Hair Depigmentation Is a Biological Readout for Pharmacological Inhibition of KIT in Mice and Humans

KATHERINE G. MOSS, GUY C. TONER, JULIE M. CHERRINGTON, DIRK B. MENDEL, and A. DOUGLAS LAIRD

Department of Preclinical Research and Exploratory Development, SUGEN, Inc., South San Francisco, California (K.G.M., J.M.C., D.B.M., A.D.L.); and Department of Medical Oncology, Peter MacCallum Cancer Institute, Victoria, Australia (G.C.T.)

Received April 2, 2003; accepted July 29, 2003

ABSTRACT

Deregulated activation of the KIT receptor tyrosine kinase has been implicated in several human cancers and in inflammation, making it an attractive target for therapeutic intervention. Conversely, deficiencies in KIT signaling have been implicated in human and animal hair pigmentation disorders, reflecting a role for KIT in the development and function of melanocytes. The goal of this study was to explore the potential utility of hair pigmentation as a biological readout for systemic inhibition of KIT by SU11248 5-[5-fluoro-2-oxo-1,2-dihydroindol-3(yli)-denemethyl]-2,4-dimethyl-1H-pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide), an oral multitargeted tyrosine kinase inhibitor with antitumor and antiangiogenic activity through targeting platelet-derived growth factor receptors, vascular endothelial growth factor receptors, KIT, and FLT3. Oral SU11248 treatment induced dose-dependent depigmentation of newly regrown hair in depilated C57BL/6 mice. Similar effects were seen after administration of a KIT-neutralizing antibody. SU11248-induced hair depigmentation was reversible with cessation of treatment. Histological and immunohistochemical evaluation of mouse skin samples supported these observations and revealed that SU11248 has no effect on levels of KIT-positive melanocytes associated with hair follicles, indicating that the inhibitory effect is at the level of melanocyte function rather than their development/survival. Similar hair depigmentation has been noted in several cancer patients receiving SU11248 in phase I trials. Strikingly, patient scalp hair exhibits bands of depigmentation and pigmentation that correspond, respectively, to periods of treatment and dosing rest periods. These data demonstrate that hair pigmentation can serve as a dose-dependent, dynamic, biological readout for KIT inhibition in mice, and, apparently, in humans.

KIT activation has been implicated in a number of human cancers, including mastocytosis, germ cell tumors, small-cell lung carcinoma, gastrointestinal stromal tumors, acute myelogenous leukemia, neuroblastoma, melanoma, ovarian carcinoma, and breast carcinoma (Turner et al., 1992; Heinrich et al., 2002). KIT activation in these tumors is generally accomplished by one or more of three mechanisms: autocrine and/or paracrine stimulation by stem cell factor (SCF, the ligand for KIT), cross-activation by other kinases, and the acquisition of activating mutations (Hirota et al., 1998; Heinrich et al., 2002). Based on these data, KIT is under active development as an oncology target. Most notably, Gleevec (Novartis, Basel, Switzerland), a selective inhibitor of Bcr-Abl, KIT, and platelet-derived growth factor (PDGF) receptor β, has been approved for the treatment of malignant metaphasic and/or unresectable gastrointestinal stromal tumors (Dagher et al., 2002). In addition to its role in cancer, KIT activation has also been implicated in inflammation (Krishnaswamy et al., 2001).

In contrast to the role of KIT up-regulation in cancer and inflammation, many white spot or band coat color patterns in mammals have been traced to loss-of-function mutations in the genetic loci dominant white spotting (W) and steel (Sl), which encode, respectively, KIT and SCF (Chabot et al., 1988; Zsebo et al., 1990; Halaban and Moellmann, 1993). Specifically, gene duplication and splice mutations in W are responsible for the white coat color in Large White pigs, whereas an inversion mutation in W presents the Rump White color pattern in mice (Stephenson et al., 1994; Marklund et al., 1998). Similarly, piebaldism, a rare human autosomal dominant disorder of melanocyte development, has been traced to a number of point, deletion, splice, and insertion mutations in the W locus (Richards et al., 2001).

The hair follicle passes through three key cyclic regeneration stages, telogen, anagen, and catagen, of which anagen is the most significant for pigmentation (Paus and Cotsarelis, 1999). Pigment for the hair shaft is generated solely by the follicular melanocytes, which reside above the dermal papilla...
next to the keratinocytes. The keratinocytes take up pigment from the melanocytes and incorporate it into the growing hair shaft (Tobin et al., 1999; Scott et al., 2002). During early anagen, SCF/KIT signaling is vitally important for both melanocyte proliferation/differentiation and proper pigment production. Without SCF/KIT signaling during anagen, melanocytes are absent or unable to pass melanin to the growing keratinocytes (Botchkareva et al., 2001). The end result is a hair follicle partially or completely devoid of color.

It has been previously demonstrated that intradermal injection of ACK45, a KIT-neutralizing monoclonal antibody, during the anagen cycle of hair follicle regeneration will prevent pigmentation of the hair shaft (Botchkareva et al., 2001). SU11248 is a small molecule inhibitor of Class III/V receptor tyrosine kinases including the vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) receptors, KIT and FLT3, and is highly selective for these kinases over other kinases evaluated (Abrams et al., 2003; Mendel et al., 2003; O’Farrell et al., 2003) Here we show that systemically administered SU11248 induces reversible hair depigmentation in mice. Moreover, SU11248 treatment has resulted in reversible loss of hair pigmentation in a number of cancer patients enrolled in Phase I studies. These data directly demonstrate that the enzymatic activity of KIT is required for development and maintenance of appropriate hair pigmentation in mice and humans. In addition, these results demonstrate the feasibility of using hair pigmentation as a biological readout for KIT inhibition in preclinical drug discovery and in the clinic.

Materials and Methods

Animals and Tissue Collection. Female C57BL/6 mice (6–8 weeks old) were purchased from Charles River (Hollister, CA) and housed in sterile filter-top cages with Aspen Chip bedding on HEPA-filtered ventilated racks. Animals received sterile rodent chow and water ad libitum and were kept under 12-h light/dark cycles. Animal experiments were conducted in accordance to Institutional Animal Care and Use Committee guidelines in the SUGEN, Inc. Animal Facility, which has been accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

To induce the hair cycle, depilation of skin on the back of mice was performed as described previously (Paus et al., 1990). Briefly, hair was removed from anesthetized mice using Nair’s cold wax hair removal kit as per kit instructions. SU11248 was administered orally as described previously (Mendel et al., 2003), with the first dose administered 4 h before depilation. The rat anti-mouse KIT-neutralizing monoclonal antibody ACK2 (Nishikawa et al., 1991) was purchased from eBioscience (San Diego, CA) and administered intraperitoneally twice weekly in a PBS vehicle. On day 21 to 28 (depending on the study), animals were sacrificed and analyzed for pigmentation of the regenerated hair shaft. Skin was harvested parallel to the vertebral line and fixed in Streck tissue fixative (Streck Laboratories, Omaha, NE) for immunohistochemical analyses. In one study, a limited number of animals were taken off treatment on day 28 and replucked on day 30 to monitor repigmentation during hair regrowth.

Immunohistochemistry. Streak-fixed skin was paraffin-embedded and sectioned. General tissue morphology was visualized by H&E staining. Slides were immunostained for KIT using a rabbit polyclonal anti-KIT antibody (A-4502 1:25; DAKO, Glostrup, Denmark). This antibody was affinity purified through antigen-bound activated thiol AvidGel F chromatography, based on package insert information supplied by the manufacturer. Secondary detection was by a horseradish peroxidase-labeled polymer conjugated to antirabbit antibodies (EnVision+ Systems; DAKO).

Results

In a pilot experiment, the ability of oral SU11248 administered once daily at 80 mg/kg to inhibit pigmentation of hair newly regrown after depilation was assessed. As a positive control (Yoshida et al., 1997), the KIT-neutralizing antibody ACK2 was administered IP twice weekly at 300 μg, 150 μg, and 50 μg per animal (n = 3 mice for all treatment groups). Three weeks later, ACK2 animals showed complete whitening of hair at 300 μg, graying at 150 μg and no depigmentation at 50 μg. SU11248-treated mice exhibited hair depigmentation comparable to animals treated with ACK2 at 300 μg (Fig. 1).

To explore the dose-responsiveness of depigmentation associated with SU11248 treatment, SU11248 was administered at 80 or 40 mg/kg once daily in this model (n = 5 mice per group). As before, SU11248 treatment at 80 mg/kg resulted in whitening of regrown coat hair. In contrast, treatment at 40 mg/kg generally resulted in moderate graying of regrown coat hair (Fig. 2). Similar results were obtained in additional studies, which also determined that treatment at 20, and 5 mg/kg once daily had no detectable effect on hair pigmentation (data not shown). Selected mice were removed from treatment on day 28 for the study shown in Fig. 2 and depilated a second time on day 30. By day 52, these mice showed regrowth of completely pigmented hair (Fig. 3), demonstrating the reversibility of this effect.

Skin samples of control and treatment animals were taken for histological evaluation. Hair pigmentation was clearly evident in hematoxylin and eosin (H&E)-stained slides from control animals; however, only translucent (white) hair was evident in skin samples from animals that had been treated with SU11248 at 80 mg/kg (Fig. 4). Gray hairs of varying shades predominated in H&E-stained slides of animals treated with SU11248 at 40 mg/kg (Fig. 4). To determine whether depigmentation was mediated via loss of KIT-positive cells in hair follicles, immunostaining for KIT was performed. The presence of KIT-positive cells, presumably melanocytes, was clearly evident in the hair follicles of both vehicle and SU11248-treated mice (Fig. 5).

Interestingly, loss of hair pigmentation has been noted in a number of patients with advanced cancer enrolled in SU11248 clinical trials. A striking example of this from a phase I trial being conducted in Australia is shown in Fig. 6, which shows an 18-year-old male patient with chemotherapy-refractory metastatic synovial sarcoma who underwent treatment with SU11248. This patient received multiple 6-week cycles of treatment, with each cycle comprising a 4-week period receiving SU11248 and a 2-week break from treatment. The patient exhibited loss of hair pigmentation while receiving SU11248, but his hair became repigmented during breaks from treatment. Five cycles of treatment resulted in his hair exhibiting multiple light/white and dark bands, with the light bands, as anticipated, being longer than the dark bands (Fig. 6). Similar reversible hair depigmentation by SU11248 has recently been reported in multiple patients from a phase I study in France (Robert et al., 2003).
We have demonstrated that treatment with SU11248 resulted in inhibition of hair pigmentation in mice and humans. This effect was fully reversible, being lost when SU11248 treatment was discontinued. In our mouse model, hair pigmentation was modulated in a dose-dependent, quasi-analog manner, i.e., individual hairs were either white, black, or varying shades of gray. Although the most spectacular effects were seen in regions of hair that had been plucked, isolated white hairs were also evident in nonplucked regions of mice, notably their muzzles (Figs. 1 and 2; data not shown). Barbering, the removal of hair and whiskers by dominant mice, may provide an explanation for this observation. Barbering entails the plucking of individual hairs, which would duplicate, on an individual level, the regional hair plucking performed in this study (Sarna et al., 2000).

Previous studies using KIT-neutralizing antibodies have demonstrated effects on both melanocyte differentiation and survival in mice (Yoshida et al., 1996; Botchkareva et al., 2001). The black mice used in all of these studies were immunocompetent, complicating interpretation of the effects of KIT neutralizing antibodies, which could potentially immunologically target cells on the basis of KIT positivity in addition to simply inhibiting KIT function. In contrast, the effects seen with SU11248 in this model are presumably mediated via inhibition of KIT kinase activity. Although it is not a specific inhibitor of KIT, SU11248 is highly selective for class III/V receptor tyrosine kinases, including KIT, over other kinases evaluated (Mendel et al., 2003).

Fig. 1. Systemic administration of SU11248 inhibits hair pigmentation in C57BL/6 mice. Daily oral administration of SU11248 at 80 mg/kg (A) was initiated immediately after hair depilation. ACK2 (KIT-neutralizing antibody) was administered intraperitoneally twice weekly at the indicated doses as a positive control. Hair depigmentation was evaluated on day 21. Daily oral administration of SU11248 at 80 mg/kg (A) results in hair whitening comparable with ACK2 at 300 μg (B). A depilated vehicle-treated animal is included in each panel for comparison.

Fig. 2. SU11248 inhibits hair pigmentation in C57BL/6 mice in a dose-dependent manner. Daily oral administration of SU11248 at 80 mg/kg (A) and 40 mg/kg (B) was initiated immediately after hair depilation. Hair depigmentation was evaluated on day 28. A depilated vehicle-treated animal is included in A and B for comparison.
continuation of SU11248 treatment after replucking of depigmented hair was associated with regrowth of a new coat of depigmented hair (data not shown).

Hair depigmentation has been reported in a number of patients receiving SU11248 in clinical trials (Raymond et al., 2003; Robert et al., 2003; Toner et al., 2003). Most commonly, this is reported as the development of white or gray hair. Depigmentation in human patients is reversible at the level of individual hair follicles, with both pigmentation and depigmentation evident on the same hair shafts, as seen in the patient shown in Fig. 6, and in at least several additional cases (Robert et al., 2003; data not shown). The presence of the pigmented bands of hair, which grow during breaks in administration of SU11248, presumably reflects the recovery of KIT activity during treatment breaks, as well as the original hair color.

In mice, depigmentation was complete when SU11248 was administered at 80 mg/kg once daily. In humans, depigmentation was evident in some patients from phase I studies treated once daily with 50 mg of SU11248 (now the recommended phase II dose) or higher doses (Robert et al., 2003; Raymond et al., 2003; Toner et al., 2003). Hair depigmentation was reversible at the level of individual hairs, with both pigmentation and depigmentation evident on the same hair shafts, as seen in the patient shown in Fig. 6, and in at least several additional cases (Robert et al., 2003; data not shown). The presence of the pigmented bands of hair, which grow during breaks in administration of SU11248, presumably reflects the recovery of KIT activity during treatment breaks, as well as the original hair color.

In mice, depigmentation was complete when SU11248 was administered at 80 mg/kg once daily. In humans, depigmentation was evident in some patients from phase I studies treated once daily with 50 mg of SU11248 (now the recommended phase II dose) or higher doses (Robert et al., 2003; Raymond et al., 2003; Toner et al., 2003). Hair depigmentation was reversible at the level of individual hairs, with both pigmentation and depigmentation evident on the same hair shafts, as seen in the patient shown in Fig. 6, and in at least several additional cases (Robert et al., 2003; data not shown). The presence of the pigmented bands of hair, which grow during breaks in administration of SU11248, presumably reflects the recovery of KIT activity during treatment breaks, as well as the original hair color.

In mice, depigmentation was complete when SU11248 was administered at 80 mg/kg once daily. In humans, depigmentation was evident in some patients from phase I studies treated once daily with 50 mg of SU11248 (now the recommended phase II dose) or higher doses (Robert et al., 2003; Raymond et al., 2003; Toner et al., 2003). Hair depigmentation was reversible at the level of individual hairs, with both pigmentation and depigmentation evident on the same hair shafts, as seen in the patient shown in Fig. 6, and in at least several additional cases (Robert et al., 2003; data not shown). The presence of the pigmented bands of hair, which grow during breaks in administration of SU11248, presumably reflects the recovery of KIT activity during treatment breaks, as well as the original hair color.

In mice, depigmentation was complete when SU11248 was administered at 80 mg/kg once daily. In humans, depigmentation was evident in some patients from phase I studies treated once daily with 50 mg of SU11248 (now the recommended phase II dose) or higher doses (Robert et al., 2003; Raymond et al., 2003; Toner et al., 2003). Hair depigmentation was reversible at the level of individual hairs, with both pigmentation and depigmentation evident on the same hair shafts, as seen in the patient shown in Fig. 6, and in at least several additional cases (Robert et al., 2003; data not shown). The presence of the pigmented bands of hair, which grow during breaks in administration of SU11248, presumably reflects the recovery of KIT activity during treatment breaks, as well as the original hair color.

In mice, depigmentation was complete when SU11248 was administered at 80 mg/kg once daily. In humans, depigmentation was evident in some patients from phase I studies treated once daily with 50 mg of SU11248 (now the recommended phase II dose) or higher doses (Robert et al., 2003; Raymond et al., 2003; Toner et al., 2003). Hair depigmentation was reversible at the level of individual hairs, with both pigmentation and depigmentation evident on the same hair shafts, as seen in the patient shown in Fig. 6, and in at least several additional cases (Robert et al., 2003; data not shown). The presence of the pigmented bands of hair, which grow during breaks in administration of SU11248, presumably reflects the recovery of KIT activity during treatment breaks, as well as the original hair color.

In mice, depigmentation was complete when SU11248 was administered at 80 mg/kg once daily. In humans, depigmentation was evident in some patients from phase I studies treated once daily with 50 mg of SU11248 (now the recommended phase II dose) or higher doses (Robert et al., 2003; Raymond et al., 2003; Toner et al., 2003). Hair depigmentation was reversible at the level of individual hairs, with both pigmentation and depigmentation evident on the same hair shafts, as seen in the patient shown in Fig. 6, and in at least several additional cases (Robert et al., 2003; data not shown). The presence of the pigmented bands of hair, which grow during breaks in administration of SU11248, presumably reflects the recovery of KIT activity during treatment breaks, as well as the original hair color.

In mice, depigmentation was complete when SU11248 was administered at 80 mg/kg once daily. In humans, depigmentation was evident in some patients from phase I studies treated once daily with 50 mg of SU11248 (now the recommended phase II dose) or higher doses (Robert et al., 2003; Raymond et al., 2003; Toner et al., 2003). Hair depigmentation was reversible at the level of individual hairs, with both pigmentation and depigmentation evident on the same hair shafts, as seen in the patient shown in Fig. 6, and in at least several additional cases (Robert et al., 2003; data not shown). The presence of the pigmented bands of hair, which grow during breaks in administration of SU11248, presumably reflects the recovery of KIT activity during treatment breaks, as well as the original hair color.
In mice, treatment with SU11248 at 40 μg/kg once daily resulted in substantial inhibition of KIT phosphorylation (Abrams et al., 2003) and hair pigmentation, whereas treatment at 80 μg/kg once daily resulted in complete inhibition of KIT phosphorylation (Abrams et al., 2003) and hair pigmentation. In contrast, 40 μg/kg given once daily is generally sufficient for full antitumor efficacy in mouse models (Mendel et al., 2003). These data are consistent with previous observations that the target plasma inhibitor concentrations for SU11248 for the VEGF and PDGF receptors (primary targets in most cancer models) is 50 to 100 ng/ml (Mendel et al., 2003), whereas the target plasma concentration for KIT may be somewhat higher (Abrams et al., 2003). Hence, although loss of hair pigmentation in treated mice and human patients should be solely indicative of inhibition of KIT, simultaneous inhibition of other class III/V receptor tyrosine kinase targets of SU11248, such as the VEGF and PDGF receptors, and FLT3, can be inferred.

Treatment with Gleevec (Novartis), a selective inhibitor of KIT, can be inferred.