BRAF in Melanoma: Current Strategies and Future Directions

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Abstract
Selective BRAF inhibitors have now been established as a standard of care option for patients diagnosed with metastatic melanoma whose tumors carry a BRAF mutation. Their successful development represents a milestone in the treatment of this disease, and has the potential to impact therapy for other malignancies as well. The use of these agents, however, has introduced a number of critical questions about the optimal use and selection of patients for BRAF inhibitor therapy. This review discusses the current status of BRAF inhibitor clinical development, the clinicopathologic features of BRAF-mutated melanoma, as well as strategies for overcoming resistance. Clin Cancer Res; 19(16); 4326–34. ©2013 AACR.

Introduction
Metastatic melanoma has proven to be an exceedingly difficult disease to treat, and long-term survivors are rare (1). It is resistant to most chemotherapeutic agents, and an understanding of the biology that drives the disease had lagged behind that of other malignancies. In the past several years, however, a number of advances have been made which have important therapeutic implications. One of these is the discovery of BRAF as a contributor to melanoma oncogenesis in a large proportion of patients (2, 3). From its discovery as an oncogene 10 years ago to the clinical development of selective BRAF inhibitors, BRAF represents a success story for translational oncology research. This review summarizes the current data about BRAF inhibitor clinical development, the clinicopathologic features associated with BRAF-mutated melanoma, and provides insight into future directions and potential combination strategies to overcome resistance to BRAF inhibitor therapy.

Pathway Overview
BRAF has garnered a great deal of attention in recent years, as a high frequency of mutations have been reported in a number of malignancies, including melanoma, papillary thyroid cancer, and most recently hairy-cell leukemia, in all accounting for 7% to 8% of all cancers (4, 5). As part of the mitogen-activated protein kinase (MAPK) pathway, it serves to transmit signals from activated cell surface growth factor receptors to numerous intracellular effectors, including transcription factors and regulators of apoptosis (Fig. 1; refs. 6 and 7). The RAF-MEK (MAP–ERK kinase)-ERK (extracellular signal-regulated kinase) signaling cascade is the best characterized MAP kinase pathway in cancer, and mutated BRAF results in constitutive activation, ultimately resulting in unregulated cell proliferation and growth (7).

Somatic BRAF mutations were first identified in a screen of cancer cell lines in 2002, where it appeared that a high
percentage of melanomas had a mutation (2). All of the mutations identified in this study were within the kinase domain at exon 11 or exon 15, with the vast majority being due to a single point mutation at amino acid position 599 (now known as 600), which resulted in the substitution of a glutamic acid for valine, V600E. A number of other mutations have been identified, including V600K, which as the second most common variant, accounts for approximately 15% to 30% of BRAF mutations in melanoma (8, 9). Interestingly, these mutations are distinct from those that would be expected from exposure to ultraviolet radiation (10). It is now known that BRAF mutations are present in approximately 50% to 60% of cutaneous melanomas, as well as at lower frequencies in other melanoma subtypes (11). Uveal melanoma, however, remains an exception, with no BRAF mutations identified to date (12, 13).

**BRAF mutation testing**

A real-time PCR–based assay using the cobas 4800 system was used to select patients for enrollment onto clinical trials with vemurafenib (14–16). This assay was subsequently approved for use in conjunction with vemurafenib as a companion diagnostic test. Specifically designed to detect the V600E mutation using formalin-fixed paraffin embedded tissue, it also has the capability to detect other V600 variants, albeit with less sensitivity and specificity. When compared to bidirectional sequencing, there seems to be 97% agreement in detecting the presence of the V600E mutation. However, in 38 patients who were confirmed to have a V600K mutation with direct sequencing, the assay detected at mutation in 25 patients, a 66% rate of agreement (17). Subsequent analyses from patients treated on phase II and phase III trials with vemurafenib show similar rates of agreement and detection of the V600K variant (18). As mutations in BRAF seem to be an early event in melanomagenesis, archival samples, or the primary lesion could potentially be used, as there seems to be a high degree of concordance between primary lesions and metastases (8, 19–21). However, out of concern for the possibility of both intra- and intertumor heterogeneity, it is preferable to determine BRAF status from the most recently available metastatic tissue (22–24).

**Overview of BRAF inhibitor development**

Targeted inhibition of this signaling cascade translates into meaningful clinical benefit for patients with metastatic melanoma, results that have now been verified in 2 phase III trials using vemurafenib and dabrafenib (25, 26). Initial attempts, however, to target BRAF were unsuccessful. Sorafenib was among the first RAF targeted therapies to be developed in melanoma, with ample preclinical data to support this strategy (27, 28). Initially developed as a RAF-1 inhibitor, early trials did not show promising results (29, 30). Subsequent studies revealed that BRAF mutations were not predictive of a clinical response to sorafenib, either alone or in combination, and cast doubt on the viability of BRAF as a target in melanoma (31–33). One potential explanation is that sorafenib has a higher affinity for other RAF isoforms as well as other pathways, thereby limiting its on target effects (34). A novel structure guided development
strategy subsequently led to the development of PLX4032, a BRAF inhibitor designed to selectively bind the mutant form of BRAF, (35). Highly selective BRAF inhibitors showed marked inhibition of ERK phosphorylation in tumors, with resultant impressive clinical responses—establishing that BRAF mutant melanomas are highly dependent on sustained BRAF kinase activity (14). The selective signaling effect of these agents seems to account for their therapeutic index in comparison to sorafenib. In a phase I/II study of the compound PLX4032, 26 of 32 BRAF mutant patients in the dose extension cohort showed a response, with median progression-free survival for all patients estimated at more than 7 months (14). A phase III study comparing vemurafenib to dacarbazine, (HR = 0.37; P < 0.001) in 36 patients with BRAFV600E mutated melanoma, treated at recommended phase II dose or higher. (36, 37). The clinical efficacy has subsequently been confirmed based on phase III study results, where patients who received dabrafenib had an improved progression-free survival when compared to dacarbazine (HR = 0.30; P < 0.0001; ref. 26). Table 1 summarizes the key findings for the monotherapy trials of dabrafenib and vemurafenib to date.

**Toxicity.** Although manageable for most patients, some toxicities related to BRAF inhibitor therapy do require coordinated multidisciplinary management. In clinical trials of vemurafenib, the most common side effects reported have been arthralgias, rash, fatigue, nausea, and alopecia. In addition to rash, photosensitivity is common and has the potential to result in severe burns. This can often be prevented with sunscreen or sun avoidance, and does not necessarily require a dose reduction. In addition, cutaneous squamous cell carcinomas (SCC) or keratoacanthomas may occur less frequently than

<table>
<thead>
<tr>
<th>Study</th>
<th>Phase</th>
<th>Trial Design</th>
<th>Patients in BRAFi arm</th>
<th>RR in BRAFi arm (%)</th>
<th>Median PFS (months)</th>
<th>Median OS (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flaherty et al. (14)</td>
<td>I/II</td>
<td>Single arm, vemurafenib dose escalation with extension cohort</td>
<td>32</td>
<td>81b</td>
<td>&gt;7</td>
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</tr>
<tr>
<td>Falchook et al. (37)</td>
<td>I/II</td>
<td>Single arm, dabrafenib dose escalation with extension cohort</td>
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<td>50</td>
<td>5.5 (95% CI, 4.1–8.3)</td>
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</tr>
<tr>
<td>Sosman et al. (15)</td>
<td>II</td>
<td>Single arm, vemurafenib 960 mg b.i.d.</td>
<td>132</td>
<td>53</td>
<td>6.8 (95% CI, 5.6–8.1)</td>
<td>15.9 (95% CI, 11.6–18.3)</td>
</tr>
<tr>
<td>Treffer et al. (36)</td>
<td>II</td>
<td>Single arm, dabrafenib 150 mg b.i.d.</td>
<td>76V600E</td>
<td>59</td>
<td>27 weeks</td>
<td>Not reported</td>
</tr>
<tr>
<td>Chapman et al. (25)</td>
<td>III</td>
<td>Randomized, vemurafenib 960 mg b.i.d. vs. DTIC 1000 mg/m² every 3 weeks</td>
<td>337</td>
<td>57</td>
<td>6.9 vs. 1.6d</td>
<td>13.6 vs. 9.7d</td>
</tr>
<tr>
<td>Hauschild et al. (26)</td>
<td>III</td>
<td>Randomized, dabrafenib 150 mg b.i.d. vs. DTIC 1000 mg/m² every 3 weeks</td>
<td>187</td>
<td>50</td>
<td>5.1 vs. 2.7 (HR 0.30; 95% CI, 0.18–0.51, P &lt; 0.0001)</td>
<td>Not reported</td>
</tr>
</tbody>
</table>

Abbreviation: b.i.d.: twice a day; DTIC, dacarbazine; PFS, progression free survival.

a Patients in extension cohort receiving recommended phase II dose.

b Unconfirmed response rate.

c Evaluable patients with BRAFV600E mutated melanoma, treated at recommended phase II dose or higher.

d The median PFS and OS are reported for the BRAFi arm compared to the control arm.
of these lesions harbor mutations in BRAF in regards to cutaneous SCCs, recent data suggests that many of additional malignant or premalignant lesions. With selective BRAF inhibitors has been the development of von Rechnitz et al. (26, 36–38). In patients treated with vemurafenib (26, 38). Grade 2 or 3 pyrexia seems to be a more common event with dabrafenib, although it was only seen in a minority of patients (26, 36–38).

Among the most concerning treatment-related events with selective BRAF inhibitors has been the development of additional malignant or premalignant lesions. With regards to cutaneous SCCs, recent data suggests that many of these lesions harbor mutations in HRAS, resulting in activation of the MAPK pathway and accelerated growth (39). This link seems to underlie the appearance the emergence of a KRAS mutant leukemia in a melanoma patient with a history of myelodysplastic syndrome (40). Updated analyses have also reported the development of atypical melanocytic lesions or new primary melanomas in some patients treated with selective BRAF inhibitors (25, 41, 42). Although a limited number of samples are available, the majority of these lesions seem to be BRAF wild type, although an NRAS mutation and a rare BRAF mutation variant have been identified (42, 43). It is therefore advisable for these patients to be followed by a dermatologist regularly while on therapy, and evaluated promptly if a concerning lesion is noted. These lesions can be adequately managed with standard local therapy, as there have been no reports of metastasis to date. In addition, with longer-term follow up, the development of colonic adenomas has been reported in patients treated with BRAF inhibitors (44). As the use of these agents is investigated in the adjuvant setting, defining guidelines for screening will become critical.

Current questions

In many ways, the successful targeting of BRAF in melanoma is translational medicine at its best: a potential oncogenic target was identified and successfully brought to clinical fruition. But resistance to BRAF inhibitor monotherapy emerges rapidly in some patients and, on average, 6 to 8 months after initiating therapy among a population of patients treated. In an effort to better define characteristics of patients who derive longer-term benefit and to devise rational combination therapy strategies, a deeper understanding of the clinicopathologic features and behavior of BRAF-mutated melanoma is needed.

Clinicopathologic features. BRAF mutations seem to be more common on body sites that are intermittently exposed to the sun compared to areas with evidence of chronic sun exposure or no sun exposure, as is the case with mucosal and acral lentiginous melanomas (45). This partially explains the observation that BRAF mutant melanomas have fewer genetic aberrations than those arising on chronically sun-exposed skin. In addition, evidence suggests that the BRAF mutation frequency and type may vary by age (9). In an analysis of 312 patients with unresectable stage IIIC or stage IV melanoma, BRAF mutations seemed to be more frequent in younger patient populations, with an incidence of around 80% in patients less than age 40. Interestingly, although the overall incidence of BRAF mutations remained at approximately 50% for older patients, the proportion V600K mutations seems to be higher with increasing age (9). This has important therapeutic implications, as patients with BRAFV600K mutations were only included unintentionally in early trials with vemurafenib if their tumor was scored positive in the assay designed for BRAFV600E. Data from this small subset of BRAFV600K patients treated with vemurafenib and purposefully enrolled on phase I and phase II studies with dabrafenib suggests that patients with BRAFV600K mutations may have lower response rates to therapy (15, 16, 36, 37). Understanding the relative efficacy of BRAF inhibitors in this subpopulation with BRAFV600K mutations will likely require an understanding of differences in tumor and host characteristics in this older patient population. Importantly, preclinical data from other tumor types suggests that an even smaller subpopulation of BRAF mutations (particularly those that affect exon 11) are likely completely unresponsive to selective BRAF inhibitors and will require a different therapeutic strategy (46).

BRAF as a prognostic marker. Although clearly a predictive marker for response to BRAF-targeted therapy, the role...
of BRAF as a prognostic marker continues to be an important consideration as treatment strategies shift toward the adjuvant setting. A body of retrospective evidence points to BRAF as a potential marker of poor prognosis. An analysis of 223 patients diagnosed with a primary melanoma at MD Anderson suggests that BRAF-mutated primary melanomas may be more frequently associated with prognostic factors that traditionally have carried a poorer prognosis, such as ulceration, when compared to wild-type tumors (47). In this analysis, patients with either BRAF or NRAS mutations, which also result in activation of the MAP kinase pathway and are found in 15% to 20% of melanomas, tended to present with a higher stage of disease when compared to patients who were wild type for both genes, although OS was similar among the genotypes when stratified by stage. In another retrospective analysis of 105 patients with stage III melanoma, median OS seemed to be worse in patients with a BRAF mutation (48). In the setting of metastatic disease, data suggests that the specific type of BRAF mutation may have prognostic implications. In an analysis of 308 patients who were prospectively followed, patients with BRAFV600E melanoma had a shorter disease-free interval (DFI) as defined from their original diagnosis to the development of first distant metastasis as compared to patients with a V600E genotype (17.4 months vs. 39.2 months; \( P = 0.048 \)), although there was no difference in DFI between the overall population of BRAF mutant versus BRAF wild type patients (9). This cohort uniquely included 3 subsets of patients: those who were BRAF wild type, as well as BRAF mutant patients who did and did not receive BRAF inhibitor therapy. In the cohort of patients with newly diagnosed melanoma, patients with BRAF mutations who received BRAF-targeted therapy had a significantly improved median OS (>18 months) when compared to those who did not receive these agents (6.5 months; \( P < 0.001 \)). Median OS for BRAF wild-type patients was not significantly different from the cohort of patients with BRAF mutant melanoma who did not receive a BRAF inhibitor. True assessments of the independent prognostic value of BRAF mutations will likely be limited in the era of effective targeted BRAF inhibitors, however, it is possible that different BRAF mutation subtypes may represent distinct subsets of metastatic disease.

**Mechanisms of resistance and combination strategies.** Although the majority of patients with a BRAF mutation will benefit from BRAF inhibitor therapy, approximately 10% of patients with a BRAFV600E mutation have tumor progression early in the course of therapy (14). Furthermore, despite seeing an initial and sometimes dramatic clinical response, the vast majority of patients have residual tumor following maximal response and most ultimately relapse in less than a year, raising important questions about the etiology of both primary and acquired resistance. It is likely a heterogeneous process across the BRAF mutant melanoma population, and a recent report suggests that distinct mechanisms may contribute to resistance at different metastatic sites even within the same patient (Fig. 2; ref. 49). This is an area of research where the full picture is still unfolding, and gaining further insight will remain critical to the development of rational strategies to effectively overcome resistance. Initially, MAPK reactivation
was proposed as a primary mechanism of resistance, but it is now becoming clear that alternative pathways also play a critical role.

In contrast to oncogene-targeted therapies that have been used with success in other malignancies, resistance to BRAF inhibition does not seem to be due to the accumulation of mutations in BRAF itself that inhibit drug binding. Although preclinical models have shown that such gatekeeper mutations are, in theory, capable of inducing resistance, de novo mutations have yet to be identified in patient tumor samples, among the relatively few samples that have been tested and reported to date (50, 51). In patient samples analyzed thus far, it seems that tumors retain the original BRAF mutation at the time of progression, although there is data to suggest that a splice variant of BRAF<sup>V600E</sup>, which lacks the RAS binding domain, results in enhanced RAF dimerization and acquired resistance to selective BRAF inhibitors (52–55).

Interestingly, mutations that are both upstream and downstream of BRAF may play a role in resistance to BRAF inhibition. A number of preclinical studies have shown that persistent activation of the MAPK pathway through oncoprotein NRAS may account for one mechanism of acquired resistance. It is thought that mutated NRAS primarily bypasses BRAF inhibition through continued activation of CRAF, resulting sustained hyperactivation of MEK and ERK (56–58). In a study using melanoma cell lines known to harbor the BRAF<sup>V600E</sup> mutation, resistant clones were generated by chronic PLX4032 exposure (51). In one resistant cell line, a de novo activating mutation in NRAS (Q61K) was identified. This same NRAS mutation was subsequently seen in 2 separate biopsies taken at disease progression from a patient treated with PLX4032. Stable NRAS knockdowns in both the resistant cell line as well as the patient sample restored PLX4032 sensitivity, further supporting this as a mechanism to bypass BRAF inhibition. Additional confirmation has also come from an analysis of a phase II trial of vemurafenib which included 132 patients. Thirteen biopsy samples were available from patients at the time of progression, with 3 NRAS mutations identified (54).

In BRAF-mutated melanoma, oncogenic signaling seems to be driven in a largely MEK-dependent manner, and alterations in MEK signaling are thought to be another potential mechanism for decreased effectiveness of BRAF-targeted therapy. In an analysis of tumor samples from patients treated with the MEK inhibitor AZD6244, a MEK mutation was identified at amino acid position 124, resulting in the substitution of a leucine for a proline in a patient who progressed on treatment (59). In ex vivo studies, cell lines derived from this tumor seemed to be resistant to MEK inhibition, with evidence of sustained p-ERK signaling even at high concentrations of MEK inhibitor. Interestingly, these cells also showed cross-resistance to the selective BRAF inhibitor PLX4720. The identification of COT, another MAP kinase, as another potential mediator of resistance to BRAF inhibitors further highlights the complexity of the MAP kinase pathway and the possibility of within-pathway bypassing of BRAF (60). Preclinical models showed sensitivity to MEK inhibition in BRAF<sup>V600E</sup> mutated cell lines, and trials showed a modest level of clinical activity for patients treated with MEK inhibitors as a single agent (61–63). The recent report that the MEK inhibitor trametinib (GSK1120212) improves survival compared to standard chemotherapy in BRAF-mutated melanoma serves as further confirmation for the potential for synergy with these agents (64). These early findings, among others, led to the development of BRAF/MEK combination therapies as a potential mechanism to overcome resistance. In a phase I/II study of the BRAF inhibitor dabrafenib combined with trametinib, the combination seemed tolerable and showed promising clinical activity (38). A randomized phase II trial enrolled 162 patients to a combination of dabrafenib and trametinib at their full doses, full-dose dabrafenib with reduced dose trametinib, or dabrafenib alone. The response rate was improved from 54% to 76% and progression-free survival was significantly improved, with 41% progression-free at 1 year on the full-dose dabrafenib/trametinib combination versus 9% on dabrafenib monotherapy (38). Interestingly, the appearance of cutaneous SCCs and other hyperproliferative skin lesions seen with BRAF inhibitor monotherapy seemed to be reduced with combination therapy, as these lesions are thought to be due to reactivation of the MAPK pathway and may be abrogated by MEK inhibition. Until further insights are gained about resistance mechanisms in certain subpopulations of the BRAF mutant melanoma population, BRAF/MEK combination therapy seems likely to become the new standard treatment for these patients.

Alternative mechanisms outside of the MAPK pathway may also prove to be important mediators of resistance to BRAF inhibition, including the PI3 kinase (PI3K) pathway with or without loss of PTEN, activation of IGF-1R, PDGFR-β, as well as hepatocyte growth factor (51, 65–68). The observation that BRAF inhibition has the potential to affect the host immune response also supports a strategy exploring the combination with immunotherapy, although which type of agent to use remains uncertain (69). A number of clinical trials testing these hypotheses are planned or are currently underway, as summarized in Table 3 (70).

Conclusions

The development of selectively targeted BRAF inhibitors represents a major breakthrough in the treatment of melanoma, as these agents have now shown an improvement in OS when compared to standard chemotherapy. These agents represent a reasonable first line option for patients with BRAF<sup>V600E</sup>-mutated melanoma, particularly those who would benefit from a rapid response to treatment. Although marked clinical improvement can be seen, responses do not seem to be durable for most patients. Significant advances, however, have already been made which provide insight into possible mechanisms of resistance. Combination strategies show early promise, with the potential to impact therapy for melanoma as well as other malignancies in which BRAF mutations can be found.
Table 3. Selected ongoing BRAF inhibitor trials (70)

<table>
<thead>
<tr>
<th>Disease setting</th>
<th>Trial design</th>
<th>Treatment plan</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single agent studies</td>
<td>Adjuvant</td>
<td>Single arm, phase II</td>
<td>Dabrafenib 150 mg b.i.d. for 4 months</td>
</tr>
<tr>
<td>Metastatic</td>
<td>Randomized, phase III</td>
<td>Vemurafenib 960 mg b.i.d. vs. placebo for 12 months</td>
<td>Enrolling</td>
</tr>
<tr>
<td>Combination with other targeted agents</td>
<td>Adjuvant</td>
<td>Randomized, phase III</td>
<td>Dabrafenib 150 mg b.i.d. + trametinib 2 mg daily vs. placebo for 12 months</td>
</tr>
<tr>
<td>Metastatic</td>
<td>Randomized, phase II</td>
<td>Vemurafenib 960 mg b.i.d. vs. vemurafenib 960 mg b.i.d. + bevacizumab 15 mg/kg every 3 weeks</td>
<td>Planned</td>
</tr>
<tr>
<td>Metastatic</td>
<td>Randomized, phase III</td>
<td>Dabrafenib 150 mg b.i.d. + trametinib 2 mg daily vs. dabrafenib 150 mg b.i.d. + trametinib placebo</td>
<td>Closed to accrual</td>
</tr>
<tr>
<td>Metastatic</td>
<td>Randomized, phase III</td>
<td>Vemurafenib 960 mg b.i.d. + GDC-073 60 mg daily (MEK inhibitor) vs. vemurafenib 960 mg b.i.d. + GDC-073 placebo</td>
<td>Enrolling</td>
</tr>
<tr>
<td>Metastatic</td>
<td>Phase I, dose escalation</td>
<td>Vemurafenib 720 mg b.i.d. + dose escalation of mTOR inhibitor (everolimus or temsirolimus)</td>
<td>Enrolling</td>
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<tr>
<td>Metastatic</td>
<td>Phase I, dose escalation</td>
<td>Vemurafenib + Hsp90 inhibitor (XL888)</td>
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<tr>
<td>Metastatic</td>
<td>Phase I, dose escalation</td>
<td>Vemurafenib + PI3K inhibitor (BKM120)</td>
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<tr>
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<td>Vemurafenib + PI3K inhibitor (PX-886)</td>
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<td>Phase I, dose escalation</td>
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<td>Vemurafenib + CDK inhibitor (P1446A)</td>
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<tr>
<td>Combination with immunotherapy</td>
<td>Metastatic</td>
<td>Phase II, single arm</td>
<td>Vemurafenib 960 mg b.i.d. × 6 wks, followed by ipilimumab 10 mg/kg i.v. every 3 wks × 4 doses, then every 12 wks</td>
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<tr>
<td>Metastatic</td>
<td>Phase I, multiple cohorts</td>
<td>Dabrafenib ± trametinib in combination with ipilimumab</td>
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<td>Uncontrolled, 2 cohorts</td>
<td>Cohort 1: vemurafenib × 6 wks + HDI-L2a</td>
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<tr>
<td>Metastatic</td>
<td>Phase I</td>
<td>Vemurafenib + anti-PDL1 antibody (MPDL3280A)</td>
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Abbreviation: b.i.d., twice a day.

References

BRAF in Melanoma


