Overexpression of the KIT/SCF in Uveal Melanoma Does Not Translate into Clinical Efficacy of Imatinib Mesylate

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Abstract

Purpose: Recently, gene amplification and overexpression of KIT as well as activating mutations in the KIT gene have been described to occur in certain subsets of melanoma. These findings suggest KIT as a potential target for therapy with imatinib mesylate in these melanomas. To date, data on the KIT status in uveal melanoma (UM) is limited.

Experimental Design: We analyzed the expression of the KIT protein (CD117, c-kit) and its ligand, stem cell factor (SCF), in primary and metastatic UM.

Results: By immunohistochemistry, SCF-positive tumor cells (>90%) were detectable in 43% of primary UM and in 58% of UM metastases. Strong expression of KIT (>90%) in tumor cells was present in 55% of primary UM and in 76% of UM metastases. This overexpression of both KIT and SCF suggests the clinical application of imatinib mesylate in metastatic UM. This notion was tested in a clinical study using Simon's two-stage design. Patients received imatinib (600 mg p.o. daily) until progress or unacceptable toxicities. The trial did not enter stage II as no objective response was observed in the first group. This observation prompted further molecular analysis, which revealed no mutations in the genomic sequence of KIT in exons 11, 13, 17, and 18. Moreover, the mitogen-activated protein kinase pathway was not activated in any of the tumors as measured by ERK phosphorylation.

Conclusions: These results show the lack of clinical effectiveness of imatinib in UM, which was originally anticipated based on the high levels of KIT and SCF expression.

Uveal melanoma (UM) is the most frequent intraocular primary tumors in Caucasian adults, having an annual incidence rate of 0.7 per 100,000 people (1). Moreover, the eye is the most common site for noncutaneous melanomas, and despite its low incidence, accounts for 13% of all deaths from melanoma (2). UM and cutaneous melanoma differ significantly in their epidemiologic, clinical, immunophenotypical, and cytogenetic features but the molecular basis for this differences has not been delineated. Although there has been a worldwide increase in cutaneous melanomas in recent decades, the incidence of UM has remained unchanged, possibly reflecting a greater effect of UV light exposure on the development of cutaneous melanoma.

During development, the interaction of stem cell factors (SCF) with its receptor, KIT, is critical for the development and survival of neural crest–derived melanocytes (3). SCF is also expressed in the adult human epidermis, in which it plays an important role in the homeostasis of KIT-expressing melanocytes. The injection of KIT or KIT-ligand blocking antibodies into human skin explants grafted on nude mice resulted in the loss of melanocytes. In contrast, injection of soluble KIT-ligand led to hyperpigmentation of the explanted skin tissue (4).

Gain-of-function mutations in KIT leading to SCF-independent signaling have been described in several human cancers (5). Recently, gene amplification and overexpression of KIT as well as activating mutations in the KIT gene have been described to occur in mucosal and acral melanomas on chronically sun-damaged skin which are otherwise characterized by a less frequent appearance of mutations (6). Mutations and/or copy number increases of KIT were observed in ~30% of these subtypes, whereas no such alterations were detected for melanoma from skin that was not chronically sun-damaged.

Despite the fact that UM lacks activating BRaf or NRas mutations, it displays high activity of the mitogen-activated protein kinase (MAPK) pathway (7). Notably, UM was not included in the abovementioned study analyzing KIT activation in melanoma (6). This is particularly puzzling, as it has been shown that 75% of primary UM exhibited KIT positivity with a significant positive association with mitotic activity (8).

The purpose of the present study was to first evaluate the expression of the KIT protein and its ligand SCF in primary UM and UM metastases, and to screen genomic DNA derived from these samples for the presence of mutations. In a second step, we addressed the clinical efficacy of targeted therapy with the tyrosine kinase inhibitor imatinib mesylate (Glivec) in 12 patients with metastatic UM.

Materials and Methods

Patients. Eligibility criteria included patients aged 18 years and older with histologically confirmed metastatic UM and at least one measurable target lesion following Response Evaluation Criteria in

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Translational Relevance

Recently, overexpression of KIT and activating mutations in the KIT gene have been described in those subsets of melanoma lacking activation of B-Raf mutations, thereby suggesting KIT as a potential target for therapy with imatinib mesylate. Because uveal melanoma does not harbor activation of B-Raf mutations and because we could detect a significant overexpression of both KIT and its ligand stem cell factor in primary and metastatic disease, we tested the efficacy of imatinib in patients suffering from advanced disease using Simon's two-stage design. After 12 weeks, the trial was stopped due to lack of any clinical benefit. Further analysis revealed not only the lack of any aberrations in the KIT gene, but also the lack of any significant activation of the mitogen-activated protein kinase pathway. These findings are in line with the clinical observation and unequivocally argue against the value of KIT inhibition in uveal melanoma.

Solid Tumors guidelines (9), no brain metastases, Eastern Cooperative Oncology Group performance status ≤2, adequate bone marrow function (leukocytes, $\geq$3,000 μL^{-1}; platelets, $\geq$100,000 μL^{-1}), and satisfactory hepatic and renal function. Patients with severe and/or uncontrolled medical diseases other than melanoma were excluded from the study. Patients' characteristics are summarized in Table 1. The study protocol was approved by the institutional review board and a written informed consent was signed by all patients prior to enrollment.

Treatment plan and evaluation. A two-stage design as described by Simon was used to ensure that the number of patients exposed to an ineffective drug was minimized (10). Before study entry, patients underwent complete medical history and physical examination including laboratory tests, echocardiogram, and radiologic assessment to determine the extent of their disease. Patients received imatinib mesylate (Glivec, Novartis) at a dose of 300 mg p.o. b.i.d. (600 mg daily) until progression or intolerable side effects. Recommended concomitant medications were metoclopramide in case of nausea and loperamide in case of diarrhea, if required. Toxicity was evaluated using common toxicity criteria and assessed at weekly intervals within the first 4 weeks of therapy, followed by 2-week intervals thereafter. Patients were followed for at least 2-month intervals. Tumor response was assessed by computed tomography and/or magnetic resonance imaging in 8-week intervals and subsequently evaluated according to the Response Evaluation Criteria in Solid Tumors guidelines. Criteria for patient's removal from the study included progression of disease or unacceptable toxicity. The frequency of treatment-related toxicity is summarized in Table 2. The primary objective of this study was to determine the overall response rate. Secondary end points were overall survival (OS), time to progression, and toxicity.

Table 1. Characteristics of patients treated with imatinib mesylate

	No. of patients (%)
Patients	12 (100)
Male	7 (58)
Female	5 (42)
Median age, y (range)	63 (47-82)
Metastatic sites*	
Liver	12 (100)
Lung	7 (58)
Skin/lymph nodes	2 (17)
Other visceral	2 (17)
Elevated serum LDH	9 (75)
Prior therapy* (in stage IV)	
Chemotherapy	3 (25)
Immunotherapy	2 (17)
1 therapy regimen	1 (8)
2 therapy regimens	2 (17)

*Multiple entries possible: AJCC, American Joint Committee on Cancer; LDH, lactate dehydrogenase.

Table 2. Frequency of treatment-related toxicity

Adverse events	Grade 3*	Grade 4*
Fatigue	Four patients	
Myalgia	Two patients	
Abdominal pain	Three patients	
Vomiting	Seven patients	One patient
Anemia		One patient
Facial edema	Two patients	

*Toxicity was graded according to the Common Toxicity Criteria (version 3.0).

Tumor material. Paraffin-embedded blocks from 21 cases derived from 10 primary and 11 metastatic tumors surgically removed from 17 individual patients with UM were used for PCR amplification and sequencing. For immunohistochemistry of KIT, we studied 28 paraffin-embedded sections derived from 11 primaries and 17 metastases. Immunohistochemistry of SCF was done on 7 primaries and 26 metastases. In addition, tissue sections were analyzed immunohistochemically for the expression pERK and RKIP. Tumors were obtained from the Departments of Dermatology and Ophthalmology, University Hospital of Würzburg, Germany.

Immunohistochemistry. Four-micron-thick sections of paraffin-embedded tumors were dried at 56°C and then treated twice with xylol for 10 min at room temperature. Subsequently, sections were washed twice with absolute ethanol and twice with 70% ethanol followed by one rinse with distilled water. For antigen retrieval, sections were incubated with citrate buffer (DAKO) at pH 6.0 (phosphorylated ERK, SCF and CD 117/c-kit) or pH 9.0 (RKIP) for 10 min at 90°C and rinsed with distilled water. Next, slides were rinsed twice with PBS (DAKO, S3024) and thereafter incubated with blocking solution (DAKO, S2023) for 10 min at room temperature. After two additional washing steps with PBS for 10 min at room temperature, the monoclonal antibody anti–phospho-p44/42 MAPK (Thr^{202}/Tyr^{204}; clone E10; Cell Signaling) or the polyclonal antibodies anti-RKIP (Upstate) or the polyclonal anti-SCF (Cell Signaling), and anti-CD117/c-kit (DAKO) were added to the sections in PBS, followed by an overnight incubation at 4°C. After washing twice for 10 min in PBS, biotinylated multispecies-specific secondary antibody (DAKO, K5003) was added to the sections for 30 min at room temperature. Slides were then washed twice in PBS/bovine serum albumin, and bound antibodies were visualized using streptavidin-horseradish peroxidase (DAKO K5003) and Vector Vip (Vector Laboratories) as peroxidase substrate according to the manufacturer's guidelines. Finally, the nuclei were stained with hemalaun. For each section, the percentage of positive melanocytic cells was estimated by two independent observers. Each section was assigned to one of the following percentage categories: negative, <1%, <10% (+), <30% (++), <60% (+++), or <90% (++++).

PCR amplification and sequencing. Genomic DNA was isolated from paraffin-embedded tumor samples using a DNA isolation kit (Qiagen). Nested PCR was done with the first 25 cycles of the PCR reaction

Table 3. PCR primers used for the amplification of the exons 11, 13, 17, and 18 of the KIT gene

	Forward primer	Reverse primer
Exon 11		
Preamplification	GTTGGGAGGTGGGGTCAGTTT	AAGGGGCGCAATTTCACAGA
Amplification	TGTTCTCTCTCCAGAGTGCTCTAA	AAACAAAGGAAGCCACTGGA
Exon 13		
Preamplification	TTTCGGGAAGGTTGTTGAG	ATACCCCATAATGATAAAAT
Amplification	CATCAGTTTGCCAGTTGTGC	AGCAAGAGAGAACAACAGTCT
Exon 17		
Preamplification	GCAAAGGCATATTAGGAACTCT	TCCCTAGACAGGATTTACATTATG
Amplification	TCATTCAAGGCGTACTTTTG	TCGAAAGTTGAAACTAAAAATCC
Exon 18		
Preamplification	TAAAATGGGGATAATGCACTTGTG	GGGGCTGCTTCCTGAGACA
Amplification	CAAGGAAGCAGGACACCAAT	CATTTCAGCAACAGCAGCAT

(20 μL) followed by a second 35 cycle PCR reaction (50 μL) in which 1 μL of the first reaction served as a template. The cycles always consisted of 30 s denaturing at 95°C, 30 s annealing at 58°C followed by 30 s elongation at 72°C. The primers used for preamplification and amplification are given in Table 3. In some cases for which this protocol did not yield a PCR amplicon suitable for sequencing, we applied a three-step PCR in which the forward preamplification primer and the reverse amplification primer were used for the intermediate step.

Sequencing was done by the company SEQLAB and analysis of the sequences was done by visual inspection of the chromatographs and alignment using the DNASTAR software.

Results

Strong expression of KIT and its ligand SCF. Immunohistochemical staining revealed a positivity of the melanoma cells for KIT (CD117, c-kit) in 10 out of 11 cases of primary UM, and in all 17 cases of metastases. Because high expression of KIT per se does not lead to activated downstream signaling, we investigated possible autocrine or paracrine stimulation of the receptor by SCF. Immunohistochemistry of SCF in UM tumors showed a diffuse and strong staining of tumor cells in 5 out of 7 primary UM and in 20 out of 26 UM metastases. Representative stainings are provided in Fig. 1. Detailed staining characteristics of KIT and SCF are given in Tables 4 and 5.

Lack of clinical efficacy of KIT inhibition. The overexpression of both KIT and its ligand SCF in UM prompted us to test the clinical efficacy of KIT inhibition by imatinib mesylate in metastatic UM. A two-stage design as described by Simon was used to ensure that the number of patients exposed to an ineffective drug was minimized (10). Between October 2003 and July 2005, 12 evaluable patients with a median age of 63 years (range, 47-82) were recruited into the study. Patients' characteristics are listed in Table 2. There were seven men (58%) and five women (42%). Liver metastases was present in all patients (100%), seven patients (58%) had additional metastases in the lungs, and two patients (17%) had additional lymph node metastases. Two patients (17%) had metastases in multiple visceral organs. Before treatment with imatinib mesylate (Glivec), nine patients (75%) had elevated serum lactate dehydrogenase levels. Three patients (25%) had received prior therapy with fotemustine, two of them in combination with immunotherapy [zoledronic acid (Zometa) and interleukin-2 (Proleukin)] and two patients (17%) had been vaccinated with survivin-2B peptide.

Abdominal pain combined with vomiting was the most common toxicity observed. These toxicities resulted in a dose reduction in two cases (17%) from 600 to 400 mg/d. One (8%) patient had facial edema. There was no significant hematologic toxicity.

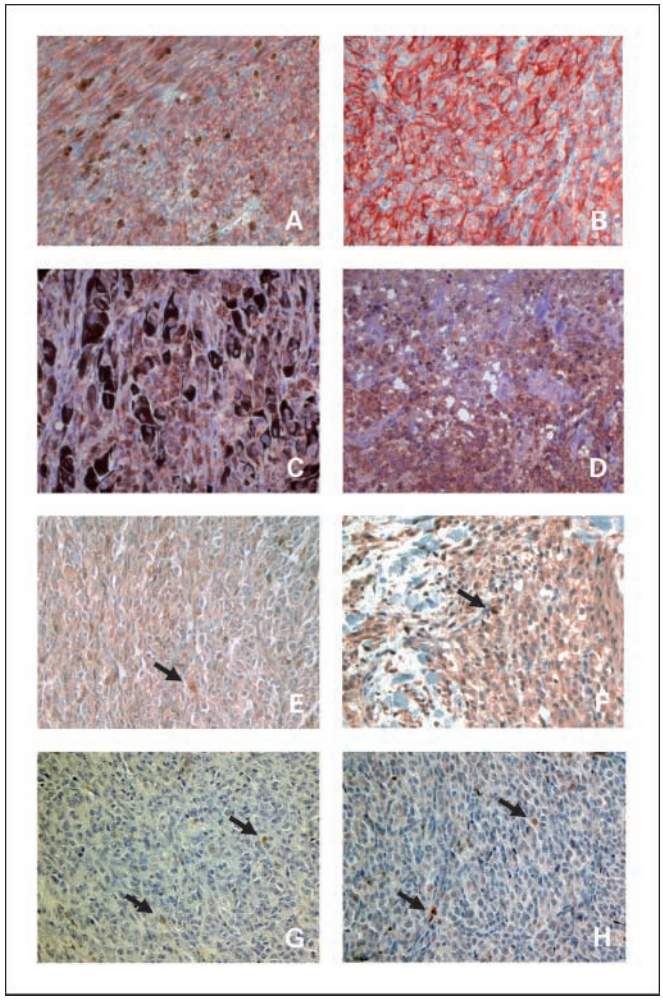

Fig. 1. Immunohistochemical staining of KIT (CD117/c-kit; *A* and *B*), SCF (*C* and *D*), RKIP (*E* and *F*), and pERK (*G* and *H*) in biopsy specimens of primary tumors (*A, C, E,* and *G*) and metastatic lesions (*B, D, F,* and *H*). Red, positive signals (magnification, ×40).

Table 4. Immunohistochemical analysis of KIT (n = 28)

	No. of cases (%)
Primary UM	11 (39)
UM metastases	17 (61)
CD117/c-kit in primary UM	
++++	6 (55)
+++	0 (0)
++	2 (18)
+	2 (18)
-	1 (9)
CD117/c-kit in UM metastases	
++++	13 (76)
+++	4 (24)
++	0 (0)
+	0 (0)
-	0 (0)

NOTE: Each section was assigned to one of the following percentage categories: negative, <1%, <10% (+), <30% (++), <60% (+++), or <90% (++++).

A total of nine patients (75%) received imatinib for ≥8 weeks. Three patients (25%) discontinued their therapy earlier because of obvious and significant disease progression. No patient achieved an objective response (complete or partial remission); the best clinical response was a stable disease in one patient (8%), which lasted for 52 weeks. Thus, the median progression-free survival was not calculated. The median OS of all patients was 6.8 months (Fig. 2A). For the eight patients (67%) who received imatinib as first line therapy, the median OS was 7.8 months (Fig. 2B). The four patients (33%) who were treated in a second line setting had a median OS of 4.9 months (data not shown).

Absence of activating KIT mutations. To understand the very poor clinical activity of imatinib mesylate in KIT-positive UM, we extended our molecular analysis of KIT signaling in clinical samples of this neoplastic disease. The exons 11, 13, 17, and 18 of the KIT gene harbor the vast majority of mutations described in human tumors. Thus, these were analyzed in 21 UM samples which were derived from 10 primary and 11 metastatic tumors from 17 individual patients. The exons and their flanking intron sequences were amplified by PCR and direct sequencing of the PCR products was done. Although we were able to obtain amplicons of exon 18 suitable for sequencing in all 21 tumor samples, we were not successful in amplifying exon 11 in two samples, exon 13 in one sample, and exon 17 in three of the samples. Sequence analysis of the successfully amplified exons revealed that no nucleotide exchanges altering the amino acid sequence of the KIT protein were present in the genomic DNA of any of the samples, indicating that KIT gene mutations are rare or absent in UM (data not shown).

A described polymorphism in exon 18 (G2586C; SNP-Nr: rs3733542), according to the National Center for Biotechnology Information single nucleotide polymorphism (SNP) database, which is present in ~20% of the European population, was detected in 7 of the 17 patients (41%). Successful detection of this SNP in our samples is an indication of the validity of our assay. Interestingly, this synonymous SNP has been suggested to be involved in miRNA-dependent regulation of KIT expression in papillary thyroid cancer (11). The presence of this and one additional SNP in the 3′-untranslated region of the KIT mRNA was associated with low levels of KIT expression and high levels of miRNAs targeting regions including these SNPs.

Absence of MAPK signaling in UM. To answer the question of whether the MAPK pathway is activated in UM due to the presence of the KIT-ligand SCF in the tumor microenvironment, we studied the presence of phosphorylated ERK in tissue sections of primary UM and metastatic primary UM by immunohistochemistry. This analysis revealed, in both primary UM and metastases, that only single tumor cells and some stromal cells infiltrating or surrounding the tumor stained positively for pERK (arrows). No differences, with respect to pERK, were evident between primary or metastatic lesions. This rather scant phosphorylation of ERK was not due to the expression of the negative regulator of MAPK signaling, RKIP. Representative stainings are provided in Fig. 1.

Discussion

Together with its ligand, SCF, the receptor tyrosine kinase KIT is a key regulator for a number of cell types, including hematopoietic stem cells, mast cells, germ cells, and melanocytes (5). SCF exists both in a secreted and a membrane-bound form. Binding of SCF to the KIT receptor induces dimerization and autophosphorylation of the receptor on tyrosine residues. KIT expression has been documented in a wide variety of human malignancies, including gastrointestinal stromal tumors, neuroblastomas, and melanomas. Gain-of-function mutations in KIT leading to SCF-independent signaling have been described in several human cancers including melanoma (5, 12). To this end, Curtin et al. have reported high frequencies (28-39%) of genetic aberrations in KIT in certain subtypes of primary melanoma including mucosal and acral melanomas (6). The authors described different mutations as well as copy number increases associated with an enhanced KIT protein expression. Eleven (69%) of 16 KIT mutations were predicted

Table 5. Immunohistochemical analysis of SCF (n = 33)

	No. of cases (%)
Primary UM	7 (21)
UM metastases	26 (79)
SCF in primary UM	
++++	3 (43)
+++	2 (29)
++	0 (0)
+	1 (14)
-	1 (14)
SCF in UM metastases	
++++	15 (58)
+++	8 (31)
++	3 (11)
+	0 (0)
-	0 (0)

NOTE: Each section was assigned to one of the following percentage categories: negative, <1%, <10% (+), <30% (++), <60% (+++), or <90% (++++).

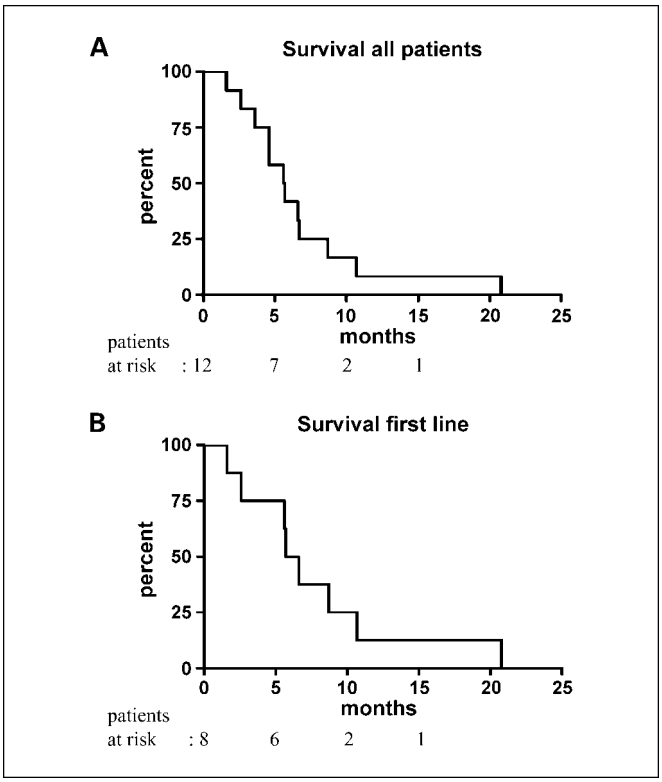

Fig. 2. OS of all 12 patients receiving imatinib mesylate (*A*) and of 8 patients who received it as first-line therapy (*B*).

to affect the juxtamembrane domain, presumably resulting in a constitutive activation of KIT. In general, UM lacks activating BRaf or NRas mutations (with the exception of a few UM cell lines; ref. 13). Notably, Weber et al. reported a high activity of the MAPK pathway in UM (7). Therefore, we studied the expression of the KIT protein and its ligand SCF in UM and screened genomic DNA derived from these samples for the presence of mutations. In concordance with recent reports, we observed that KIT is expressed in the majority of tumor cells in primary and metastatic UM (8, 14, 15).

To date, data on activating KIT mutations in UM are limited. Notably, sequence analysis of 21 UM samples did not reveal any mutations in exons 11, 13, 17, and 18. These results are in agreement with recently published data by other investigators (14, 16). The observation of high expression of KIT in the absence of activating KIT gene mutations in UM raises the question of other mechanisms such as autocrine or paracrine stimulation of the receptor, e.g., by SCF may be operational in activating KIT signaling. Indeed, Lefevre et al. have shown *in vitro* that stimulation of KIT in UM cell lines occurs by autocrine-produced SCF. The growth-inhibitory effect of imatinib mesylate was highest in UM cells harboring the SCF/KIT autocrine loop and lowest in KIT-negative cell lines (17). In the present study, we brought these observations forward into the clinical situation: immunohistochemistry revealed a diffuse and strong staining for KIT in >90% of 43% of primary UM, and in 58% of UM metastases; SCF was expressed in 70% of primary and in >75% of metastatic lesions. Binding of SCF to the KIT receptor induces dimerization and autophosphorylation of the receptor on tyrosine residues. The phosphorylated tyrosine residues serve as docking sites for a number of signal transduction molecules containing Src homology 2 domains, which are recruited to the receptor and initiate downstream signaling events including activation of the MAPK pathway (18). Indeed, constitutive activation of MEK and ERK has been described in UM (19). However, when we scrutinized MAPK activation by staining for phosphorylated ERK after the disillusioning clinical effect of KIT inhibition by imatinib, only a sparse presence of ERK phosphorylation was observed, which was mainly restricted to the inflammatory infiltrate or stromal cells. A possible explanation for this lack of MAPK pathway activation might be the expression of inhibitory molecules. RKIP, which binds to RAF and has the ability to interrupt interactions between RAF and MEK, has been shown to interfere with downstream events such as activation of ERK, when overexpressed (20). However, in our series, only single tumors expressed RKIP irrespective of the origin of the samples, e.g., primary or metastatic lesions.

Malignant melanoma is a disease typified by exceedingly poor response to therapy. Promising preclinical data implicated imatinib mesylate, which inhibits the enzymatic activity of several tyrosine kinases including KIT and platelet-derived growth factor-α, as therapy in a subset of human melanomas (14, 15, 17). Recently, Hodi et al. reported a patient with metastatic anal melanoma harboring a seven-codon duplication in exon 11 of KIT, who had a complete resolution and/or significant improvement of tumor mass reduction within 4 months of treatment with imatinib mesylate (21). Three clinical phase II trials testing imatinib in metastatic melanoma revealed no objective responses and poor survival rates (22–24). However, in these trials, neither KIT expression, mutation, nor activation status were evaluated (25). In agreement with our data, Penel et al. recently reported no objective response of imatinib mesylate (400 mg p.o. b.i.d.) in 10 assessable patients with metastatic UM (26). Several possible explanations for these disappointing observations exist. First, the inhibition of KIT alone is certainly insufficient to prevent the growth of malignant melanoma (27). Other signaling pathways might be implied in UM proliferation (13, 14). Second, drugs of greater specificity or potency may be necessary. Recent studies investigating tyrosine kinase inhibitors showed that the outcome of patients treated with targeted therapies does not necessarily correlate with the expression of its target molecule (28, 29). Third, the critical requirement for effective tyrosine kinase therapy is that the target protein must indeed be activated, which, at least in our series, seems to be the main explanation of the failure of imatinib mesylate in UM.

In conclusion, the presented data unequivocally confirmed the prevalent overexpression of KIT and its ligand SCF in primary and metastatic UM. However, albeit this overexpression is highly suggestive for an autocrine activation loop resulting in constitutive KIT signaling, the absence of ERK phosphorylation as well as the lack of clinical efficacy of the KIT inhibitor imatinib strongly argues against this notion.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Prescher G, Bornfeld N, Friedrichs W, Seeber S, Becher R. Cytogenetics of twelve cases of uveal melanoma and patterns of nonrandom anomalies and isochromosome formation. Cancer Genet Cytogenet 1995;80:40–6.
2. Albert DM, Niffenegger AS, Willson JK. Treatment of metastatic uveal melanoma: review and recommendations. Surv Ophthalmol 1992;36:429–38.
3. Wehrle-Haller B. The role of Kit-ligand in melanocyte development and epidermal homeostasis. Pigment Cell Res 2003;16:287–96.
4. Grichnik JM, Burch JA, Burchette J, Shea CR. The SCF/KIT pathway plays a critical role in the control of normal human melanocyte homeostasis. J Invest Dermatol 1998;111:233–8.
5. Ronnstrand L. Signal transduction via the stem cell factor receptor/c-Kit. Cell Mol Life Sci 2004;61:2535–48.
6. Curtin JA, Busam K, Pinkel D, Bastian BC. Somatic activation of KIT in distinct subtypes of melanoma. J Clin Oncol 2006;24:4340–6.
7. Weber A, Hengge UR, Urbanik D, et al. Absence of mutations of the BRAF gene and constitutive activation of extracellular-regulated kinase in malignant melanomas of the uvea. Lab Invest 2003;83:1771–6.
8. Mouriaux F, Kherrouche Z, Maurage CA, Demailly FX, Labalette P, Saule S. Expression of the c-kit receptor in choroidal melanomas. Melanoma Res 2003;13:161–6.
9. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92:205–16.
10. Simon R. Optimal two-stage designs for phase II clinical trials. Control Clin Trials 1989;10:1–10.
11. He H, Jazdzewski K, Li W, et al. The role of microRNA genes in papillary thyroid carcinoma. Proc Natl Acad Sci U S A 2005;102:19075–80.
12. Rubin BP, Heinrich MC, Corless CL. Gastrointestinal stromal tumour. Lancet 2007;369:1731–41.
13. Calipel A, Lefevre G, Pouponnot C, Mouriaux F, Eychene A, Mascarelli F. Mutation of B-Raf in human choroidal melanoma cells mediates cell proliferation and transformation through the MEK/ERK pathway. J Biol Chem 2003;278:42409–18.
14. All-Ericsson C, Girnita L, Muller-Brunotte A, et al. c-Kit-dependent growth of uveal melanoma cells: a potential therapeutic target? Invest Ophthalmol Vis Sci 2004;45:2075–82.
15. Pereira PR, Odashiro AN, Marshall JC, Correa ZM, Belfort R, Jr., Burnier MN, Jr. The role of c-kit and imatinib mesylate in uveal melanoma. J Carcinog 2005;4:19.
16. Pache M, Glatz K, Bosch D, et al. Sequence analysis and high-throughput immunohistochemical profiling of KIT (CD 117) expression in uveal melanoma using tissue microarrays. Virchows Arch 2003;443:741–4.
17. Lefevre G, Glotin AL, Calipel A, et al. Roles of stem cell factor/c-Kit and effects of Glivec/STI571 in human uveal melanoma cell tumorigenesis. J Biol Chem 2004;279:31769–79.
18. Linnekin D. Early signaling pathways activated by c-Kit in hematopoietic cells. Int J Biochem Cell Biol 1999;31:1053–74.
19. Zuidervaart W, van Nieuwpoort F, Stark M, et al. Activation of the MAPK pathway is a common event in uveal melanomas although it rarely occurs through mutation of BRAF or RAS. Br J Cancer 2005;92:2032–8.
20. Yeung K, Seitz T, Li S, et al. Suppression of Raf-1 kinase activity and MAP kinase signalling by RKIP. Nature 1999;401:173–7.
21. Hodi FS, Friedlander P, Corless CL, et al. Major response to imatinib mesylate in KIT-mutated melanoma. J Clin Oncol 2008;26:2046–51.
22. Ugurel S, Hildenbrand R, Zimpfer A, et al. Lack of clinical efficacy of imatinib in metastatic melanoma. Br J Cancer 2005;92:1398–405.
23. Eton O, Billings L, Kim KB. Phase II trial of imatinib mesylate (STI-571) in metastatic melanoma (MM). J Clin Oncol 2004;22:717s (suppl; abstr 7528).
24. Wyman K, Atkins MB, Prieto V, et al. Multicenter phase II trial of high-dose imatinib mesylate in metastatic melanoma: significant toxicity with no clinical efficacy. Cancer 2006;106:2005–11.
25. Becker JC, Brocker EB, Schadendorf D, Ugurel S. Imatinib in melanoma: a selective treatment option based on KIT mutation status? J Clin Oncol 2007;25:e9.
26. Penel N, Delcambre C, Durando X, et al. O-Mel-Inib: A Cancero-pole Nord-Ouest multicenter phase II trial of high-dose Imatinib mesylate in metastatic uveal melanoma. Invest New Drugs 2008;26:561–5.
27. Becker JC, Ugurel S, Schrama D. Strategies to optimize the use of targeted agents for tumor therapy. J Dtsch Dermatol Ges 2008;6:281–5.
28. Thatcher N, Chang A, Parikh P, et al. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). Lancet 2005;366:1527–37.
29. Chung KY, Shia J, Kemeny NE, et al. Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. J Clin Oncol 2005;23:1803–10.