KIT as a Therapeutic Target in Metastatic Melanoma

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Context Some melanomas arising from acral, mucosal, and chronically sun-damaged sites harbor activating mutations and amplification of the type III transmembrane receptor tyrosine kinase KIT. We explored the effects of KIT inhibition using imatinib mesylate in this molecular subset of disease.

Objective To assess clinical effects of imatinib mesylate in patients with melanoma harboring KIT alterations.

Design, Setting, and Patients A single-group, open-label, phase 2 trial at 1 community and 5 academic oncology centers in the United States of 295 patients with melanoma screened for the presence of KIT mutations and amplification between April 23, 2007, and April 16, 2010. A total of 51 cases with such alterations were identified and 28 of these patients were treated who had advanced unresectable melanoma arising from acral, mucosal, and chronically sun-damaged sites.

Intervention Imatinib mesylate, 400 mg orally twice daily.

Main Outcome Measures Radiographic response, with secondary end points including time to progression, overall survival, and correlation of molecular alterations and clinical response.

Results Two complete responses lasting 94 (ongoing) and 95 weeks, 2 durable partial responses lasting 53 and 89 (ongoing) weeks, and 2 transient partial responses lasting 12 and 18 weeks among the 25 evaluable patients were observed. The overall durable response rate was 16% (95% confidence interval [CI], 2%-30%), with a median time to progression of 12 weeks (interquartile range [IQR], 6-18 weeks; 95% CI, 11-18 weeks), and a median overall survival of 46.3 weeks (IQR, 28 weeks-not achieved; 95% CI, 28 weeks-not achieved). Response rate was better in cases with mutations affecting recurrent hotspots or with a mutant to wild-type allelic ratio of more than 1 (40% vs 0%, P=.05), indicating positive selection for the mutated allele.

Conclusions Among patients with advanced melanoma harboring KIT alterations, treatment with imatinib mesylate results in significant clinical responses in a subset of patients. Responses may be limited to tumors harboring KIT alterations of proven functional relevance.

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See also Patient Page.
and CSD sites infrequently have BRAF mutations, but commonly have amplifications or activating mutations of KIT.8,9 KIT is a type III transmembrane receptor tyrosine kinase.10 Binding of its ligand, stem cell factor, results in receptor dimerization, autophosphorylation, and activation of several signaling pathways; thereby, mediating cancer cell growth, proliferation, invasion, metastasis, and inhibition of apoptosis.

The importance of KIT in normal melanocyte development is well established11-13; however, its role as an oncogene and therapeutic target in melanoma has only recently become clear. Although KIT is expressed in some melanomas, loss of expression is observed with progression of disease from superficial to invasive to metastatic stages, suggesting that KIT possesses tumor suppressive functions.14-18 Furthermore, 3 phase 2 studies of metastatic melanoma treated with imatinib mesylate, an orally available ATP-competitive inhibitor of several tyrosine kinases including KIT, did not demonstrate clinical activity.19-21 These trials accrued before the discovery of activating mutations of KIT in melanoma and did not select patients based on the presence of KIT mutations or amplification.

KIT is an established therapeutic target in cancers with activating mutations of KIT, such as gastrointestinal stromal tumors (GIST), and significant benefit is achieved with various small molecule inhibitors of KIT including imatinib mesylate.22 Several melanoma cell lines with KIT mutations are highly sensitive to imatinib mesylate.23-25 Furthermore, several patients with melanoma harboring KIT alterations, including a K642E mutation as well as a 7-codon duplication of exon 11, have been reported to achieve major durable responses to imatinib mesylate.26,27

Given the preclinical and anecdotal clinical activity of imatinib mesylate observed in KIT mutant melanoma, we conducted this study to test the hypothesis that inhibition of KIT in a molecularly selected subgroup of patients with melanomas harboring mutations or amplification of KIT will result in objective regression and disease control. We further explored whether the identification of functionally relevant KIT alterations would allow us to better select patients most likely to respond to KIT inhibition.

**METHODS**

**Patients**

Between April 23, 2007, and April 16, 2010, 328 patients were enrolled from 1 community and 5 academic oncology centers in the United States for molecular screening and determination of eligibility. Eligible patients included those patients aged 18 years or older with metastatic melanoma arising from acral, mucosal, and body sites with signs of CSD harboring mutations or amplification of KIT. Patients required an Eastern Cooperative Oncology Group performance status of 0 (able to be fully active and perform all predisease activities without restriction), 1 (unable to perform physically strenuous activity but ambulatory and able to perform work of a light or sedentary nature, such as light housework or office work), or 2 (ambulatory and capable of all self-care, but unable to perform any work activities),26 life expectancy of 3 months or longer, measurable disease according to the Response Evaluation Criteria in Solid Tumors (RECIST),20 and adequate bone marrow, hepatic, and renal function. Prior systemic therapy and treated stable brain metastases were permitted.

All patients were informed of the investigational nature of the study and provided written informed consent in accordance with institutional and federal guidelines. The protocol was approved by each center’s institutional review board. Race/ethnicity was self-reported by the participants.

**Molecular Screening**

Tumor specimens were tested for KIT mutations and amplification. DNA for mutation analysis was extracted from formalin-fixed, paraffin-embedded specimens as previously published.20 Polymerase chain reaction assays using primers specific for KIT exons 9, 11, 13, 17, and 18; NRAS exons 1 and 2; BRAF exon 15; and GNAQ exon 5 were used, followed by Sanger sequencing. Polymerase chain reaction products were purified using ExoSAP-IT (USB Corporation, Cleveland, Ohio) and directly sequenced in the forward and reverse directions using the Applied Biosystems 3730 capillary DNA analyzer (Applied Biosystems, Foster City, California).

Fluorescence in situ hybridization was performed on formalin-fixed, paraffin-embedded sections as previously described.23 Human BAC clones RP11-722F1 and CTD-3180G20 spanning KIT in 4q12 (Invitrogen, Carlsbad, California), reference clones RP11-365H22 (Wellcome Trust Sanger Institute, Hinxton, Cambridge, England) and RP11-799E21 (BACPAC Resources, Oakland, California) for proximal 4q, and RP11-19F13 (Wellcome Trust Sanger Institute) and RP11-461G20 (BACPAC Resources) proximal 4p were used.23 BAC DNA was labeled by nick translation with red dUTP (KIT), orange dUTP (4q reference), or green dUTP (4p reference) from Enzo Life Sciences Inc, Plymouth Meeting, Pennsylvania. Analysis used a Zeiss Axioplan epifluorescence microscope with motorized stage and Isis 5 imaging software (MetaSystems Group, Waltham, Massachusetts). Amplification was defined as a KIT-to-centromere ratio of more than 2.5, with a ratio of more than 10 representing high-level amplification. Polysomy was defined as the presence of discrete red, orange, and green signal groups.

**Study Procedures**

Patients whose tumors harbored a KIT alteration were eligible to receive imatinib mesylate, 400 mg orally twice daily in 6-week cycles until unacceptable toxicity or disease progression. Imatinib mesylate was supplied by the Division of Cancer Treatment and Diagnosis at the National Cancer Institute. Imatinib mesylate was reduced to 400 mg/d in the setting of toxicity, with one further reduction to 300 mg/d permitted. Study participation was discontinued if adverse effects persisted.
Safety evaluations were conducted within 14 days of treatment initiation, every 2 weeks for 18 weeks, and every 6 weeks thereafter. Evaluation included physical examination, complete blood cell count, and clinical chemistry panel, including lactate dehydrogenase. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 (available at http://www.ctep.info.nih.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf).

Radiographic imaging and tumor measurements were performed within 4 weeks of treatment initiation, every 6 weeks for 18 weeks, and every 12 weeks subsequently. Response was judged according to RECIST version 1.0. Secondary end points included time to progression, overall survival, and correlation of molecular alterations and clinical response.

A Simon 2-stage mini-max design was used to assess the primary end point of response, with the following parameters: 10% not promising response rate, 30% promising response rate, and .10 type I and II error rates. If 1 or more durable responses were observed in the first 16 evaluable patients, accrual continued until 25 evaluable patients were identified. If 5 or more durable responses were observed, imatinib mesylate would be considered worthy of further testing in this patient population.

Survival time was defined as the time from initiation of imatinib mesylate to the date of death or last follow-up. Time to progression was defined as the time from initiation of imatinib mesylate to the date of progression or last follow-up. Survival distributions were estimated using the Kaplan-Meier method. Comparisons were made by log-rank test. Base-line patient characteristics and adverse events were reported using summary statistics and frequency tables. Adverse events were reported as the most severe manifestation of each event category during any cycle of treatment. Fisher exact test was used to compare molecular alteration and response. Statistical analysis was conducted using SAS version 9.2 (SAS Institute Inc, Cary, North Carolina). All significance tests were 2-sided and used a 5% level of significance.

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Table 1. Frequency of Alterations Identified in KIT, BRAF, NRAS, and GNAQ by Melanoma Subtype

<table>
<thead>
<tr>
<th>Melanoma</th>
<th>KIT Mutation</th>
<th>KIT Amplification</th>
<th>Both</th>
<th>Either</th>
</tr>
</thead>
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<tr>
<td>Acral (n = 65)</td>
<td>18/84 (21.4)</td>
<td>8/83 (9.6)</td>
<td>6/82 (7.3)</td>
<td>20/84 (23.8)</td>
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<tr>
<td>Mucosal (n = 93)</td>
<td>17/93 (18.3)</td>
<td>1/91 (1.1)</td>
<td>3/91 (3.3)</td>
<td>21/93 (22.6)</td>
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<tr>
<td>CSD, centrally reviewed (n = 32)</td>
<td>5/32 (15.6)</td>
<td>3/30 (10.0)</td>
<td>2/30 (6.7)</td>
<td>6/32 (18.8)</td>
</tr>
</tbody>
</table>

Abbreviation: CSD, chronically sun-damaged.

Among the 87 patients with melanoma arising from sun-damaged skin confirmed histopathologically by central review, only 6 cases harbored a KIT mutation or amplification. Overall, 51 cases harboring a mutation or amplification of KIT were identified; however, the subset of cases described in this Table includes only 49 such cases.

RESULTS

Molecular Screening

Three hundred twenty-eight patients consented to molecular screening. The flow of patients and tumor testing is shown in eFigure 1 (available at http://www.jama.com). Mutation status for KIT, BRAF, NRAS, and GNAQ, and KIT amplification status was determined in 295 cases, including 85 acral melanomas, 93 mucosal melanomas, and 87 melanomas clinically determined to arise from CSD sites.
KIT AS A THERAPEUTIC TARGET IN METASTATIC MELANOMA

Fifteen melanomas arising from skin without clinical signs of CSD, 1 primary meningeal melanoma, and 14 melanomas of unknown primary site were inadvertently included for screening. KIT mutations or amplification were found in 23.8% (20 of 84 cases) of acral melanomas, 24.7% (23 of 93 cases) of mucosal melanomas, and 9.2% (8 of 87 cases) of melanomas clinically determined to arise from CSD sites. Histopathological confirmation of grade 2 or higher solar elastosis defining CSD was performed in 39 of 87 melanomas (44.8%) with clinical signs of CSD.

Clinical and histopathological assessments of CSD were concordant in 32 cases (82%). The proportion of histopathologically confirmed CSD melanomas harboring KIT mutations or amplification was 18.8% (6 of 32 cases), twice that observed in melanomas clinically determined to arise from CSD sites (Table 1). No KIT alterations were identified in the meningeal melanoma, non-CSD melanomas, or melanomas of unknown primary tested.

Fifty-one cases harbored KIT alterations, including 26 cases (51%) harboring KIT mutations only, 9 (18%) harboring KIT amplification only, and 16 (31%) concurrently harboring both KIT mutations and amplification. The distribution and frequency of mutations identified is shown in eFigure 2. Forty-eight of 51 cases with KIT alterations had no concomitant mutations in BRAF, NRAS, or GNAQ. In total, 162 of 295 samples (54.9%) tested had mutations in BRAF, NRAS, or GNAQ, or a mutation or amplification of KIT (eTable 1).

**Figure 1. Treatment Response Over Time by Melanoma Subtype and Genetic Alteration of KIT.**

<table>
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<tr>
<th>Subtype</th>
<th>Mutation</th>
<th>Amplification (Ratio)</th>
<th>Amplified Pattern</th>
<th>Mutant: Wild-Type Ratio</th>
<th>Progression of disease</th>
<th>Stable disease</th>
<th>Transient partial response</th>
<th>Durable partial response</th>
<th>Durable complete response</th>
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</table>

CSD indicates melanoma arising from chronically sun-damaged skin; NA, not applicable. The melanoma subtype, KIT mutational and amplification status, the ratio of mutant to wild-type alleles as determined by their respective electropherogram peak heights, and the RECIST (Response Evaluation Criteria in Solid Tumors) response achieved for each patient treated with imatinib mesylate is shown. Amplification was defined as a KIT-to-centromere ratio of 2.5 or more, with the pattern of amplification described as uniform (homogeneous) or mixed (heterogeneous) across the tumor specimen. The best response as determined by RECIST is identified by the color of the bar. The length of the bar represents the duration of time in weeks that each patient remained receiving therapy.

<sup>a</sup>Mutations previously identified in melanoma<sup>33,34</sup> and gastrointestinal stromal tumors.<sup>15,34</sup>  <sup>b</sup>Tumors with mutant to wild-type allelic ratios of more than 1:1, which are those hypothesized most likely to respond to therapy with KIT inhibition.  
<sup>c</sup>Patients receiving treatment at the time of this report.

**Treatment**

Twenty-eight of 51 patients whose tumors harbored KIT mutations or amplification were treated during the study (13 [46%] with mucosal melanoma, 10 [36%] with acral melanoma, and 5 [18%] with melanoma arising from CSD sites—3 arising from the head and neck and 2 arising from the trunk). The remaining 23 patients were not treated during the study because of no evidence of active disease (n=9), deceased (n=5), receiving other therapy (n=5), ineligible due to brain metastasis (n=1), ineligible due to increased creatinine (n=1), ineligible due to active human immunodeficiency virus infection (n=1), and ineligible due to the inability to wean off methadone, a contraindicated medication per protocol due to CYP3A4 interactions with imatinib mesylate (n=1). None of the patients who were treated had mutations in BRAF, NRAS, or GNAQ. Baseline patient characteristics are shown in Table 2. Twenty-five patients were evaluable for the primary end point of response. Three were evaluable for toxicity only (2 patients stopped treatment within 2 weeks of starting therapy due to rapid disease progression and 1 stopped treatment due to the development of drug rash with eosinophilia and systemic symptoms syndrome within 10 days of initiating imatinib mesylate).

The most common adverse events observed are shown in eTable 2. Thirteen of 28 patients (46%) who were treated required a dose reduction to 400 mg/d, with 2 requiring a second dose reduction to 300 mg/d.

**Clinical Outcomes**

Two patients achieved durable complete responses, 2 achieved durable partial responses, 2 achieved transient partial responses, and 5 achieved stable disease lasting 12 or more weeks. No significant association between clinical melanoma subtype (mucosal: 23%; 95% confidence interval [CI], 8%-50%; acral: 38%; 95% CI, 14%-69%; and CSD: 0%; 95% CI, 0%-49%; P=.36) or lactate dehydrogenase (normal: 38%; 95% CI, 18%-61%; vs increased: 0%; 95% CI, 0%-30%; P=.06) and response was observed. RECIST response, time to progression,
Representative radiographic images of patients achieving responses are shown in Figure 2. Because 2 of the 6 responses were transient and not sustained on a follow-up scan performed 6 weeks after the initial response was documented, the predetermined end point of 5 responses of 25 evaluable patients who were treated was not met.

The median time to progression was 12 weeks (interquartile range [IQR], 6-18 weeks; 95% CI, 11-18 weeks). The 4 patients who achieved durable responses maintained disease control for more than 1 year. Durable disease control was observed in patients achieving stable disease, with 2 such patients maintaining stability for more than 6 months. Median survival from the time of initiation of therapy was 46.3 weeks (IQR, 28 weeks—not achieved; 95% CI, 28 weeks—not achieved), with 10 patients (40%) alive at the time of analysis (Figure 3).

Five patients underwent optional serial tumor biopsies to determine histopathological and immunohistochemical effects of therapy. One case demonstrating decreased cellularity and reduced tumor proliferation in a clinically responding metastasis compared with both pretreatment sample as well as a clinically progressing lesion is shown in eFigure 3.

**Association of Molecular Alterations and Clinical Response**

We observed that KIT mutations are widely distributed over the coding region as shown in eFigure 2. Many KIT mutations identified in our study have not previously been reported in melanoma or GIST and were present only in individual cases, suggesting that some may represent passenger mutations rather than bona fide driver alterations. Specific amino acid changes encountered repeatedly in cancer are likely to be of functional relevance. Interestingly, all 6 responses occurred in tumors with L576P or K642E mutations, the most common mutations in melanoma. The proportion of responders

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observed in the 13 cases whose tumors harbored mutations recurrently found in GIST \( ^{37,38} \) or melanoma \( ^{8,9} \) (V559C, L576P, V642E, and N822K) is 46%, higher than in the group as a whole.

Another method to separate driver from passenger mutations is to identify evidence of positive selection for the mutation. We investigated whether relative abundance of mutant KIT influenced response to imatinib mesylate in 2 ways. First, we assessed whether patients with tumors harboring KIT that was both mutated and amplified were more likely to achieve tumor response than those patients whose tumors harbored either alteration alone. Although a greater likelihood of response was observed in these cases, the difference was not statistically significant (36%; 95% CI, 15%-65%; vs 14%; 95% CI, 4%-40%; \( P = .35 \)).

We then evaluated whether the ratio of the mutant to wild-type KIT allele influenced response to imatinib mesylate. Mutations associated with a mutant to wild-type allele ratio of more than 1 are likely to be pathogenetically relevant. In a pure population of diploid tumor cells with a heterozygous mutation, this ratio is expected to be 1. As tumor samples typically contain normal cell contamination, the allelic ratio of heterozygous samples is often smaller than 1. Allelic ratios of more than 1 indicate the presence of an independent genetic event, such as amplification of the mutant allele, deletion of the wild-type allele, or loss of heterozygosity, which shifts the ratio in favor of the mutant allele. Such positive selection of the mutated allele suggests that the mutation is

**Figure 3.** Overall Survival and Time to Progression of All 25 Patients and Those With Recurrent or Sporadic Mutations

Kaplan-Meier curves for overall survival (15 deaths) and time to progression (22 progression events) of the 25 evaluable patients are shown. The median overall survival was 10.7 months (95% confidence interval [CI], 6.5 months—not achieved) and the median time to progression was 2.8 months (95% CI, 2.5-4.0 months). Mutations repeatedly identified in melanoma or gastrointestinal stromal tumors and those associated with a mutated to wild-type allele ratio of more than 1 are those most likely to be pathogenetically relevant. The subset of patients with tumors harboring such alterations achieved an overall survival and time to progression greater than that achieved in cases without such characteristics.
a functionally relevant event. Subgroup analysis reveals a higher response rate in cases with an allelic ratio of more than 1 (71% vs 6%; P = .002), with a longer median time to progression (18.0 weeks [IQR, 12.0-95.4 weeks; 95% CI, 11.6-95.4 weeks] vs 11.0 weeks [IQR, 5.0-16.0 weeks; 95% CI, 5.0-12.0 weeks]; P = .01) and extended median survival (median not achieved [IQR, 46.5 weeks-not achieved; 95% CI, 34.8 weeks-not achieved] vs 31.3 weeks [IQR, 18.1-80.5 weeks; 95% CI, 18.1-61.5 weeks]; P = .03).

**COMMENT**

These results demonstrate that a subset of melanomas with genetic alterations of KIT respond to treatment with imatinib mesylate. Durable responses were observed in 16% (95% CI, 2%-30%) of these patients, with all sustained for more than 1 year. Two additional responses were observed but not sustained on a follow-up scan performed 6 weeks after the initial response was documented. Therefore, this study did not meet its predetermined end point. Nevertheless, the responses achieved in this population preselected based on the presence of KIT mutations or amplification compare favorably to that observed in prior trials of molecularly unselected patients, in which response was limited to 1 of 62 evaluable participants treated.

This study additionally highlights the challenges in identifying appropriate patients for treatment with KIT inhibition. Seventy-four percent of BRAF mutant melanomas harbor a substitution of glutamic acid for valine at amino acid 600 (the V600E mutation). By contrast, mutations in KIT are more widely distributed over the coding region. Treatment of patients with melanoma harboring the oncogenic V600E BRAF mutation with an effective inhibitor of RAF, such as PLX4032 (Plexxikon, RG7204; Roche Pharmaceuticals, Basel, Switzerland), results in tumor response in 81% of cases. The more modest results in this study suggest that only select KIT alterations are truly oncogenic and indicative of an effective therapeutic target.

By using more selective molecular criteria, we may better identify patients who will respond to imatinib mesylate. Response to imatinib mesylate is predicted to be dependent on the region of the protein affected by a mutation, with some mutations affecting the binding affinity of imatinib mesylate to KIT as previously demonstrated in vitro and clinical studies of GIST.

Prior observations in GIST demonstrated the sensitivity of K642E and N822K mutations and the resistance of V654A and D820Y mutations to imatinib mesylate. Concordant with these findings, patients with melanoma harboring these resistant mutations progressed, although disease stability and responses were observed in those patients whose tumors harbored K642E and N822K mutations (Figure 1). Imatinib mesylate—resistance in GIST commonly results from the development of secondary KIT mutations, including V654A, D820Y, N822K, and A829P. These mutations were identified as primary mutations in several melanomas and may also predict a lower probability of response with imatinib mesylate. Indeed, the best response we observed in such cases was stable disease. Importantly, although an in vitro study demonstrated poor sensitivity of a melanoma cell line harboring an L576P mutation to imatinib mesylate, we observed dramatic in vivo responses in patients with melanomas harboring this mutation.

To separate bona fide driver from passenger alterations of KIT, we sought evidence for tumor selection of specific mutations. We observed that imatinib mesylate has greater activity in tumors harboring recurrent KIT mutations found in melanoma or GIST, as well as in tumors with a mutant KIT allele in greater abundance than the wild-type allele. When combining those patients whose tumors have an allelic ratio of more than 1 with those whose tumors harbor recurrent primary mutations found in GIST or melanoma, we observed a better response rate, time to progression, and overall survival compared with other cases (Figure 3). These criteria may serve as indicators of genetic events relevant to oncogenesis and should be investigated further as predictive markers of response to KIT inhibition.

In conclusion, our data show that imatinib mesylate therapy in patients with melanoma harboring specific KIT alterations results in clinical responses, consistent with the paradigm of oncogene addiction. Prediction of response to KIT inhibition can be improved beyond what we report herein by identifying tumors that harbor KIT alterations of functional relevance.

**Author Contributions:** Dr Carvajal had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Carvajal, Wolchok, Takebe, Bastian, Schwartz.

Acquisition of data: Carvajal, Antonescu, Wolchok, Chapman, Roman, Tchetter, Busam, Chmielowski, Lutzky, Pavlick, Fusco, Cage, Takebe, Vemula, Bastian, Schwartz.


Statistical analysis: Carvajal, Panageas.

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Study supervision: Carvajal, Wolchok, Takebe, Bastian, Schwartz.

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Online-Only Material: eFigures 1 through 3 and eTables 1 and 2 are available at http://www.jama.com.

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REFERENCES


