV600 kinase in mechanistic and antiproliferative cellular assays.

The effect of three doses of PLX4032 on antitumor activity and survival was determined in vivo using the murine LOX melanoma xenograft model.¹⁰ PLX4032 100 mg/kg twice daily for 12 days grew at similar rates to tumors treated with vehicle, with no tumor growth inhibition reported, while V600E-positive tumors were largely eradicated.¹⁴ These observations, together with the data generated in BRAFV600E-expressing xenograft models, have shown the in vivo selectivity of PLX4032 for BRAFV600E.

Mode of Action

In vitro and in vivo studies provide evidence for the mode of action for PLX4032.¹³⁻¹⁷ As a potent and selective inhibitor of mutant BRAF, PLX4032 was designed to suppress MAPK signaling through suppression of BRAF activity. The only known substrate of BRAF is MEK kinase. Phosphorylation of MEK by BRAF results in activation of MEK; pMEK in turn phosphorylates ERK, and pERK translocates into the nucleus where it activates transcriptional factors that are responsible for upregulating cell proliferation and cell survival (Fig. 2). The studies described above show that PLX4032 potently inhibits MEK phosphorylation and activation, which consequently inhibits ERK phosphorylation and ultimately cell proliferation in tumor cells expressing the mutant BRAF gene.

Phase I Clinical Trial with Extension Phase

Clinical trials began in 2007 and after reformulation allowed adequate blood levels to be achieved, striking clinical activity was seen in the patients with V600E-mutated melanoma. A total of 87 patients (including 81 with melanoma) were recruited to a phase I study and received doses of up to 1,120 mg twice daily.¹⁷ Patients were exposed to relatively constant levels at a steady state that was between

six and nine times the mean level on day 1 at the 960 mg twice-daily dose. The mean half-life of PLX4032 was approximately 50 hours and the area under the plasma concentration–time curve (AUC) for the 960 mg twice-daily dose was $1,741 \pm 639 \mu\text{mol/L/hour}$.²³ The 960 mg twice-daily dose was selected for phase II and phase III evaluation. Divided dosing was continued, despite the long half-life in order to avoid a potentially unacceptable number of capsules per dose.

Phase I pharmacodynamic analyses showed that tumor levels of phosphorylated ERK, cyclin D1, and Ki-67 were markedly reduced at day 15 compared with baseline, indicating that PLX4032 inhibited the MAPK pathway, resulting in decreased cell proliferation.¹⁷ Additionally, there was a clear relationship between AUC, pharmacodynamic changes, and clinical responses.^{8,17} Responses were largely seen only when patients demonstrated over 90% phosphorylated ERK inhibition intratumorally (Fig. 2).

Of the 16 patients with V600E BRAF melanoma in the higher-dose levels with reformulated PLX4032, 11 patients demonstrated an objective Response Evaluation Criteria in Solid Tumors (RECIST) clinical tumor regression. The phase I study included an expansion phase of 32 patients with V600E BRAF mutant melanoma who were all receiving the recommended phase II dose. The response rate continued to be remarkable with 26 of 32 evaluable patients (81%) demonstrated a RECIST based objective clinical responses (Fig. 3).¹⁷ Among patients with metastatic disease, responses were recorded at all metastatic sites and there was an improvement in symptoms for those with symptomatic disease, including a reduction in the requirement for narcotic pain relief within 1 to 2 weeks (although it should be noted these data were anecdotal and were not systematically collected). Evaluation of patients in this extension arm is ongoing; a confirmed response rate of 59% and a PFS of 7.61 months were recorded as of September 30, 2010. The most frequent adverse events (AEs) were arthralgia, rash, nausea, photosensitivity, fatigue, pruritus, and palmar-plantar dysesthesia. Most events were mild to moderate in severity and easily manageable. Cutaneous squamous cell carcinoma occurred at a rate of 31% (10/32 patients) during the extension phase of the study. The median time to occurrence of these lesions was 8 weeks; the majority were resected and none led to discontinuation of treatment. The majority of excised lesions reviewed by a central pathology laboratory were classified as squamous cell carcinoma, keratoacan-

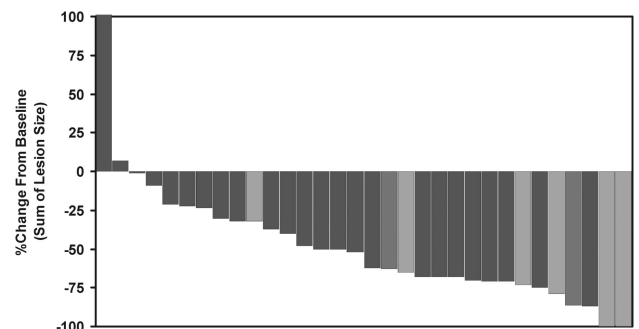


Fig. 3. Phase I extension waterfall curve of best percent tumor regression by RECIST for PLX4032. Tumor responses occurred in the majority (81%) of patients in V600E plus melanoma extension cohort (960 mg twice a day). These were not always confirmed as required by RECIST and were not independently reviewed.

Tumor Regression (Target Lesions) Occurred in Majority of Patients (IRC)

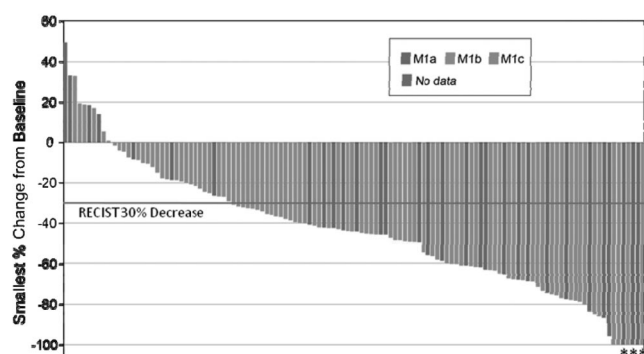


Fig. 4. Phase II waterfall curve of best percent decrease in tumor for PLX4032. Seven patients had 100% tumor shrinkage, three of whom had confirmed complete response; one patient had unconfirmed complete response; and three patients had nontarget lesions present. One hundred twenty-two patients had baseline and one or more post-baseline scan with measurable disease.

thoma subtype, with a low likelihood of invasive or metastatic potential.¹⁸ These uniformly could be easily excised with wide margins. There was no need to even interrupt therapy and certainly no dose reduction was performed.

Phase II Clinical Trial

A phase II (BRIM 2) study of PLX4032 in patients with metastatic melanoma was recently reported.¹⁹ In all, 344 patients were screened of whom 132 were eligible for treatment. The rate of BRAFV600E mutation in the screened population (344 patients) was 56%. Preliminary best overall response rate (BORR, primary study endpoint) was greater than 52% as assessed by an independent review committee (IRC) at only 6 months after completion of accrual (Fig. 4). Another updated assessment will occur in February 2011. The median response duration was nearly 7 months and median PFS was greater than 6 months for the entire cohort. Side effects (AEs, serious AEs, dose reductions) were manageable, the most common being arthralgia, rash, photosensitivity, fatigue, and alopecia. As previously reported in the phase I/II trial, squamous cell carcinoma primarily of keratoacanthoma type was reported in 32 patients, representing approximately 25% of patients. Thus BRIM-2 met its primary endpoint demonstrating a BORR (IRC) whose lower limit of 95% confidence interval was greater than 40%. As of the clinical cutoff data of September 27, 2010, 50 patients remained on protocol. The full results will be reported later this year at the 2011 ASCO Annual Meeting.

A phase III study (BRIM3; NCT01006980) comparing the efficacy of PLX4032 with that of dacarbazine in patients with previously untreated metastatic melanoma has completed accrual. Primary outcome data (coprimary endpoints of OS and PFS) have been met with demonstration of clinical benefit in the first-line setting for both PFS and OS with hazard ratio (HR) 0.65 for OS and HR 0.55 for PFS reached. As described in detail below, paradoxical activation of ERK by RAF inhibitors has been reported in cells that lack a BRAF mutation. These reports emphasize the importance of selecting patients with BRAFV600E mutations for treatment with PLX4032.

Another Selective RAF Kinase Inhibitor: GSK2118436

GSK2118436 is a selective RAF inhibitor. It is an ATP-competitive; reversible inhibitor with selectivity demonstrated against 270 kinase panel. Only 10 of 270 kinases are inhibited at IC₅₀ 10 to 100 nmol/L, and the others are all inhibited at IC₅₀ from 100 nmol/L to greater than 10,000 nmol/L. As demonstrated in Fig. 5, this is especially evident with IC₅₀ for the three most common V600E BRAF mutations of 0.5 to 1.9 nmol/L. This selectivity was also demonstrated for V600 BRAF mutant cell lines and corresponding inhibition of V600E melanoma xenografts. In a large-dose expansion phase I trial where doses of 150 and 200 mg orally daily of GSK2118436 were administered, 20 of 26 (77%) patients with V600E mutations had objective responses.²⁰ Results on durability are not available because of the short follow-up. All of these responses have not been confirmed. Of great interest is the clinical activity observed in patients with brain metastases and those patients with non-V600E BRAF mutations (V600K or V600D). In patients with brain metastases, eight of 10 demonstrated clinical responses in brain lesions, while of the nine patients with V600K BRAF mutations in their melanoma, four of nine had objective responses.

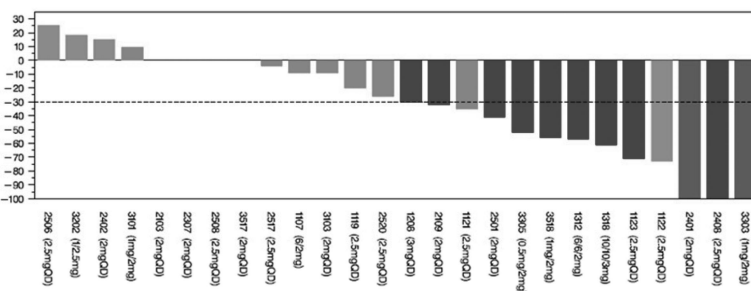
MEK Inhibitors: Clinical Experience

Preclinical data has correlated the presence of activating mutations in BRAF with sensitivity to non-ATP competitive MEK inhibitors, AZD6244 and CI-1040.^{21,22} Initial phase I trials with an active MEK inhibitor, PD3250901 demonstrated potent inhibition of ERK phosphorylation and clinical responses in a number of patients with melanoma with V600E BRAF-mutated tumors. However, early phase II trials were marred by some neuromuscular toxicities and retinal vein thromboses, leading to early closure and abandonment of this MEK inhibitor. Another drug developed by Array Pharmaceuticals, AZD6244, showed promise in a phase I dose-escalation study based on pharmacodynamic evidence for target inhibition and clinical benefit. This led to further study with a randomized phase II clinical trial of AZD6244 compared with temozolomide. Six patients receiving AZD6244 had a confirmed partial response (PR), of which five were BRAF positive (12% of BRAF-positive patients). Nine patients receiving temozolomide had a confirmed PR, three of which were BRAF positive (12% of patients who were BRAF positive).²³ Finally, at the 2010 ASCO Annual Meeting and later at the 2010 ESMO Congress, results in a phase I dose-escalation study were pre-

ENZYME	STATUS	IC ₅₀ (nM)
B-RAF	V600E	0.6
B-RAF	V600K	0.5
B-RAF	V600D	1.9
B-RAF	WT	12
C-RAF	WT	5

Fig. 5. In vitro kinase inhibition by GSK2118436 (BRAF inhibitor). GSK2118436 is a selective RAF inhibitor.

Fig. 6. Phase I of GSK 1120212 waterfall curve of best percent tumor regression by RECIST. Twenty-eight patients with BRAF-mutant melanoma: 90% are M1c; 48% had history of brain metastases. Two complete responses and partial responses; response rate 41% (95% CL, 24–61%).



sented for a MEK inhibitor, GSK1120212.²⁴ It is a reversible, selective, allosteric inhibitor of MEK1/MEK2 activation and kinase activity. Primary toxicities were rash (acneiform), diarrhea, and, rarely, left ventricular cardiac dysfunction. A central serous retinopathy was observed at higher doses, but when patients were screened before treatment, this did not occur at or below the RP2D, which was 2.0 mg q day. There was a half life of 4.5 days with pharmacodynamic evidence for potent target inhibition at tumor sites. Finally responses were observed in both BRAF-mutant and BRAF WT melanomas. Final results demonstrated that at active doses at or above the RP2D, of 28 patients with BRAF-mutated melanoma, there was a response rate of 41% (Fig. 6). This has led to rapid development of combination regimens with GSK 2118436 (BRAF inhibitor) and GSK1120212 (MEK inhibitor)—which have been found to be safe—and even more recently GSK1120212 with a PI3K inhibitor in collaboration with Novartis. As described below, these combinations will be critical to moving therapy forward and transforming high response rates to more durable and complete responses with a longer-lasting effect on patients. Other MEK inhibitors have entered the clinic in phase I trials, including a compound, TAK-733, jointly being developed by Takeda and Millennium Pharmaceuticals.

Mechanisms of Acquired Resistance: BRAF Inhibitors

Initial investigations into potential mechanisms of acquired resistance suggest that there are several genetic and signal transduction alterations that can circumvent BRAF inhibition. The number of cell lines that have been treated chronically with PLX4032 to generate acquired resistance is small, and the number of tumor samples acquired at the time of clinical disease progression and thoroughly characterized is even smaller. Nonetheless, a few key observations have been reported recently that support reactivation of the MAP kinase pathway or RAS effector pathways as resistance mechanisms for which there is support from both in vitro and ex vivo analyses. Paradoxical activation of ERK by RAF inhibitors has been reported in cells that lack a BRAF mutation. Three recent reports have explored the potential mechanism(s) for this activation by showing that selective BRAF inhibitors, such as PLX4720 (an analog of PLX4032), 885-A, and GDC-0879 (selective BRAF inhibitors of different chemical series), stimulate MEK-ERK signaling through CRAF activation in the presence of an upstream activator (e.g., activated RTK, RAS mutation) in melanoma and other cell lines lacking BRAF mutations.²⁵⁻²⁷ These studies support a model in which BRAF-specific inhibitors enhance RAS-GTP-dependent CRAF activation through the formation of BRAF-CRAF heterodimers or CRAF homodimers. This is followed by recruitment of CRAF to the plasma

membrane, triggering activation of the MEK-ERK pathway. RAS mutations would additionally stimulate alternative downstream pathways. In several cases, the appearance of an activating NRAS mutation coexisting with the BRAFV600E mutation at the time of disease progression has been documented (personal communication from Lo and Solit).²⁵ In other cases, a marked increase in the expression of PDGFR beta has been observed and appears to mediate upregulation of PI3 kinase pathway signaling.²⁷ Recently, Villanueva and colleagues reported that a combination of flexible RAF isoform switching and enhanced IGF-1R/PI3K signaling was involved in mediating resistance to the BRAF inhibitors.²⁸ This could be overcome by combined IGF-1R and MEK inhibition. Loss of the phosphatase and tensin homolog (a negative regulator of the PI3-Akt signaling pathway) was also found in a relapsed patient sample, further suggesting that combined inhibition of the MAPK and PI3K pathways could improve tumor control. Lastly, signaling through COT/TPL2—which has previously been described as an activator of MEK signaling—can promote resistance in previously sensitive cell lines, and increased expression of this molecule in tumor samples derived from patients progressing following initial response supports the potential relevance in vivo.²⁹ In another study, acquisition of KRAS mutation was observed in an in vitro PLX4032-acquired resistance model, implying that multiple mechanisms leading to increased pathway activity are at play (Su, personal communication). Importantly, in all of these analyses, acquired secondary mutations in BRAF have not been observed, suggesting that compensatory signaling to other molecules is the primary mode of resistance.

Pathway Forward for MAP Kinase Pathway Inhibition in Melanoma

As of 2011, we have established that drugs with potent and selective inhibitory activity against activated mutated BRAF kinase can induce rapid, clinically meaningful responses in a majority of patients with melanoma whose tumors express the V600 BRAF mutation (E/K/D). On the other hand, these responses are not nearly as durable as hoped for with a median progression-free survival of less than 8 months and near universal resistance less than 24 months. These responses have already translated into improved survival compared with previously standard chemotherapy (phase III BRIM release). Furthermore, we now have clinical activity from a MEK inhibitor, GSK2110212 in V600E BRAF-mutant melanoma with a response rate of greater than 40% without intolerable toxicity. Finally, the availability of resistant tumors from patients receiving the BRAF inhibitors already suggest different strategies to pursue to both overcome and prevent resistance. Combina-

tion therapy will likely be critical to convert the responses into long-term remissions. The availability of not only MEK inhibitors, but also PI3K inhibitors, AKT inhibitors, mammalian target of rapamycin complex 1 (mTORC1)/TOR complex 2 inhibitors, and PI3K/mTORC inhibitors in the clinic obviously provides additional strategies to target resistance either before or after it occurs. Additionally drugs targeting PDGFR and IGF1R kinases are in clinical trials or approved

therapy for other cancers. The spectrum of agents also provides strategies that may be effective against another 20% of mutated melanoma tumors, those carrying a NRAS mutation either at codon 12, 13, or 61, which are all activating. Finding approaches that can more effectively target BRAF mutations and effectively target NRAS mutations could translate into effective therapy for 60% to 70% of all melanoma cases.

Author's Disclosures of Potential Conflicts of Interest

Author	Employment or Leadership Positions	Consultant or Advisory Role	Stock Ownership	Honoraria	Research Funding	Expert Testimony	Other Remuneration
Jeffrey A. Sosman		AstraZeneca, Millennium, Roche					

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