A Retrospective Evaluation of Vemurafenib as Treatment for BRAF-Mutant Melanoma Brain Metastases

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Melanoma • Brain metastasis • BRAF • Vemurafenib

ABSTRACT

Background. RAF inhibitors are an effective therapy for patients with BRAF-mutant melanoma and brain metastasis. Efficacy data are derived from clinical studies enriched with physiologically fit patients; therefore, it is of interest to assess the real-world experience of vemurafenib in this population. Tumor-specific genetic variants that influence sensitivity to RAF kinase inhibitors also require investigation.

Methods. Records of patients with BRAF-mutant melanoma and brain metastases who were treated with vemurafenib were reviewed. Clinical data were extracted to determine extracranial and intracranial objective response rates, progression-free survival (PFS), overall survival (OS), and safety. A bait-capture, next-generation sequencing assay was used to identify mutations in pretreatment tumors that could explain primary resistance to vemurafenib.

Results. Among patients with intracranial disease treated with vemurafenib, 27 were included in survival analyses and 22 patients were assessable for response. The extracranial and intracranial objective response rates were 71% and 50%, respectively. Discordant responses were observed between extracranial and intracranial metastatic sites in 4 of 19 evaluable patients. Median PFS was 4.1 months (95% confidence interval [CI]: 2.6–7.9); median intracranial PFS was 4.6 months (95% CI: 2.7–7.9), median OS was 7.5 months (95% CI: 4.3–not reached), with a 30.4% 1-year OS rate. Outcomes were influenced by performance status. Vemurafenib was tolerable, although radiation-induced dermatitis occurred in some patients who received whole-brain radiotherapy. Adequate samples for next-generation sequencing analysis were available for seven patients. Melanomas categorized as “poorly sensitive” (>20% tumor growth, new lesions, or ≤50% shrinkage for >4 months) harbored co-occurring mutations in genes predicted to activate the phosphatidylinositol 3-kinase-AKT (PI3K-AKT) pathway.

Conclusion. Vemurafenib is highly active in BRAF-mutant melanoma brain metastases but has limited activity in patients with poor performance status. The safety and efficacy of concurrent radiotherapy and RAF inhibition requires careful clinical evaluation. Combination strategies blocking the MAPK and PI3K-AKT pathway may be warranted in a subset of patients.

Implications for Practice: Vemurafenib is active for BRAF-mutant intracranial melanoma metastases in an unselected patient population typical of routine oncologic practice. Patients with poor performance status appear to have poor outcomes despite vemurafenib therapy. Preliminary data indicate that co-occurring or secondary alterations in the phosphatidylinositol 3-kinase-AKT (PI3K-AKT) pathway are involved in resistance to RAF inhibition, thus providing a rationale for dual MAPK and PI3K-AKT pathway inhibition in this patient population.

INTRODUCTION

Central nervous system (CNS) involvement in patients with metastatic melanoma remains a major therapeutic challenge associated with a significant impact on quality of life and frequently results in melanoma-related death. The prognosis for patients with melanoma that has metastasized to the brain is poor, with estimated median overall survival (OS) of 4–6 months from the time of diagnosis of intracranial disease [1–4]. Surgical resection and stereotactic radiosurgery (SRS)
local control of small-volume CNS disease [5]. For patients not amenable to localized approaches, chemotherapy [6, 7], immunotherapy [8], and whole-brain radiotherapy (WBRT) [9] result in objective responses in only 7%–16% of patients.

The RAF kinase inhibitors vemurafenib and dabrafenib have marked activity against BRAF-mutant melanomas, with response rates of ~50% [10–13]. As opposed to dacarbazine, both vemurafenib and dabrafenib have been shown to extend progression-free survival (PFS) in patients with unresectable or metastatic BRAF-mutant melanoma, and vemurafenib extends their OS [12, 13]. A phase II study with dabrafenib showed an intracranial response rate of 30.8% in patients who had progressed after prior therapy for brain metastases and 39.2% in patients who had no prior therapy for brain metastases [14]. Prospective data indicate that vemurafenib is also active against melanoma brain metastasis, with a similar response rate [15, 16]. The inclusion criteria for the three prospective studies of RAF inhibitors were restrictive, limiting the size of intracranial lesions in some instances and excluding those patients with poor performance and or leptomeningeal disease. Consequently, there are few meaningful published data to guide clinicians in routine clinical practice on the use of vemurafenib in patients with advanced CNS disease.

We conducted a retrospective analysis of 27 patients with BRAF-mutant melanoma and brain metastasis treated with vemurafenib at a single center. In this article, we report the clinical activity, outcomes, and toxicity of this approach in a patient population that might be more representative of the general population [1–4]. For the purposes of hypothesis generation, we also describe initial results of a next-generation sequencing assay performed on pretreatment metastatic tumors that aimed at defining somatic mutations that predict sensitivity to vemurafenib.

**Methods**

**Patient Selection and Clinical Characteristics**

The database of Memorial Sloan Kettering Cancer Center (MSKCC) was queried for all patients with unresectable or metastatic melanoma with a BRAFV600 mutation who were treated with vemurafenib sometime between 2007 and 2013. Tumors were genotyped by standard amplification and sequencing of exon 15 of the BRAF gene [10] or by using a mass spectrometry-based assay (Sequenom Inc., San Diego, CA, https://www.sequenom.com) [17]. We extracted demographic information, characteristics of the primary melanoma, Eastern Cooperative Oncology Group performance status (ECOG PS), lactate dehydrogenase, and extent of intracranial disease prior to initiation of vemurafenib, history of prior or concurrent intracranial therapy, and history of ipilimumab treatment. This retrospective analysis was approved by the MSKCC institutional review board.

**Efficacy**

All patients received vemurafenib at an initial dose of 960 mg orally twice a day. Antitumor efficacy was retrospectively assessed by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [18]. The best overall response was determined separately for intracranial and extracranial disease sites and calculated as the maximum change in the sum of the largest diameter of up to five target lesions. Imaging modalities used were magnetic resonance imaging (MRI) for CNS lesions and computed tomography of the chest, abdomen, and pelvis for extracranial disease. Each patient was then designated as having complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) for both intracranial and extracranial disease at each radiographic assessment. Patients who died or were lost to follow-up prior to first assessment or who had incomplete imaging throughout the treatment period were graded as not evaluable. The intracranial and extracranial objective response rates were defined as the proportion of CR plus PR in evaluable extracranial or intracranial target lesions. Discordant responses were defined as >20% tumor growth or new tumors at intracranial sites in the setting of tumor shrinkage of >20% at extracranial sites or vice versa.

Specific criteria were used for selection of intracranial target lesions. For those patients who did not receive prior intracranial therapy, target lesions were selected by the investigators on the basis of measurability per RECIST version 1.1. Lesions treated previously with resection or SRS were omitted as targets. In the case of the former, residual disease after resection was considered an acceptable target. For patients who underwent WBRT, lesions were selected based on measurability and only after progression was documented on serial imaging by the treating physician, unless otherwise noted.

**Toxicity Assessment**

Toxicity was determined by investigator-assessed chart review and graded using the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0.

**Tumor Genotyping and Exon-Capture Sequencing**

In patients who had sufficient pretreatment formalin-fixed, paraffin-embedded (FFPE) tissue from either an intracranial or extracranial metastatic site and accompanying matched normal DNA obtained from peripheral blood, we profiled genomic alterations in 300 cancer-associated genes using the IMPACT assay (Integrated Mutation Profiling of Actionable Cancer Targets). This assay uses solution phase hybridization-based exon capture and massively parallel DNA sequencing to capture all protein-coding exons and selected introns of 300 genes of interest (oncogenes, tumor suppressor genes, and members of pathways deemed actionable by targeted therapies). The analysis was performed as reported previously [17, 19]. A mean unique sequence coverage of 669 was achieved. For this report, we focused our analysis on a subset of 35 genes selected on the basis of the frequency of mutations reported in melanoma samples or on their ability to activate the MAPK and/or phosphatidylinositol 3-kinase (PI3K) signaling pathway. For the purposes of correlating somatic genetic changes, tumor sensitivity to vemurafenib was scored as highly sensitive (≥50% tumor shrinkage for ≥7 months or any shrinkage for ≥12 months), poorly sensitive (≥20% tumor growth, new lesions, or ≤50% shrinkage for <4 months), or intermediate (not highly or poorly sensitive). This scoring system was based on the median duration of response to vemurafenib and average tumor shrinkage according to the published data [10–13].

**Biostatistics**

Continuous variables were summarized with descriptive statistics, such as median and range. Categorical variables...
were tabulated as counts and percentages. Response rates were calculated along with a 95% confidence interval (CI). The chi-square test was used for intergroup comparisons. PFS was defined as the time from the start of vemurafenib until the date of last documented contact (censored), date of progression (overall progression or intracranial progression, whichever occurred first), or date of death. OS was defined as the time from the start of vemurafenib until the date of last documented contact (censored) or date of death. PFS and OS were estimated using Kaplan-Meier methodology with the log-rank test for comparison between subgroups. A p value of < .05 was considered significant. Genomic data are reported in a descriptive fashion. All analyses were completed using GraphPad Prism version 6 (GraphPad Software, Inc., La Jolla, CA, http://www.graphpad.com).

**Results**

**Patient Characteristics**

Of 140 patients with unresectable or metastatic BRAF-mutant melanoma in the database, 27 (19.3%) had brain metastasis prior to the start of treatment with vemurafenib and were assessable for survival and toxicity. Clinical characteristics of the patients are reported in Table 1. The majority of the melanomas harbored a BRAF\(^{V600E}\) mutation; 15% harbored a BRAF\(^{V600K}\) mutation.

Notably, patients had an extensive burden of intracranial disease. Eleven patients (41%) patients had ≥5 lesions, and 6 (22%) of these patients had >10 lesions. Three patients (11%) had leptomeningeal disease documented by cross-sectional imaging and/or cerebrospinal fluid cytology in addition to measurable targets by RECIST. The median diameter of the largest brain metastases was 19.5 mm (range: 4–56 mm). In general, patients had functional limitations due to disease, with 15 of 27 patients (56%) having an ECOG performance status of 1 and 5 patients (18.5%) having a performance status of 2 or worse.

The median time from the diagnosis brain metastases to the start of vemurafenib was 6 weeks (range: 0.3–24.4 weeks) for all patients. Seventeen patients (55%) had received prior WBRT, SRS, debulking craniotomy, or a combination of these treatment modalities. No patients had been previously treated with different BRAF or MEK inhibitors. One patient received WBRT concurrently with vemurafenib therapy. Fourteen patients (52%) received ipilimumab at some time during their treatment with stereotactic radiosurgery or WBRT.

**Antitumor Activity**

Table 1. Patient characteristics (n = 27)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, n (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>15 (56)</td>
</tr>
<tr>
<td>Female</td>
<td>12 (44)</td>
</tr>
<tr>
<td>Age, median (range)</td>
<td>53 (25–86)</td>
</tr>
<tr>
<td>Primary site, n (%)</td>
<td></td>
</tr>
<tr>
<td>Head/extremity</td>
<td>10 (37)</td>
</tr>
<tr>
<td>Trunk</td>
<td>11 (41)</td>
</tr>
<tr>
<td>Unknown</td>
<td>6 (22)</td>
</tr>
<tr>
<td>BRAF mutational status, n (%)</td>
<td></td>
</tr>
<tr>
<td>V600E</td>
<td>23 (85)</td>
</tr>
<tr>
<td>V600K</td>
<td>4 (15)</td>
</tr>
<tr>
<td>ECOG PS, n (%)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>7 (26)</td>
</tr>
<tr>
<td>1</td>
<td>15 (55.5)</td>
</tr>
<tr>
<td>≥2</td>
<td>5 (18.5)</td>
</tr>
<tr>
<td>LDH, U/L median (range)(^a)</td>
<td>382.5 (205–742)</td>
</tr>
<tr>
<td>Intracranial disease burden, leptomeningeal disease, n (%)</td>
<td>3 (11)</td>
</tr>
<tr>
<td>Number of lesions, n (%)</td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>16 (59)</td>
</tr>
<tr>
<td>5–10</td>
<td>5 (19)</td>
</tr>
<tr>
<td>&gt;10</td>
<td>6 (22)</td>
</tr>
<tr>
<td>Maximum tumor diameter, mm (range)</td>
<td>19.5 (4–67)</td>
</tr>
<tr>
<td>Prior intracranial treatment, n (%)</td>
<td></td>
</tr>
<tr>
<td>WBRT(^b)</td>
<td>9 (33.5)</td>
</tr>
<tr>
<td>Stereotactic radiosurgery only</td>
<td>1 (3.5)</td>
</tr>
<tr>
<td>Craniotomy only</td>
<td>3 (11)</td>
</tr>
<tr>
<td>Multimodality therapy(^c)</td>
<td>2 (7)</td>
</tr>
<tr>
<td>None</td>
<td>12 (44)</td>
</tr>
<tr>
<td>Treatment with ipilimumab, n (%)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>13 (48)</td>
</tr>
<tr>
<td>Prior</td>
<td>9 (33)</td>
</tr>
<tr>
<td>Subsequent</td>
<td>5 (19)</td>
</tr>
</tbody>
</table>

\(^a\)Only available in 10 of 27 patients.
\(^b\)One patient received vemurafenib and concurrent WBRT.
\(^c\)Two patients received multimodality therapy consisting of craniotomy with stereotactic radiosurgery or WBRT.

Of 27 patients identified, 24 patients were radiographically evaluable for extracranial response, intracranial response, or both. Supplemental online Figure 1 shows the frequency of interval imaging over the disease course for each patient until progression of disease, death, or last follow-up. Three patients who were treated at MSKCC and other hospitals had incomplete imaging throughout the course of therapy, including the one patient who received concurrent WBRT and vemurafenib therapy. Consequently, adequate assessment of best change from baseline target lesion was not possible for these patients.

Of 24 patients with adequate cross-sectional imaging, 21 were evaluated for determination of the extracranial objective response rate. Three patients were excluded because measurable extracranial disease was not present. There was 1 patient with CR, 14 with PR, 5 with SD, and 1 with PD (Fig. 1A).

Consistent with prior reports, the extracranial objective response rate for evaluable patients was 71% (15 of 21 patients; 95% CI: 48%–88%); the median time to response was 7.1 weeks [12].

Twenty-two patients were radiographically evaluable for objective intracranial response. Two patients were not evaluable because follow-up brain imaging was not available. Both patients had initial evidence of extracranial response to vemurafenib. In one case, the patient died 2 months after the start of therapy, before intracranial assessment. In the second case, gastrointestinal hemorrhage occurred due to visceral progression of disease, leading to the discontinuation
of vemurafenib prior to brain reimaging. The median intervals to first extracranial and intracranial response assessments were 7.5 weeks and 5.8 weeks, respectively. Twelve patients had restaging scans done simultaneously or less than 7 days apart.

Assessment of intracranial disease revealed three patients with CR, eight with PR, seven with SD, and four with PD (Fig. 1B). The intracranial objective response rate (intracranial CR plus PR) for evaluable patients was 50% (11 of 22 patients; 95% CI: 29%–71%). In four patients with evaluable intracranial disease who received prior WBRT, progression of disease could not be confirmed prior to the start of vemurafenib; however, exclusion of these patients did not alter the intracranial response rate (50%). We did not observe a significant difference in intracranial objective response rates in patients who had received prior cranial irradiation compared with those who had not.

Interestingly, 4 of 19 patients who had both extracranial and intracranial disease had discordant responses (Fig. 2). In all 4 cases, there was progression in the brain (new brain metastases or growth of target lesions of at least 20%) at the first interval assessment despite shrinkage of visceral disease.

**Patient Outcomes**

The median follow-up for surviving patients was 10.8 months (range: 2.0–18.9 months). The estimated median PFS for the entire cohort was 4.1 months (Fig. 3A). The estimated median OS for all patients was 7.5 months, and the estimated 1-year OS was 30.4% (95% CI: 13.2%–50.1%) (Fig. 3B). In univariate analysis, only ECOG performance status affected OS (median OS: not reached for ECOG PS 0, 8.8 months for ECOG PS 1, 2.0 months for ECOG PS ≥2; p = .0006). The estimated median intracranial PFS was 4.6 months (95% CI: 2.7–7.9 months) and was calculated based on survival data of 26 patients; 1 patient treated with concurrent WBRT and vemurafenib was excluded from intracranial PFS analysis.

**Tolerability**

The majority of treated patients required at least one dose modification (19 of 27 patients, 70.4%), although no patient permanently discontinued treatment because of adverse events. The spectrum and severity of adverse events to vemurafenib were similar to available prospective data in patients without brain metastases (Table 2) [12]. We noted that 3 of 10 patients who had WBRT developed either grade 2 radiation dermatitis or radiation recall (Table 2; supplemental online Fig. 2). In a patient receiving vemurafenib concurrent with WBRT, a painful cutaneous reaction began 1 week into combined therapy and reached maximum severity 3 weeks after the initiation of treatment (supplemental online Fig. 2A). The reaction, which was limited to the radiation field, was characterized by an erythematous hyperkeratotic plaque. Tortuous skin folding and hypertrophy was also recognized and consistent with acquired cutis verticis gyrata. After 3 months of topical emollients and topical high-potency corticosteroids, the dermatitis resolved completely (described in detail in [20]). A second patient started vemurafenib 4 days after completing WBRT. Within 1 week, the patient complained of pain, erythema, edema, and desquamation of the scalp and ears (supplemental online Fig. 2B). This grade 2 radiation dermatitis resolved with a vemurafenib dose modification and topical corticosteroids after 4 weeks. One additional patient developed radiation recall after 3 weeks of vemurafenib therapy, which was 6 weeks after the completion of WBRT. The reaction was characterized by erythema; hyperkeratosis, and acquired cutis verticis gyrata (supplemental online Fig. 2C) [20]. These symptoms persisted but did not worsen until she died 11 weeks after the start of vemurafenib.

**Genomic Profiling and Sensitivity to Vemurafenib**

Sufficient DNA from pretreatment metastatic melanoma sites and matched normal DNA were available for next-generation sequencing analysis in seven patients. Besides the BRAF V600E mutation, which was observed in all 7 patients, no other recurrent mutations were identified in the evaluated 300 cancer-associated genes. Mutation status of 35 genes of interest in recurrent mutations were identified in the evaluated 300 cancer-associated genes. Mutation status of 35 genes of interest in two pretreatment tumors from 7 patients is shown in Figure 4.

Three patients (patients 1–3) had poorly sensitive tumors and progressed after 2–3 months of therapy. We noted that alterations in the PI3K-AKT pathway due to activating mutation in PIK3CA or PTEN inactivation were present in these tumors. The PIK3CA E545K mutation observed in patient 1 (Fig. 4B) is a hot spot mutation observed in several cancers [21]. It occurs within the helical domain (exon 9) and induces transformation of chicken embryo fibroblasts in vitro. The transforming potential of this point mutation is associated with increased lipid kinase activity and increased phosphorylated AKT [22]. In
addition, a missense mutation in \textit{ERBB4} and a nonsense mutation in \textit{RB1} (R455*) that abrogates RB function [23] were observed in patient 1 (Fig. 4A). Tumors with loss of RB1 appear to be less dependent on RAF signaling for cellular proliferation, indicating a role for alterations in this pathway in RAF inhibitor resistance [24]. Highly activating \textit{ERBB4} mutations are observed in melanoma, but no evidence shows that such mutations induce resistance to RAF inhibition [25].

A \textit{PTEN} mutation is due to a frameshift insertion of four base pairs (Fig. 4C) in patient 2. Using the ExPASy tool (Swiss Institute of Bioinformatics, Lausanne, Switzerland, http://www.expasy.org), we mimicked the mutation and discovered that it leads to the generation of a new stop codon soon after insertion, generating a truncated protein. The functionality of the truncated protein requires further investigation, but the alteration is predicted to lead to an inactive protein. In patient 3, two separate alterations in \textit{PTEN} were observed and have been reported previously: a nonsense mutation (\textit{PTEN}^G249X) and a missense mutation (\textit{PTEN}^G160R). The former leads to a truncated protein, whereas the latter disrupts the IT loop catalytic site, resulting in a nonfunctional protein [26]. Additional alterations of unclear significance in critical genes in the PI3K-AKT pathway in this patient included \textit{PIK3CA}^{V344G}, \textit{PREX2}^{R663S}, and \textit{PREX2}^{R1263K} mutations.

Figure 2. Discordant intracranial and visceral response. (A): A 2-by-2 table of 19 patients with extracranial and intracranial imaging demonstrating 15 concordant responses and 4 discordant responses. (B, C): Representative imaging of a discordant intracranial and extracranial response. A pretreatment magnetic resonance imaging brain scan and computed tomography (CT) scan of the abdomen demonstrate a left high frontoparietal operative cavity with enhancing tissue and mild edema, consistent with residual disease, and a large right adrenal metastasis with invasion to the inferior vena cava (B). Approximately 6 weeks later, a CT scan of the head shows disease progression with extensive edema and midline shift, whereas the retroperitoneal disease shrank significantly (C).

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Figure 3. Patient outcomes. Progression-free survival (A) and overall survival (B) calculated by the Kaplan-Meier method. Ticks represent censored patients (n = 27).

Three highly sensitive tumors were also identified in the cohort. Patients 5 and 6 had PR for 7 and 8 months, respectively. Patient 7 attained an intracranial complete response and an extracranial partial response to vemurafenib. The patient has been free of disease for more than 19 months following a metastasectomy at the only site of residual extracranial disease, which did not reveal viable tumor on pathologic examination. Activating mutations in the PI3K-AKT pathway were not identified in these highly sensitive tumors. Mutations of unclear significance include a splice variant of \textit{RB1}, a missense mutation in \textit{NOTCH4} (G1005R), and a missense mutation in \textit{PREX2} (W375L).

**DISCUSSION**

We have reported our single-institution experience using vemurafenib in patients with BRAF-mutant melanoma metastatic to the CNS. We observed an intracranial objective response rate of 50% among 22 evaluable patients, indicating that the activity of vemurafenib for brain metastases is meaningful and relevant in an unselected patient population typical of routine oncology practice. Our results are in line with those published by Dzienis and Atkinson in a similar series of 22 patients treated with vemurafenib [27]. Kefford and colleagues recently reported early results from a phase II trial of vemurafenib in BRAF-mutated melanoma patients with brain metastases; the intracranial response rate was 26%, as assessed by the investigators [15]. A prior phase II study of dabrafenib in patients with BRAF-mutated melanoma and brain metastasis reported an overall intracranial objective response rate of 31.4%; there was little difference between patients who progressed after prior CNS therapy and patients who were treatment naïve [14]. It appears from these trials that prior
treatment with radiotherapy or surgery does not significantly affect the proportion of patients who respond intracranially to RAF inhibitors. In addition, the activity of BRAF inhibitors appears to be superior to ipilimumab and temozolomide. Ipilimumab resulted in intracranial response rates of 16% and 11.7% in a phase II trial and an expanded access program, respectively [8, 28]. The results of available literature addressing different systemic treatments for patients with metastatic melanoma and CNS involvement are summarized in Table 3.

It is unclear why we observed a response rate at least twice as high as prior studies, but this could reflect differences in patient populations and bias due to small sample size. In addition, a central independent review was not performed owing to the study design, and discrepancies regarding the assessment of intracranial disease burden were reported previously [14]. In the aforementioned trial by Long et al. [14], investigator and review committee assessments were discordant in 42% of the cases, and discordances were also reported in the prospective trial by Kefford et al. [15]. These findings highlight the difficulties involved in evaluation of intracranial disease in metastatic melanoma.

An appealing feature of our report is the assessment of the corresponding extracranial response rate. We observed discordant responses to vemurafenib, with intracranial growth and extracranial disease shrinkage in 4 of 19 patients. Similar findings were reported by Azer et al. in 23 patients treated with dabrafenib in phase I or II trials [29] in which intracranial and extracranial responses were concordant in 71% of the cases. It is interesting to speculate on factors that might account for this apparent observation. Pharmacokinetic variables may affect the ability of vemurafenib and dabrafenib to enter the brain in a subset of patients. In a murine model, the blood-brain barrier restricts the efflux of these compounds through the action of p-glycoprotein and breast cancer resistance protein [30, 31], and by blocking these transporters, higher intracranial concentrations of vemurafenib are obtained in vivo [32]. Moreover, it has also been suggested that size and vascular composition of brain lesions may influence the ability of drugs to penetrate the brain [33]. Intertumoral heterogeneity of mutations in BRAF and other genes also may account for varying sensitivity to RAF inhibitors among different metastatic sites. Colombino et al. showed a 20% discordance between NRAS or BRAF mutational status between the primary tumor and brain metastases [34]. However, if loss of the BRAF mutation was a common mechanism of resistance, BRAFWT tumors would be expected to be found frequently at relapse during RAF inhibitor treatment, but this does not seem to be the case. Finally, technical factors relating to the assessment of intracranial disease, such as variable image quality, cystic or necrotic lesions, or the presence of edema and hemorrhage, may also influence discordance in clinical response rates [35].

Although survival data with RAF inhibitors are favorable compared with historical controls [1–4], it is disappointing that overall survival was not longer, given the high intracranial response rate in this population. A critical observation is that patients with BRAF-mutant melanoma brain metastasis and poor ECOG performance status did poorly despite vemurafenib therapy, with median overall survival of 2 months. This suggests that patients with neurologic compromise and symptomatic CNS disease are unlikely to be rescued with RAF inhibitor therapy.

Approximately 50% of patients did not achieve at least a partial intracranial response, and 4 of 22 of patients had primary progression of intracranial disease. We found that poorly sensitive melanomas had mutations that were predicted or known to activate the PI3K-AKT pathway. Such alterations were not seen in highly sensitive melanomas. Six unique mutations in genes of the PIK3-AKT pathway were identified in the patients with poorly sensitive tumors. Two tumors contained mutations expected or known to inactivate PTEN. Mutations in the tumor suppressor PTEN are frequent in melanoma, occurring in 44% of human samples [36], and deletions in PTEN are necessary for malignant transformation in a murine model [37]. Preclinical data suggest that PTEN loss renders BRAF-mutant melanoma cell lines less sensitive to the RAF inhibitors; however, additional genetic changes (i.e., RB1 loss) are probably required for innate resistance [24]. In a small cohort of melanoma patients treated with dabrafenib, alternations in PTEN trended (p = .059) toward shortened PFS compared with melanomas without such mutations [38]. Two patients with poorly sensitive tumors also harbored PIK3CA mutations (the melanoma from patient 3 harbored mutations in both PIK3CA and PTEN). PIK3CA mutations co-occur with BRAF in ∼5% of melanomas [36]. PIK3CAE545K has been identified previously, is known to constitutively activate AKT signaling, and is predicted to confer resistance to RAF inhibition [22]. PIK3CAV546E has been reported in other solid tumors, although its functional relevance has not been formally explored in vitro (COSMIC database). Finally, mutations in PREX2, a gene encoding a PTEN regulator, were observed in patients with both highly and poorly sensitive tumors. This gene is mutated in 14% of melanomas, and some mutations can have oncogenic potential [39]. None of the PREX2 mutations reported in this study have been described previously, and it is unclear if these mutations are implicated in RAF inhibitor resistance.

Gene expression profiling indicates that melanoma brain metastases are biologically distinct from the corresponding primary tumor [40]. Others have observed, using immunohistochemistry staining, that activation of the PI3K-AKT pathway is more common in melanoma brain metastases than visceral lesions [41, 42]. Furthermore, brain metastases that progress on vemurafenib appear to exhibit AKT activation and loss of...
PTEN, whereas paired responding extracranial lesions show no such activation of the PI3K-AKT pathway [42]. Inhibition of AKT with or without concomitant RAF inhibition leads to growth inhibition in vitro of melanoma cell lines that are derived from RAF inhibitor-resistant intracranial disease. Taken together, these observations suggest a role for targeting both the PI3K-AKT and MAPK pathways in this population.

Three of 10 patients in this series who received prior or concurrent WBRT developed significant cutaneous adverse events within the irradiated field. Preclinical data indicate that RAF inhibitors may be radiosensitizers [43, 44], and several clinical anecdotes suggest that vemurafenib is a radiosensitizer capable of enhancing radiation-induced toxicities such as radiation necrosis and radiation recall [20, 45, 46]. Consequently, a hurdle to combination strategies with radiotherapy may be enhanced toxicity, possibly the result of paradoxical activation of ERK signaling in normal cells in response to concurrent RAF inhibition and radiotherapy [47–49]. Although no patients were treated with concurrent stereotactic radiosurgery and vemurafenib, this may be a promising strategy to deliver combined treatment that could potentially result in fewer additive side effects.

This retrospective series has several limitations. The timing of radiographic assessments was not prospectively predefined, thus inherent variability in measurement of disease among investigators can introduce a bias with regard to outcomes, particularly for assessment of progression-free survival. Nevertheless, as observed in supplemental online Figure 1, all patients underwent regular screening, and small differences in the time to interval reimaging do not affect the estimation of response and overall survival. Another limitation is that patients were not required to progress following prior whole-brain therapy to receive vemurafenib. That being said, given the extremely low response rate to WBRT, it is highly unlikely that any of these patients would have responded; however, we excluded these few patients from the response analysis. In addition, because of the retrospective nature of this trial, symptoms were not assessed in a structured way, the doses of steroids were not uniform, and this information was missing for a number of patients.

Figure 4. Schematic illustration of representative genes altered in the five melanoma patients analyzed by the IMPACT assay. (A): Heat map showing the mutational state of 35 selected genes in the 7 melanoma patients analyzed. All patients had a BRAFV600E mutation. (B): Representation of PIK3CAE545K mutation in DNA from FFPE tumor (left upper panel) from patient 1. Multiple reads from tumor-derived DNA show a G to A transition in 41% of reads. This indicates a glutamic acid to lysine mutation in PIK3CA. No such mutation is detected in germline DNA (left lower panel) (C). A 4-bp CGTT insertion in exon 7 of PTEN was found in 110 reads of an FFPE tumor (right upper panel) of patient 2. This frameshift insertion was not observed in the normal DNA of the same patient (right lower panel). The low frequency of the PTEN mutation observed (3.9%) is due to high stroma contamination of the FFPE tumoral sample.

Abbreviations: bp, base pair; FFPE, formalin-fixed, paraffin-embedded; Ins, insertion; Pt, patient.
patients. Consequently, analyses regarding some additional relevant variables involved in the clinical care of patients with CNS metastases were not possible. Finally, genomic data could not be obtained for all patients, and in this regard, results should be considered preliminary and hypothesis generating.

CONCLUSION

Vemurafenib is an active and effective therapy for melanoma brain metastases characterized by a BRAF(V600E) or BRAF(V600K) mutation. Vemurafenib is associated with a higher response rate than whole-brain radiotherapy. Further investigation is required to determine whether it should be considered as a first-line therapy option for patients with BRAF-mutated brain metastases not amenable to stereotactic radiosurgical techniques and how it should be combined with radiotherapy. Clinicians should be aware that there is a signal of enhanced toxicity from radiotherapy given concomitantly with vemurafenib. We believe concomitant radiotherapy and vemurafenib should be avoided, if possible. Given the frequency of PI3K-AKT pathway activation in melanoma brain metastases, especially in those resistant to single-agent RAF inhibitors, it seems rational to consider dual RAF and PI3K-AKT pathway inhibition in this patient cohort.

ACKNOWLEDGMENTS

This work was funded by the Conquer Cancer Foundation of the American Society of Clinical Oncology (J.J.H.), the John Figgie Fund (P.B.C.), and the Melanoma Research Alliance (M.F.B., D.B.S., P.B.C.).

REFERENCES


Table 3. Summary of available studies including patients with metastatic melanoma and central nervous system involvement treated with systemic agents

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Design</th>
<th>Drug</th>
<th>Sample</th>
<th>ORR, intracranial (%)</th>
<th>mOS (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agarwala et al. [7]</td>
<td>2004</td>
<td>Phase II</td>
<td>Temozolomide</td>
<td>151</td>
<td>6</td>
<td>13.9</td>
</tr>
<tr>
<td>Long et al. (BREAK-MB) [14]</td>
<td>2012</td>
<td>Phase II</td>
<td>Dabrafenib</td>
<td>Total: 172</td>
<td>Cohort A, V600E: 74</td>
<td>39.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cohort A, V600K: 15</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cohort B, V600E: 65</td>
<td>30.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cohort B, V600K: 18</td>
<td>22.2</td>
</tr>
<tr>
<td>Margolin et al. [8]</td>
<td>2012</td>
<td>Phase II</td>
<td>Ipilimumab</td>
<td>Total: 72</td>
<td>Cohort A: S1</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cohort B: 21</td>
<td>5</td>
</tr>
<tr>
<td>Queirolo et al. [28]</td>
<td>2013</td>
<td>EAP</td>
<td>Ipilimumab</td>
<td>146</td>
<td>11.7</td>
<td>18.0</td>
</tr>
<tr>
<td>Kefferd et al. [15]</td>
<td>2013</td>
<td>Phase II</td>
<td>Vemurafenib</td>
<td>Total: 146</td>
<td>Cohort 1: 90</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cohort 2: 56</td>
<td>20</td>
</tr>
<tr>
<td>Dummer et al. [16]</td>
<td>2014</td>
<td>Pilot</td>
<td>Vemurafenib</td>
<td>24</td>
<td>16</td>
<td>23.0</td>
</tr>
<tr>
<td>Dzienis and Atkinson [27]</td>
<td>2014</td>
<td>Retrospective</td>
<td>Vemurafenib</td>
<td>Total: 22</td>
<td>Cohort A: 16</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cohort B: 6</td>
<td>50</td>
</tr>
</tbody>
</table>

Abbreviations: EAP, Expanded Access Program; mOS, median overall survival; NR, not reported; ORR, objective response rate.

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DISCLOSURES

Mario E. Lacouture: Bayer, Bristol-Myers Squibb, Genentech, EMD Serono, Merck, Novocure, Helsinn, Novartis, Boehringer Ingelheim, Reata (C/A), Berg Pharma (RF), Threshold Pharmacy, Nerre Therapeutics, Advancell (H); Richard D. Carvajal: Novartis (C/A), Novartis, Morphoteq, Bristol-Myers Squibb (RF); Neal Rosen: AstraZeneca, Novartis (C/A); Paul B. Chapman: Bristol-Myers Squibb, GlaxoSmithKline, Roche/Genentech (C/A, RF). The other authors indicated no financial relationships.

(C/A) Consulting/advisory relationship; (RF) Research funding; (E) Employment; (ET) Expert testimony; (H) Honoraria received; (OI) Ownership interests; (IP) Intellectual property rights/inventor/patent holder; (SAB) Scientific advisory board

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