

## Review

# c-KIT signaling as the driving oncogenic event in sub-groups of melanomas

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**Summary.** As we enter the era of targeted therapy for melanoma, attempts are being made to sub-group tumors on the basis of their driving oncogenic mutations, with the hope of developing truly personalized therapeutic regimens. c-KIT is a receptor tyrosine kinase whose aberrant activation is implicated in the progression of gastrointestinal stromal tumors and some acute myeloid leukemias. The role of c-KIT signaling in melanoma has been controversial; although c-KIT activity is critical to melanocyte development, its expression tends to be lost in most melanomas. Some reports have even shown that the re-expression of c-KIT induces apoptosis in melanoma cell lines.

The recent publication of work showing the presence of activating c-KIT mutations in acral and mucosal melanomas, as well as melanomas arising on skin with chronic sun damage, has renewed interest in c-KIT signaling in melanoma. Recent work from our own laboratory has further identified melanomas with constitutive c-KIT signaling activity resulting from c-KIT receptor overexpression. Although the initial clinical trials of the c-KIT inhibitor imatinib mesylate in melanoma were negative, some dramatic responses have been seen in patients with very high c-KIT expression and/or documented activating mutations, fostering the belief that focused studies in patients selected on the basis of c-KIT mutational status will yield more encouraging results. The current review discusses the role of c-KIT signaling in melanoma progression and how this new information can be applied to the targeted therapy of melanoma.

**Key words:** c-KIT, Melanoma, Imatinib, BRAF, Ocular melanoma

### c-KIT receptor structure, function and signaling

The receptor tyrosine kinase (RTK) c-KIT is a critical regulator of growth, differentiation, migration and proliferation in the hematopoietic, germ cell and melanocytic systems (Lennartsson et al., 2005; Grichnik, 2006). The *KIT* gene was originally identified as the viral oncogene *v-KIT*, derived from the feline sarcoma virus HZ 4-FeSV, and then subsequently as the proto-oncogene form *c-KIT*. Structurally, c-KIT is a member of the platelet-derived growth factor (PDGF) family of kinases and is composed of five immunoglobulin-like motifs in the extracellular portion and a 70-100 residue hydrophilic kinase insert domain that forms the intracellular portion. The c-KIT receptor ligand is the glycoprotein Stem Cell Factor (SCF), which also known under a variety of other names including mast cell growth factor and steel factor (SF). KIT signaling occurs following the binding of SCF to c-KIT, a process that leads to receptor dimerization and autophosphorylation. This reveals docking sites for several Src homology-2 (SH2) domains. SH2 domains are found on a number of signaling proteins, and thus c-KIT is known to recruit and activate a number of intracellular signaling pathways implicated in tumor progression, such as phosphoinositide-3 kinase (PI3K)/AKT, Src, mitogen-activated protein kinase (MAPK), janus kinase (JAK)/signal transducers and activators of transcription (STAT) and phospholipase-C (PLC)- $\gamma$  (Ali and Ali, 2007). The PI3K/AKT pathway is among the signaling pathways known to be important for melanoma progression, and its activation occurs as a consequence of the direct binding of the p85 subunit of PI3K to phosphorylated Tyr-721 of c-KIT (Fig. 1). This in turn leads to the activation of AKT and the inhibition of the pro-apoptotic protein BAD (Serve et al., 1994). In addition, PI3K also activates the small GTP binding protein Rac1, which has been implicated in the SCF-induced proliferation of

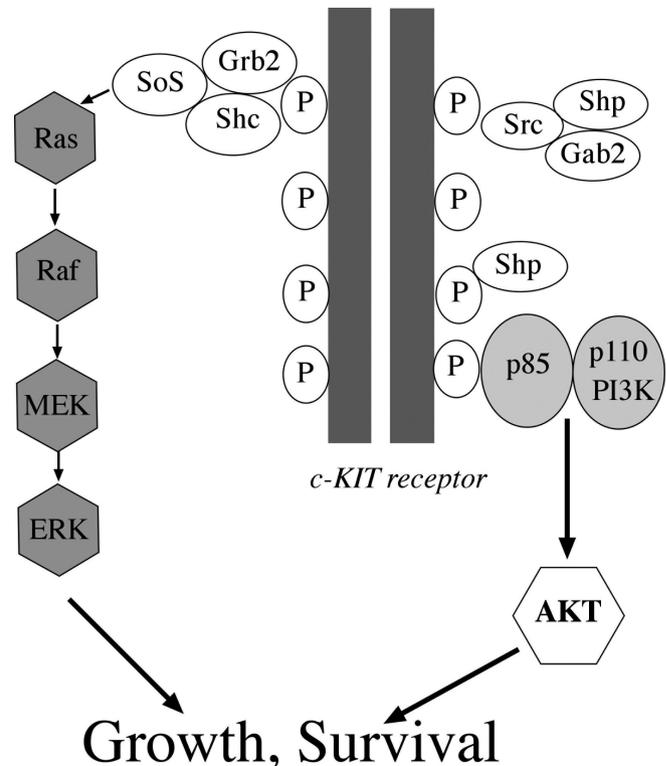
bone marrow-derived mast cells (Timokhina et al., 1998). Rac1 is also a key regulator of the actin cytoskeleton in melanoma and contributes to both motility and invasion (Friedl and Wolf, 2003). The Src family of kinases are also known to be involved in the motile behavior of melanoma cells (Smalley and Herlyn, 2005). Activation of Src occurs following the direct recruitment of the kinase to c-KIT following phosphorylation at Tyr-568/Tyr-570 (Price et al., 1997) (Fig. 1). The MAPK pathway is also known to be critical for melanoma initiation and progression (Smalley, 2003), and this too is a downstream target of c-KIT receptor activity. KIT mediated MAPK activation occurs following the recruitment of the adaptor protein Grb2 to phosphorylated Tyr-703/Tyr-936 of activated c-Kit (Thommes et al., 1999), this then recruits the SOS protein, which in turn activates Ras/Raf and the rest of the MAPK cascade (Fig. 1).

### The role of c-KIT in cancer

As c-KIT/SCF signaling stimulates pathways known to be critical for cell proliferation, invasion and motility, it is perhaps no surprise that KIT signaling is implicated in the initiation and progression of many types of cancer. KIT signaling has been most widely studied in gastrointestinal stromal tumors (GIST) (Kitamura and Hirota, 2004), a family of mesenchymal tumors arising in the gastrointestinal tract. Of these, 60-70% of GIST arise in the stomach, 20-30% develop in the small intestine and a further 5% in the colon or rectum. Histologically, most GIST seem to develop from the interstitial cells of Cajal, a spindle-type cell responsible for synchronizing gut activity. Most GIST (88.2%) have been reported to harbor gain-of-function mutations in c-KIT, which allow for receptor activation in the absence of SCF (Heinrich et al., 2003). Most of the common activating mutations are located within an 11 amino acid stretch between Lys-550 and Val-560, which forms part of the juxtamembrane domain encoded by exon11. Although many GIST tumors are cured through surgery, 20-40% of cases will either recur or relapse, and these are now amenable to treatment with the small molecule c-KIT inhibitor imatinib mesylate (Gleevec<sup>®</sup>, Novartis). In pre-clinical studies, primary GIST cell cultures and established GIST cell lines were found to have constitutive signaling activity in the MAPK, AKT, phospho-S6-Kinase (S6K), STAT1 and STAT3 pathways (Duensing et al., 2004). Treatment of GIST cell lines with imatinib led to the inhibition of both AKT and MEK signaling, and these effects were associated with cell cycle arrest and apoptosis induction (Tuveson et al., 2001; Frolov et al., 2003). Interestingly, imatinib treatment was found to only partly reduce STAT1/3 signaling activity, suggesting that these particular pathways were driven through other oncogenic mutations/RTKs (Duensing et al., 2004). In an in vivo mouse model of GIST, imatinib was strongly pro-apoptotic and led to tumor regression associated with the

inhibition of MAPK and mammalian target of rapamycin (mTOR) signaling (Rossi et al., 2006). In addition to direct effects upon cell growth and apoptosis in GIST, imatinib also has other indirect activities on tumor survival. It has been shown that increased c-KIT activity can stimulate glucose uptake in GIST cells, with clinical studies demonstrating a reduction in glucose uptake in GIST patients following imatinib treatment, as measured by 18fluoro-deoxyglucose positron emission tomography (FDG-PET). The effects of imatinib upon nutrient availability are AKT dependent and involve the direct modulation of glucose transporter 4 (Glut-4) expression (Tarn et al., 2006). There is also some suggestion that imatinib treatment may also have unintended beneficial activity upon cells of the immune system, with one report demonstrating that imatinib stimulates dendritic cells to enhance natural killer (NK) cell-mediated oncolytic activity (Borg et al., 2004).

In addition to GIST, c-KIT expression has also been reported in a wide variety of cancers, including 84% of seminomas, 65% of adenoid cystic carcinomas and 17% of large cell carcinomas of the lung (Went et al., 2004)



**Fig. 1.** Scheme showing some of the key pathways involved in melanoma progression that may be stimulated through c-KIT receptor activity. Upon phosphorylation, the KIT receptor stimulates the MAPK pathway (Ras/Raf/MEK/ERK) through recruitment of an adaptor complex consisting of Grb2/Sos/Shc. Other phosphorylation sites on the KIT receptor are also known to recruit Src family kinases and the p85 subunit of PI3K. Activation of all of these pathways leads to increased growth and survival of melanoma cells.

as well as mast cell leukemias (Kajiguchi et al., 2008). In mast cell leukemia cells, c-KIT signaling is shown to regulate the tyrosine phosphorylation and nuclear localization of  $\beta$ -catenin leading to enhanced transcription of cyclin D1 and c-Myc (Kajiguchi et al., 2008). Currently, imatinib treatment is under clinical investigation for a wide range of malignancies.

### The role of c-KIT in melanocyte development

Melanocytes are the classic migratory cell, and during development melanocyte precursors, so-called melanoblasts, migrate from the neural crest to the epidermis and the bulge of the hair follicle (Nishikawa et al., 1991). The SCF/c-KIT axis plays an important role in this migratory process, and it has been shown that SCF treatment stimulates the migratory behavior of melanocytes grown on fibronectin and increases their expression of pro-invasive integrins (Scott et al., 1994, 1996). The likely source of SCF during this process is the tissue microenvironment of the neural crest/skin, with both dermal fibroblasts and human epidermal keratinocytes being shown to express SCF. Consistent with these findings, the *c-KIT* and *SCF* genes are located at genetic loci associated with pigmentary defects, with c-Kit being located at the W (white spotting) locus on human chromosome 4 and mouse chromosome 5 (Chabot et al., 1988) and SCF being located at the steel locus on human chromosome 12 and mouse chromosome 10 (Witte, 1990). Naturally occurring inactivating mutations at either of these loci leading to impaired c-KIT signaling are associated with depigmented patches of skin/fur characteristic of piebaldism in humans and white spotting in mice (Spritz et al., 1992). In these instances, c-KIT signaling appears to be critical at the level of the melanocyte precursor and promotes the survival and proliferation of c-KIT-positive neural crest-derived cells, where it works in concert with other soluble factors such as endothelin-3 (ET-3) (Kawa et al., 2000). Although c-KIT signaling is essential for melanocyte development, recent work has shown that it may in fact be dispensable for the survival/proliferation of mature pigmented melanocytes. The forced expression of a constitutively active c-KIT (D814Y) mutant into mouse melanocytes led to decreased pigmentation and increased migratory activity (Alexeev and Yoon, 2006). The decrease in pigmentation seen was likely the consequence of the increased degradation of the melanocyte-specific transcription factor microphthalmia-associated transcription factor (MITF). It is known that c-KIT stimulation leads to the MAPK/RSK-1-mediated Ser-73/Ser-409 phosphorylation of MITF followed by subsequent polyubiquitination and proteasomal degradation (Hemesath et al., 1998). This study further showed that constitutive levels of c-KIT signaling led to increased N-cadherin expression in the melanocytes, a finding consistent with both increased motile behavior and increased oncogenic potential in melanoma cells (Li et

al., 2001). The lack of proliferation seen in the c-KIT mutated melanocytes was unexpected, but is likely to be a consequence of c-KIT constituting only one oncogenic "hit". It is well known that overexpression of single activating oncogenes, such as mutated BRAF, typically lead to growth arrest and eventual oncogene-induced senescence (Michaloglou et al., 2005). This data suggests that c-KIT can only oncogenically transform melanocytes when accompanied by genetic lesions in other pathways, the nature of which is currently unknown.

### The role of c-KIT in melanoma progression

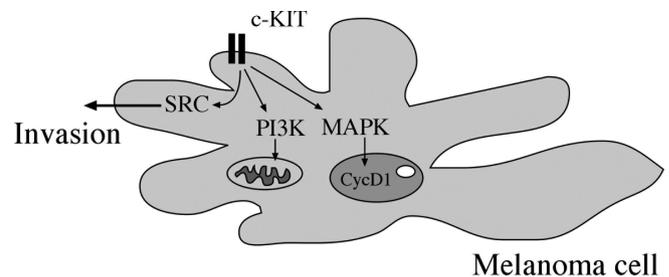
For many years, the possible role for c-KIT/SCF signaling in melanoma initiation and progression was largely dismissed. A number of early pathological studies had shown that c-KIT receptor expression was progressively lost during local melanoma growth and invasion (Lassam and Bickford, 1992; Natali et al., 1992). It was further demonstrated that over 70% of melanoma cell lines and tumor samples lacked any c-KIT expression (Lassam and Bickford, 1992). On the basis of these results it was assumed that c-KIT was primarily a regulator of melanocyte behavior and therefore dispensable for melanoma growth. Some studies went even further and showed that overexpression of c-KIT in previously metastatic melanoma cell lines led to significant reductions in tumor growth and suppression of metastasis (Huang et al., 1996, 1998). It was also demonstrated that the exposure of c-KIT-expressing melanoma cells to the c-KIT ligand SCF led to the induction of apoptosis. Mechanistically, it was shown that loss of the AP-2 transcription factor was the likely mechanism underlying the downregulation of c-KIT receptor expression (Huang et al., 1998).

Recent advances in molecular profiling and high-throughput genotyping have renewed interest in the role of c-KIT in melanoma. These studies have led to the realization that melanomas are not genetically and histologically homogeneous and may follow different paths to oncogenic transformation. One of the major findings in recent years has been the identification of activating oncogenic mutations in BRAF (the V600E mutation) in the majority (~60%) of melanomas (Davies et al., 2002). Most of the transforming activity of the V600E BRAF mutation is thought to result from the activation of the MAPK pathway (Davies et al., 2002). Under physiological situations, the MAPK pathway is stimulated through the interaction of growth factors with their respective cell surface receptors followed by the transmission of their signals to the interior of the cell through the small GTPase Ras (Robinson and Cobb, 1997). When active in its GTP-bound state, Ras activates a number of downstream effectors, one of which is the Raf family of serine/threonine kinases. There are three isoforms of Raf: A-Raf, BRAF and C-Raf (also called Raf-1). Once active, Raf activates the MAPK cascade,

resulting in the sequential activation of MEK1 and MEK2, which in turn activate extracellular-signal regulated kinase (ERK)1 and ERK2 (Crews et al., 1992; Kyriakis et al., 1992). Upon activation, the ERKs can either regulate cytoplasmic targets or can migrate to the nucleus, where they phosphorylate a number of transcription factors as well as driving proliferation through regulation of cyclin D1 expression. The incidence of BRAF mutations is higher in melanomas arising on skin in the absence of chronic sun damage (as defined by the lack of solar elastosis), and although the BRAF V600E mutation is not an ultraviolet (UV)-signature mutation there is some suggestion that UV-exposure may play some role in the acquisition of these mutations (Thomas et al., 2006). Not all melanoma harbor mutations in BRAF, at least 15% have oncogenic mutations in NRAS (Padua et al., 1984) and there is a smaller but significant group with activating mutations and gene amplification in *c-KIT* (Curtin et al., 2006). Melanomas on sites with little UV exposure, such as the skin on the palms of the hands, the soles of the feet or subungual sites (acral melanomas) and on mucous membranes (mucosal melanomas) have a very low incidence of BRAF mutations (Curtin et al., 2006). In addition to this, BRAF mutations are also known to be rare on skin that exhibits signs of chronic sun damage (solar elastosis). Unlike typical cutaneous melanomas, the incidence of acral and mucosal melanomas is similar across all racial groups. Recent studies have shown that 21% of mucosal melanomas, 11% of acral melanomas and 17% of melanomas arising on sun-damaged skin harbor activating mutations in *c-KIT*, with most of these occurring at the imatinib-sensitive juxtamembrane position (Curtin et al., 2006). Sequencing of *c-KIT* exons 11, 13, 17 and 18 revealed the most prevalent mutations to be K642E, L576P, D816H and V559A. It was additionally shown that, in most cases, *c-KIT* mutation was accompanied by an increase in copy number and genomic amplification as identified by array comparative genomic hybridization (aCGH). There were also instances where *c-KIT* was amplified in the absence of a mutation and thus it was reported that the total number of *c-KIT* aberrations (either amplification and/or mutation) were 39% for mucosal, 36% for acral and 28% for melanomas arising on sun-damaged skin (Curtin et al., 2006). Subsequent studies have shown that *c-KIT* is expressed in 88% of oral mucosal melanomas, and that at least 22% of these harbored activating mutations (Rivera et al., 2008). In these instances, most of the *c-KIT* expression was in the in situ component, with strong expression reported in the invasive portion in only 22% of cases (Rivera et al., 2008). Another recent paper also reported the presence of the activating L576P mutation in *c-KIT* in 15% of anal melanomas, a mutation that the authors showed to be imatinib sensitive in vitro (Antonescu et al., 2007).

Our group recently identified another subset of melanomas that lacked BRAF mutations but expressed high levels of *c-KIT* and cyclin dependent kinase

(CDK)-4 (Smalley et al., 2008a). These melanoma cell lines lacked activating *c-KIT* mutations and showed no evidence of an SCF/*c-KIT* autocrine loop. They were, however, found to have constitutive *c-KIT* receptor signaling as shown by the presence of high level phospho-*c-KIT* expression, suggesting that the signaling activity may have arisen as a consequence of very high receptor expression levels leading to spontaneous dimerization. Similar findings have been reported in non-small cell lung cancer where very high epidermal growth factor (EGF) receptor expression levels lead to constitutive signaling activity (Zandi et al., 2007). It was further found that the melanoma cell lines with high *c-KIT* receptor expression were highly sensitive to imatinib in both in vitro preclinical organotypic cell culture models and in human melanoma xenograft experiments (Smalley et al., 2008a). In this instance, the anti-melanoma effects of imatinib were mediated both through apoptosis induction and cell cycle arrest. Mechanistically, imatinib was found to inhibit MEK/ERK signaling in these *c-KIT* dependent melanomas and was associated with increased expression of the cyclin dependent kinase inhibitor (CDKi) p27KIP1 (Smalley et al., 2008a) (Fig. 2). This is in marked contrast to the treatment of melanoma cell lines harboring BRAF V600E mutations with either BRAF or MEK inhibitors, where only cytostatic effects are observed (Haass et al., 2008). It is therefore suggested that imatinib may impact upon multiple pathways in addition to MAPK in *c-KIT*-driven melanomas (Fig. 2). Interestingly, the mere presence of *c-KIT* protein was not predictive of response in our cell line panel. Some of our melanoma cell lines that maintained *c-KIT* receptor expression, harbored a BRAF V600E mutation and lacked phospho-*c-KIT* receptor expression were not found to respond to imatinib (Smalley et al., 2008a). It is therefore highly likely that not all melanomas that express *c-KIT* protein necessarily rely on *c-KIT* receptor signaling to drive their progression, particularly if this occurs in the context of an activating BRAF mutation. The restriction of *c-KIT*



**Fig. 2.** Signaling scheme showing how *c-KIT* activated pathways may contribute towards melanoma progression. Constitutive activity through *c-KIT* activates Src leading to enhanced invasion and motility. Increased PI3K and MAPK activity may also increase survival and lead to enhanced growth through cyclin D regulation.

mutations and c-KIT protein expression to minor subgroups of melanomas helps to explain the earlier studies largely refuting a role for c-KIT signaling in melanoma progression. Our studies have shown that most melanoma cell lines (>98%, (Smalley et al., 2008b)) harbor either an activating *BRAF* or *NRAS* mutation (most are *BRAF* mutated), and there are very few melanoma cell lines available that rely on c-KIT signaling. Indeed, the very high prevalence of *BRAF* mutations in cell lines compared to patient samples suggests that *BRAF*-driven melanomas may in fact be highly suited to current cell culture techniques.

### The role of c-KIT signaling in uveal melanoma

Another subtype of melanoma that seems to rely on c-KIT signaling are the ocular melanomas. Uveal melanoma is the most common primary eye tumor in adults; these derive from the melanocytes of the choroid, ciliary body and iris. Although cutaneous and ocular melanocytes share a very similar embryological origin, the biological behavior of the resultant melanomas is very different. Ocular melanomas tend to spread via the circulatory system and preferentially metastasize to the liver, whereas cutaneous melanomas metastasize via the lymph nodes and often result in nodal, lung and brain metastasis. The mechanism of oncogenic transformation between the two melanocyte populations is also likely very different, with uveal melanomas generally lacking activating *BRAF* mutations (Rimoldi et al., 2003), and alterations at the *CDKN2A* locus (Hearle et al., 2003). Recent studies have shown that *c-KIT* expression is maintained in 87% of uveal melanomas, although these typically lack activating c-KIT mutations at exons 11 and 13 (Pache et al., 2003). Further work showed that 61% of uveal liver metastases also expressed c-KIT and that the presence of high c-KIT receptor expression at the immunohistochemical level correlated with a worse prognostic outcome (All-Ericsson et al., 2004). Cell culture experiments have demonstrated that uveal melanoma cell lines harbor phospho-c-KIT expression (indicative of receptor activity) and that they can be growth-arrested following imatinib treatment (All-Ericsson et al., 2004). This study also showed a lack of *c-KIT* mutations in uveal melanoma, again suggesting that either high receptor expression or an autocrine SCF/c-KIT loop was responsible for the kinase activity observed. Unfortunately, these promising pre-clinical results have not translated into clinical success. A recent phase II clinical trial of uveal melanoma patients showed no objective responses in 10 patients treated with high dose imatinib (400 mg twice daily) and only one incidence of stable disease lasting 5 months (Penel et al., 2008).

### Clinical studies on c-KIT inhibition in melanoma

A number of small molecule RTK inhibitors have been developed that target KIT activity, the best studied

of which being imatinib mesylate, an RTK inhibitor with activity against Bcr-Abl, PDGFR and c-KIT (Heinrich et al., 2000; Druker et al., 2001). Although imatinib is now routinely used in the treatment of patients with chronic myeloid leukemia and GIST, its activity in non-selected groups of melanoma patients has been very disappointing (Ugurel et al., 2005). Phase II clinical trials of imatinib in patients with metastatic melanoma revealed no objective responses, poor survival rates and significant toxicity (Ugurel et al., 2005). Although the initial round of clinical studies on imatinib in melanoma were negative, the careful selection of melanoma patients with activating mutations in c-KIT is now underway at a number of centers, including our own. Two case reports have recently been published detailing remarkable clinical responses of individual patients to imatinib. The first patient to be reported was a 79-year old woman with metastatic mucosal melanoma. Immunohistochemical staining revealed the tumor to have very strong staining for c-KIT and mutational analysis demonstrated the presence of a seven-codon duplication in exon 11 (Hodi et al., 2008). After four weeks of treatment with imatinib (400 mg daily), regression of the metastatic lesions was noted on a PET/CT scan, and at time of publication the patient was continuing to respond to therapy. Another recent case report described a patient with a mucosal melanoma that stained strongly for c-KIT, harbored an activating exon 13 (K642E) mutation and showed increased copy number (Lutzky et al., 2008). Following surgical removal of the primary tumor the patient manifested metastatic spread; imatinib therapy ultimately led to the resolution of all of the metastatic nodules. At the time of publication the patient continues to be disease-free and is being maintained on imatinib (Lutzky et al., 2008). The most recent report on the therapeutic utility of imatinib in melanoma is a phase II trial of patients selected on the basis of their tumors expressing one of the molecular targets of the inhibitor by immunohistochemistry (PDGFR, c-KIT, c-abl or abl-related gene) (Kim et al., 2008). Although the trial was negative, one significant response was observed in a patient with acral-lentiginous melanoma. Analysis of the patient's tumor showed it to have the highest c-KIT expression of the entire cohort, albeit lacking a c-KIT receptor mutation. It was also shown that imatinib treatment induced a significant level of apoptosis in this tumor and in the surrounding endothelial cells (Kim et al., 2008). In an ongoing phase II study at our institution, patients with mucosal, acral lentiginous and sun-induced melanoma with measurable metastatic disease and an activating exon 11 or 13 c-KIT mutations are being treated with imatinib.

### Future perspectives

Although it was long thought that c-KIT was not a viable therapeutic target in melanoma, there is accumulating evidence that there may be small groups of patients with melanomas arising from acral or mucosal

sites or skin sites with chronic sun exposure that may benefit from imatinib treatment. At this stage we only have limited clinical evidence to support the use of imatinib in melanoma, and larger phase II studies are needed to confirm these initial positive case reports. It also remains to be seen whether c-KIT overexpression will predict for response in the clinical setting. Our pre-clinical data suggest that it will, but this will likely also depend on what other oncogenic mutations the tumor possesses. It is, however, encouraging that a limited number of patients with c-KIT amplification respond to imatinib, as this offers some of the first proof-of-principle that targeted therapy strategies may succeed in this extremely recalcitrant and therapy resistant tumor.

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