SU14813: a novel multiple receptor tyrosine kinase inhibitor with potent antiangiogenic and antitumor activity

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Abstract

Receptor tyrosine kinases (RTK), such as vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), stem cell factor receptor (KIT), and fms-like tyrosine kinase 3 (FLT3), are expressed in malignant tissues and act in concert, playing diverse and major roles in angiogenesis, tumor growth, and metastasis. With the exception of a few malignancies, seemingly driven by a single genetic mutation in a signaling protein, most tumors are the product of multiple mutations in multiple aberrant signaling pathways. Consequently, simultaneous targeted inhibition of multiple signaling pathways could be more effective than inhibiting a single pathway in cancer therapies. Such a multitargeted strategy has recently been validated in a number of preclinical and clinical studies using RTK inhibitors with broad target selectivity. SU14813, a small molecule identified from the same chemical library used to isolate sunitinib, has broad-spectrum RTK inhibitory activity through binding to and inhibition of VEGFR, PDGFR, KIT, and FLT3. In cellular assays, SU14813 inhibited ligand-dependent and ligand-independent proliferation, migration, and survival of endothelial cells and/or tumor cells expressing these targets. SU14813 inhibited VEGFR-2, PDGFR-β, and FLT3 phosphorylation in xenograft tumors in a dose- and time-dependent fashion. The plasma concentration required for *in vivo* target inhibition was estimated to be 100 to 200 ng/mL. Used as monotherapy, SU14813 exhibited broad and potent antitumor activity resulting in regression, growth arrest, or substantially reduced growth of various established xenografts derived from human or rat tumor cell lines. Treatment in combination with docetaxel significantly enhanced both the inhibition of primary tumor growth and the survival of the tumor-bearing mice compared with administration of either agent alone. In summary, SU14813 inhibited target RTK activity *in vivo* in association with reduction in angiogenesis, target RTK-mediated proliferation, and survival of tumor cells, leading to broad and potent antitumor efficacy. These data support the ongoing phase I clinical evaluation of SU14813 in advanced malignancies. [Mol Cancer Ther 2006;5(7):1774–82]

Introduction

Receptor tyrosine kinases (RTK) are cell surface receptors that initiate growth, differentiation, and survival of various cell types, including endothelial and tumor cells. Each receptor comprises an extracellular ligand-binding domain, a transmembrane domain, and a cytoplasmic catalytic domain. Binding of ligand activates the protein kinase catalytic activity, initiating downstream signaling cascades that result in altered gene expression and cellular function (1). Aberrant RTK signaling, due to overexpression or activating mutation of receptors and/or their ligands, frequently underlies diverse facets of tumor pathobiology, including hyperproliferation, migration, survival, differentiation, neoangiogenesis, and invasion (2, 3), and thus provides an attractive target for cancer therapy.

Among targeted cancer therapies in recent clinical development, there has been a proliferation of agents designed to inhibit single RTKs in solid tumors, including those directed against stem cell factor receptor (KIT, imatinib mesylate), epidermal growth factor receptor (EGFR, erlotinib), HER2/neu (trastuzumab), and those targeting RTK ligands, including vascular endothelial growth factor (VEGF, bevacizumab; refs. 4–7). Their shown clinical activity has validated the continued pursuit of molecular targeted therapeutics. However, because tumors and their supporting vasculature generally express multiple RTKs, which regulate cell survival, proliferation, and angiogenesis (3), inhibition of several rather than a single

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RTK may elicit increased or more rapid biological effect, and calls for the development of agents that simultaneously target multiple RTKs.

The class III/IV ''split kinase domain'' subgroup of RTKs, consisting of receptors for VEGF, platelet-derived growth factor (PDGF), SCF/KIT ligand, macrophage colony-stimulating factor/colony-stimulating factor 1 receptor (M-CSF/CSF-1), and fms-like tyrosine kinase 3 (FLT3) ligand, trigger signaling pathways required for cell growth and survival, and are implicated either in tumor cell growth and/or in angiogenesis. Pathway activation is triggered by local ligand overexpression, or amplification and/or activating mutations in the RTKs, including VEGF receptors (VEGFR) in melanoma (8), PDGF receptors (PDGFR) in gliomas (9), and (potentially) KIT in small-cell lung carcinoma (10, 11). Mutations of split kinase domain RTKs, such as internal tandem duplications in the juxtamembrane region of FLT3 in acute myelogenous leukemia (AML; ref. 12), activating mutations in FLT3 and KIT in AML (12, 13), and activating translocations of PDGFR-β in chronic myelomonocytic leukemia (14), are particularly significant in driving cell growth in hematologic malignancies (3).

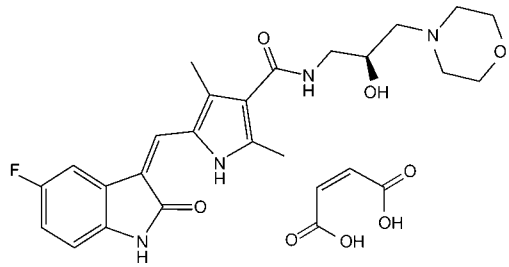
Split kinase domain RTKs and their ligands also play a central role in tumor neoangiogenesis. VEGFRs promote endothelial cell proliferation, survival, migration/invasion, and differentiation leading to angiogenesis (15). PDGFR-β mediates proliferation and survival of smooth muscle cells and pericytes that support tumor neovasculature, and of fibroblasts in the tumor stroma, which supply growth factors to endothelial and cancer cells (3). Simultaneous inhibition of both targets is expected to suppress tumor neoangiogenesis to a greater extent than inhibition of either target alone, and this indeed seems to be the case (16, 17). In addition, use of such a multitargeted strategy might delay or prevent the emergence of resistance to therapy (3).

We report results of preclinical studies for SU14813, a potent multitargeted RTK inhibitor that selectively inhibits multiple split kinase domain RTKs, including VEGFRs, PDGFR-α and PDGFR-β, KIT, and FLT3. SU14813 is similar in terms of structure and target profile to sunitinib (SU11248; SUTENT; Pfizer, Inc., New York, NY; refs. 18, 19), which has shown broad antitumor activity in preclinical studies and has shown clinical activity in patients with gastrointestinal stromal tumor (20, 21), metastatic renal cell carcinoma (22), and breast carcinoma (23). Sunitinib was recently approved by the Food and Drug Administration for the treatment of advanced renal cell carcinoma and for the treatment of gastrointestinal stromal tumor after disease progression on, or intolerance to, imatinib mesylate therapy (24). These results further validate the use of active multitargeted inhibitors, such as SU14813, for the treatment of a broad number of malignancies.

Materials and Methods

Compound

SU14813 (Fig. 1) was synthesized at SUGEN, Inc. (South San Francisco, CA). It has the molecular formula $C_{23}H_{27}FN_4O_4$ (free base) or $C_{23}H_{27}FN_4O_4{\cdot}C_4H_4O_4$ (salt), and its chemical name is 5-[(*Z*)-(5-fluoro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)methyl]-*N*-[(2*S*)-2-hydroxy-3-morpholin-4-ylpropyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxamide maleate.



5-[(Z)-(5-fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl]-N-[(2S)-2-hydroxy-3-morpholin-4-ylpropyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide maleate

Figure 1. Chemical structure of SU14813 maleate.

In vitro Studies

Colo205 (human colon carcinoma), HT29 (human colon carcinoma), A375 (human melanoma), SF767T (human glioma), and C6 (rat glioma) cells were obtained and cultured as previously described (25, 26). MV522 (human colon carcinoma), Lewis lung carcinoma (LLC), 786-O (renal cell carcinoma), U118MG (human glioblastoma) cells, and NIH-3T3 cells were obtained from and cultured as recommended by the American Type Culture Collection (Manassas, VA). RS4;11 and MV4;11 (human leukemia) cells were obtained from American Type Culture Collection, and propagated as described by O'Farrell et al. (19). OC1-AML5 (human AML) cells were obtained and cultured as previously described (19). Human umbilical vein endothelial cells were purchased from Clonetics (San Diego, CA).

Biochemical Kinase Assays. The method for obtaining biochemical IC_{50} values for SU14813 against its RTK targets, as well as other selected kinases, was determined as described previously (27, 28) using glutathione *S*-transferase fusion proteins containing the complete cytoplasmic domains of RTKs.

Cellular Receptor Phosphorylation Assays. Cellular assays to directly determine the ability of SU14813 to inhibit ligand-dependent and autonomous RTK phosphorylation, cell proliferation, or mitogenic responses were done as described previously (10, 19, 27–29). Porcine aortic endothelial cells overexpressing full-length human VEGFR-2, PDGFR-β, and KIT were generated as described in a previous report (30). NIH-3T3 cells overexpressing murine VEGFR-2 and PDGFR-α were generated as described previously (18). Receptor phosphorylation measurements by ELISA were done according to a previously published protocol (31), with some modifications. An appropriate amount of transfected cells were seeded in 96-well plates in growth medium for 6 to 8 hours before the medium was replaced with serum-reduced medium [0.1% fetal bovine serum (FBS)] and the cells were incubated overnight at 37°C. Compound was diluted in 100% DMSO and added to the cells at a final DMSO concentration of 0.1% (v/v). After incubation at 37°C for 1 hour, the cells were stimulated with 50 to 100 ng/mL VEGF-A, 20 to 50 ng/mL PDGF-BB, 50 to 100 ng/mL PDGF-AA, or 80 ng/mL SCF (all from R&D Systems,

Minneapolis, MN), followed by an additional 5- to 10-minute incubation. The medium was removed and the cells were lysed with 80 μL lysis buffer (Cell Signaling Technology, Danvers, MA) for 8 minutes at room temperature. Cellular IC_{50} values were calculated by curve fitting using a four-variable analysis in an Excel-based statistical data-analyzing template.

Growth Factor–Stimulated Endothelial Cell Survival Assay. The effect of SU14813 on endothelial cell survival was evaluated. Passages 4 to 5 human umbilical vein endothelial cells were grown to subconfluency in EGM2 medium containing 10% FBS, endothelial cell growth supplement, and 10 μg/mL sodium heparin (Sigma Chemical, St. Louis, MO). The cells were seeded in 96-well plates at 10,000 per well in F12K medium and 10% FBS. The next day, cells were starved for 18 hours in F12K + 1% FBS and then incubated with SU14813 in various concentrations. Forty-five minutes later, 20 ng/mL growth factor [VEGF or basic fibroblast growth factor (bFGF)] was introduced into the assay. Three days later, cell numbers were determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (Promega, Madison, WI).

Anchorage-Independent and Anchorage-Dependent Growth Inhibition Assays. To assess inhibition of anchorage-independent growth, 24-well culture dish wells were coated with 0.5 mL bottom agar mixture (10% FBS, DMEM, 0.6% agar). After the bottom layer had solidified, 0.5 mL top agar mixture (10% FBS, DMEM, 0.3% agar) containing 1,500 per well was added into each well with 2× concentration of SU14813. Plates were incubated for 3 weeks and stained overnight at 37°C, 5% CO_2 in a solution of 1 μg/mL iodonitrotetrazolium salt (Sigma Chemical) in 50% ethanol. Colonies were visualized at ×400 magnification, manually counted, and the average number of colonies per well was calculated.

To assess inhibition of anchorage-dependent growth, cells were grown in the presence of 10% FBS and DMEM. SU14813 was added, and the cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 2 days. Total cell protein was determined by staining cells with sulforhodamine B (Sigma Chemical), and IC_{50} values were determined for quadruplicate wells as described previously (32).

In vivo Efficacy Studies

Animals. Female *nu/nu* mice (8–12 weeks old, weighing 25 g) were obtained from Charles River Laboratories (Wilmington, MA) or Harlan (Indianapolis, IN). Animals were housed under specific pathogen-free conditions in Lab Products (Seaford, DE) ventilated cages in barrier rooms. Animals received sterile rodent chow or Lab Diet 5061 (PMI Nutrition International, St. Louis, MO), and water was available *ad libitum* via the Edstrom automatic system. Animal experiments were done in observance with SUGEN Institutional Animal Care and Use Committee guidelines in an animal facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, or in compliance with Pfizer Institutional Animal Care and Use Committee guidelines in the Laboratory Animal Research Center of Pfizer Global Research & Development (La Jolla, CA). All studies were done in accordance with the Institute of Laboratory Animal Research (NIH, Bethesda, MD) Guide for the Care and Use of Laboratory Animals (33).

***In vivo* Antitumor Efficacy Studies in s.c. Xenograft Models.** SU14813 was evaluated for its efficacy in s.c. tumor xenograft models. Before implantation, tumor cells were harvested during exponential growth and washed once with sterile phosphate-buffered saline. For some models, 50% Basement Matrigel Matrix (BD Biosciences, Bedford, MA) was added to tumor cells to aid initial tumor formation. In general, 2×10^6 to 6×10^6 tumor cells were implanted s.c. into the hind flank region of mice on day 0, and tumors were allowed to attain a size of 100 to 350 mm^3 before institution of treatment with SU14813, formulated as a carboxymethyl cellulose–based suspension and administered via gavage at the indicated doses and regimens. Tumor growth was evaluated by twice-weekly measurement of tumor volume. Studies were typically terminated when tumors in vehicle-treated animals reached an average size of 1,500 mm^3, or when the tumors were judged to adversely affect the well-being of the animals. Because tumors were established and often large at the time treatment was initiated, percentage of inhibition of tumor growth after initiation of treatment with SU14813 was calculated as $100 \times (1 - [(\text{tumor volume}_{\text{final}} - \text{tumor volume}_{\text{initial}}$ for the compound-treated group) / (tumor $\text{volume}_{\text{final}}$ − tumor $\text{volume}_{\text{initial}}$ for the vehicle-treated group)]). Where applicable, percent tumor regression for each group was calculated as $100 \times$ (tumor $\text{volume}_{\text{initial}}$ − tumor $\text{volume}_{\text{final}}$) / (tumor $\text{volume}_{\text{initial}}$).

***In vivo* Antitumor Efficacy in a Bone Marrow–Engrafted AML Model.** SU14813 efficacy was evaluated using a survival model generated by engraftment of MV4;11 human AML cells into the bone marrow of cyclophosphamide-pretreated nonobese diabetic/severe combined immunodeficient mice, as described previously (19).

***In vivo* Combination Therapy Antitumor Efficacy Studies.** SU14813 was evaluated for its efficacy and synergism in combination with the microtubule inhibitor docetaxel in docetaxel-resistant murine LLC model. SU14813 was administered p.o. twice daily (BID) at doses of 10, 40, 80, or 120 mg/kg beginning on day 5 after tumor implantation. Docetaxel 40 mg/kg (mouse maximum tolerated dose) was administered i.v. thrice weekly also beginning on day 5 after tumor implantation. Responses were assessed by tumor growth inhibition, evaluated on day 21, the last day of dosing.

***In vivo* Target Modulation Studies.** *In vivo* target modulation studies to determine the effect of SU14813 on the phosphorylation of target RTKs expressed on cancer cells were done as described previously (18, 19). Mouse plasma samples were stored at −80°C until they could be analyzed for drug concentration using liquid chromatography with tandem mass spectrometric detection, as described by Mendel et al. (18).

Results

In vitro Studies

Biochemical Kinase Assays. SU14813 was developed as part of a program targeting multiple split kinase domain

Table 1. ***In vitro*** **enzymatic inhibitory activity and cellular target activity of SU14813**

	Target RTKs (biochemical IC_{50}, μmol/L)				Nontarget tyrosine kinases (biochemical IC_{50}, μmol/L)			
	VEGFR-2	VEGFR-1	PDGFR-β	KIT	FGFR-1	EGFR	Src	cMet
SU14813	0.05	0.002	0.004	0.015	3.5	>20	2.5	9

	Cell target phosphorylation assay (cellular IC_{50}, μmol/L)					Ligand-dependent proliferation (cellular IC_{50}, μmol/L)			Ligand-independent proliferation (cellular IC_{50}, μmol/L)
	VEGFR-2	PDGFR-β	KIT	FLT3-ITD	c-fms	PDGF-BB	FL	EGF	FLT3-ITD
SU14813	0.04	0.02	0.006	0.05	0.05	0.03	0.07	46	0.02

Abbreviations: FGFR-1, FGF receptor 1; FL, FLT3 ligand; EGF, epidermal growth factor.

RTKs at SUGEN, Inc. SU14813 inhibits kinase activity of VEGFR-1 and VEGFR-2, PDGFRs, KIT, FLT3, and CSF1R/FMS. In biochemical assays, SU14813 inhibited these RTK targets with IC_{50} values ranging from 0.002 to 0.05 μmol/L (Table 1). SU14813 was tested against a total of 38 kinases in biochemical assays and was found to exhibit a high degree of selectivity for its target RTKs. Nontarget tyrosine kinases exhibited IC_{50} values in the range of ~1 to >200 μmol/L, so selectivity was generally in the range of ~100- to 10,000-fold for target RTKs over other kinases evaluated (selected kinase cross-selectivity data for FGF receptor 1, EGFR, Src, and cMet are included in Table 1). In addition, SU14813 did not inhibit enzymatic activity or ligand binding of a panel of 23 nonkinase enzymes and receptors (data not shown).

Cellular Assays. SU14813 inhibited cellular ligand-dependent phosphorylation of VEGFR-2 (transfected NIH 3T3 cells), PDGFR-β (transfected NIH 3T3 cells), KIT (Mo7e cells), and FLT3-internal tandem duplication (FLT3-ITD; MV4;11 cells) as well as FMS/CSF1R (transfected NIH 3T3 cells), another split-kinase RTK (Table 1). Furthermore, SU14813 inhibited VEGFR-2, PDGFR-β, and KIT phosphorylation in porcine aorta endothelial cells overexpressing these targets, with cellular IC_{50} values of 5.2, 9.9, and 11.2 nmol/L, respectively. Consistent with its cellular RTK inhibitory activities, SU14813 inhibited PDGF-dependent proliferation of NIH-3T3 cells overexpressing PDGFR-β, FLT3 ligand–dependent proliferation of OC1-AML5 human AML cells expressing wild-type FLT3, and autonomous proliferation of MV4;11 human AML cells expressing constitutively active mutant FLT3-ITD (Table 1). In contrast, SU14813 did not potently inhibit epidermal growth factor–dependent DNA synthesis in NIH 3T3 cells overexpressing EGFR, a nontarget RTK, verifying that its effects are RTK target specific (Table 1).

Growth Factor–Stimulated Endothelial Cell Survival Assay. At the cellular function level, SU14813 showed potent activity against VEGF-stimulated but not bFGF-stimulated human umbilical vein endothelial cell survival (Fig. 2). The selectivity window between the two receptors was >270-fold, consistent with the biochemical data demonstrating that SU14813 only weakly inhibited FGF receptor 1 activity.

Anchorage-Independent and Anchorage-Dependent Growth Inhibition Assays. Anchorage-independent growth is a hallmark of transformation and is mediated via autocrine growth factor production (34). Human glioblastoma U-118MG cells exhibit elevated basal PDGFR-β phosphorylation in culture (presumably reflecting autocrine PDGF production), which is highly responsive to SU14813 treatment (data not shown). In the soft agar assay (anchorage-independent growth), SU14813 inhibited the growth of U-118MG with an IC_{50} of 50 to 100 nmol/L. Conversely, SU14813 had no effect on growth in soft agar of HT-29 human colon adenocarcinoma cells (IC_{50} > 1,500

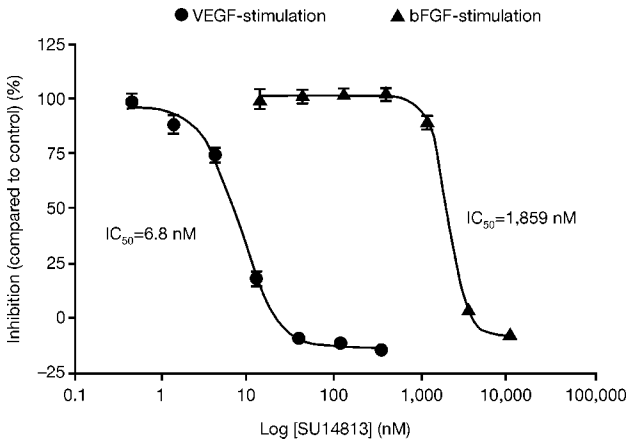


Figure 2. SU14813 inhibited VEGF-, but not bFGF-stimulated human umbilical vein endothelial cell survival. Subconfluent human umbilical vein endothelial cells were starved in 1% FBS overnight and treated with SU14813 over a range of concentrations. Cells were incubated in the presence of 20 ng/mL of either VEGF (●)or bFGF (▲) for 3 d. Cell numbers were determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (see Materials and Methods for experimental details). The IC_{50} values were obtained from curves generated by a nonlinear regression curve fit. *Points,* percentage inhibition; *bars,* SD.

Table 2. SU14813 is efficacious in s.c. tumor xenograft models

Cell line	Initial tumor volume (mm^3)	Dose (mg/kg)	Percent inhibition, % (days dosing)	Percent regression, % (days dosing)	*P*
MV4;11 (human AML, FLT3-ITD)	350	10 BID	64 (18)		<0.001
		20 BID		68 (25)	<0.001
		40 BID		89 (32)	<0.001
786-O (human renal, VHL mut)	300	20 BID		35 (9)	0.02
		40 BID		50.5 (9)	<0.001
		80 QD		46.5 (9)	<0.001
C6 (rat gliomas, PDGFR-β)	300	40 BID	67 (14)		<0.001
		80 BID	70 (14)		<0.001
C6 (rat glioma)	100	20 BID	57 (17)		<0.001
		40 BID	64 (17)		<0.001
C6 (rat glioma)	150	10 BID	13 (16)		0.4
		20 QD	21 (16)		0.08
		20 BID	29 (16)		0.015
		40 QD	26 (16)		0.05
		40 BID	50 (16)		<0.001
		80 QD	50 (16)		<0.001
Colo205 (human colon, EGFR)	300	40 BID	43 (10)		0.008
		80 BID	54 (10)		0.001
MV522 (human colon)	150–200	3 BID	20 (15)		0.30
		10 BID	23 (15)		0.24
		20 BID	30 (15)		0.12
		40 BID	71 (15)		<0.0001
		80 BID	80 (15)		<0.0001

NOTE: Percentage growth inhibition compared with vehicle-treated control groups is indicated in all cases, except where tumor regression was seen. In this case, maximum percentage regression relative to starting size is indicated. Statistical analyses were done using the two-tailed Student's *t* test. $n = 10$ to 20 animals for vehicle groups, $n = 8$ to 10 animals for treatment groups.

nmol/L), which express high levels of EGFR, but which do not express detectable levels of type III and IV RTKs. In addition, SU14813 had no effect on U-118MG cells grown on plastic (anchorage-dependent growth; $IC_{50} > 12{,}000$ nmol/L), indicating that the soft agar result does not reflect general cytotoxicity of SU14813 toward these cells. These data indicate that SU14813 can directly and selectively inhibit tumor cells expressing, and dependent upon, its target RTKs.

Overall, there is general agreement between the biochemical and cellular RTK activities of SU14813, supporting the conclusion that the compound crosses cellular membranes, and that the cellular responses are a result of the activity of SU14813 against its targets.

Pharmacokinetic Studies

Pharmacokinetic properties of SU14813 were studied in mice, the species used in several nonclinical *in vivo* efficacy models. SU14813 showed moderate systemic clearance (46 mL/min/kg) corresponding to 50% of hepatic blood flow in mice, and moderate volume of distribution (1.5 L/kg) was observed, suggesting good distribution into tissues. The plasma half-life ($t_{1/2}$) was relatively short (1.8 hours) in mice compared with other nonrodent species (data not shown), suggesting a BID dosing regimen for antitumor efficacy studies. Linear pharmacokinetics were observed in mice, with rapid absorption and good exposure after p.o. administration (30–50 mg/kg). Oral bioavailability was ~40%. Plasma protein binding of SU14813 in mice was similar to that in humans, with an unbound fraction ranging from 4% to 6%.[11]

In vivo Efficacy Studies

***In vivo* Antitumor Efficacy Studies.** In xenograft models using human and rat tumor cells implanted in athymic or nonobese diabetic/severe combined immunodeficient mice, SU14813 exhibited dose-dependent antitumor efficacy, resulting in regression of 786-O (PDGFR, VEGFR-expressing) and MV4;11 (activated FLT3-dependent) tumors, growth arrest of Colo205 tumors, and growth delay of C6 (PDGFR-expressing) and MV522 tumors (Table 2). Representative study results using C6, MV4;11, and 786-O models are shown in Fig. 3.

These data indicate that SU14813 is efficacious against a tumor driven by activated mutant FLT3-ITD (MV4;11 AML tumors). Moreover, SU14813 is efficacious in the renal carcinoma cell line 786-O, which harbors a loss-of-function mutation in the von Hippel-Lindau tumor suppressor gene (35), thought to cause dysregulated production of VEGF and PDGF (36). Furthermore, SU14813 is also efficacious against a tumor expressing an activated wild-type target RTK (PDGFR-β in C6 rat glioma tumor cells), and against tumor types (Colo205 and MV522) that do not express active RTK targets of SU14813.[12] In Colo205 and MV522 tumors, the compound most likely targets VEGFRs and/or PDGFRs residing mainly/exclusively in the tumor vasculature and stroma.

[11] Pfizer, Inc., data on file, New York, NY, 2006, unpublished data.
[12] D. Hu-Lowe, in preparation.

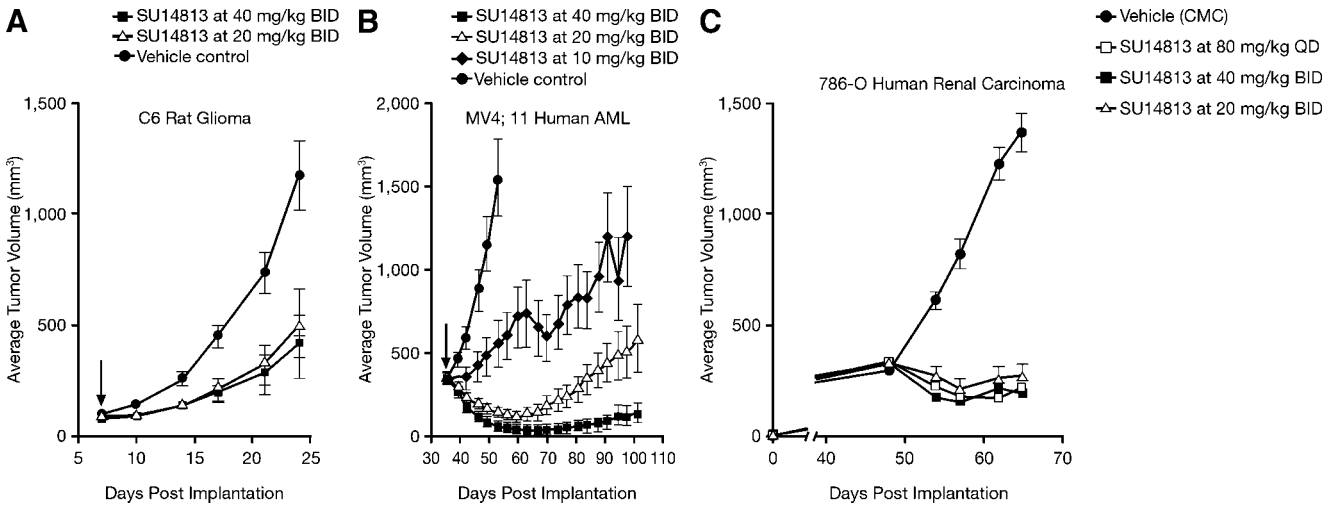


Figure 3. SU14813 treatment is efficacious in the C6 rat glioma (**A**), MV4;11 human AML (**B**) and 786-O human renal carcinoma (**C**) s.c. xenograft tumor models. Cancer cells were cultured, harvested, and implanted s.c. into athymic mice, as described in Materials and Methods. SU14813 treatment was initiated at the indicated doses and regimens. *Points,* mean tumor volume; *bars,* SE. *Arrows,* start of treatment. $n = 10$ animals for all groups except 786-O vehicle, where $n = 20$.

SU14813 was evaluated in three C6 tumor efficacy studies (Table 2). The first two studies explored efficacy against small (100 mm^3) and medium-sized (300 mm^3) established tumors. The third study was designed to more thoroughly explore the consequences of varying the dosing regimen in this model [once daily (QD) versus BID]. In these experiments, the fully efficacious dose was 40 mg/kg BID; 20 mg/kg BID was still efficacious and performed comparably to 40 mg/kg BID in some studies; and 80 mg/kg QD exhibited efficacy comparable with 40 mg/kg BID. In general, considering all the disease models, SU14813 at 40 mg/kg BID seemed to be sufficient to produce full antitumor efficacy (Table 2).

SU14813 was also evaluated in a physiologically relevant leukemia model in mice, in which MV4;11 human AML cells engraft and proliferate in the bone marrow. In this model, mice were euthanized at the appearance of hind limb paralysis stemming from disease progression. Treatment with SU14813 (10 or 20 mg/kg BID) was initiated 30 days following injection of MV4;11 cells. SU14813 treatment conferred an approximate doubling of survival time from 23 to 42 days at these dose levels (Table 3).

***In vivo* Combination Therapy Antitumor Efficacy Studies.** Targeted agents such as SU14813 have the potential to combine with conventional chemotherapeutic agents in an additive or synergistic fashion, based on their distinct mechanisms of action. This possibility was tested for SU14813 in combination with docetaxel in the murine LLC model. The LLC model is resistant to docetaxel treatment. SU14813 administered as a single agent at 10, 40, 80, and 120 mg/kg BID inhibited 25%, 48%, 55%, and 63% of tumor growth, respectively, compared with the control group. Docetaxel given alone at the mouse maximum tolerated dose (40 mg/kg) inhibited 21% of tumor growth. When SU14813 was combined with docetaxel, tumor growth inhibition was enhanced to 38%, 65%, 77%, and 76%, respectively (Fig. 4). Thus, SU14813 dose-dependently inhibited LLC tumor growth in mice, and tumor growth inhibition seemed to have been enhanced when SU14813 was combined with docetaxel. SU14813 was well tolerated as a single agent up to a dose of 40 mg/kg BID, as indicated by minimal body weight loss, whereas treatment with docetaxel resulted in an 8% average body weight loss. When the two agents were administered in combination, body weight loss increased to 13% to 17% for all groups independent of the dose levels of SU14813. The enhanced body weight loss is likely to be primarily due to docetaxel treatment.

***In vivo* Target Modulation Studies.** To show *in vivo* target inhibition, a series of phosphorylation experiments was conducted using tumor tissues from several mouse xenograft models that express either VEGFR-2 (A375 human melanoma), FLT3-ITD (MV4;11 human acute myeloleukemia), or PDGFR-β (SF767T human glioma). Administration

Table 3. SU14813 is efficacious in a bone marrow myeloproliferative disease model

Treatment	Dose (mg/kg)	Time to progression (d ± SE)	*P* vs vehicle
Vehicle	—	23.3 ± 2.6	—
SU14813	10 BID	41.6 ± 4.4	0.006
SU14813	20 BID	42.7 ± 4.2	0.004

NOTE: MV4;11 human AML cells were infused i.v. into cyclophosphamide-pretreated nonobese diabetic/severe combined immunodeficient mice. Thirty days later, p.o. administration of SU14813 at the indicated dosages and regimens was initiated. Average time to progression (as assessed by the appearance of hind limb paralysis) is indicated. Statistical analyses were done using the two-tailed Student's *t* test. $n = 15$ animals per group. —, not applicable.

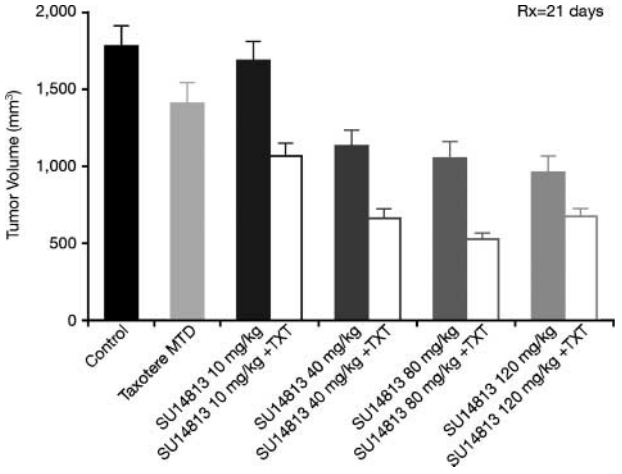


Figure 4. Antitumor efficacy of SU14813 and docetaxel administered alone and in combination in the LLC model. SU14813 was administered p.o., BID, starting on day 5 after tumor implant and randomization. Tumor growth inhibition was assessed after 21 d of treatment. SU14813 treatment dosages are indicated above. Docetaxel (Taxotere, *TXT*) was dosed at 40 mg/kg (mouse maximum tolerated dose) thrice weekly, i.v., and the first dose was given on day 5. Twelve animals were used in each group. *Columns,* tumor volume; *bars,* SE.

of a single p.o. dose of SU14813 to mice bearing these tumors produced substantial inhibition of VEGFR-2, FLT3-ITD, and PDGFR-β phosphorylation, respectively, compared with the amount of receptor phosphorylation in tumors in untreated or vehicle-treated mice (Fig. 5). Target inhibition was also dose dependent (Fig. 5, *center*) and the duration of target inhibition by SU14813 increased with dose (data not shown). In these and similar studies, substantial inhibition of VEGFR-2, FLT3-ITD, and PDGFR-β phosphorylation in tumors occurred when the total plasma concentration (free plus protein-bound) of SU14813 was in the range of 100 to 200 ng/mL (data not shown). Thus, based on the results of these *in vivo* target modulation studies, 100 to 200 ng/mL has been identified as the target plasma concentration range required to achieve inhibition of target RTKs in mice. Analyses of SU14813 plasma concentration/time curves generated from a mouse acute dose pharmacokinetic study or from the end of repeat-dose efficacy studies indicated that plasma concentrations of SU14813 persist at or above 100 to 200 ng/mL for ~12 h/d at the fully efficacious dose of 40 mg/kg BID (Fig. 6). Based on plasma protein binding measurements, the free concentration of SU14813 in blood at the 100 to 200 ng/mL target plasma concentration range would be in the single-digit nanomolar range, which is similar to the *in vitro* cellular IC_{50} values reported in Table 1. Collectively, these data established a target plasma concentration range important for translation to clinical studies.

Discussion

The lack of specificity, limited efficacy, and extensive adverse side effects of cytotoxic chemotherapy have led to the rational design and development of cancer therapies targeting molecular pathways responsible for tumor growth and survival. However, with the exception of a few malignancies that seem to be driven by a single genetic mutation in a gene encoding a signaling protein, most tumors are the product of multiple mutations in multiple aberrant signaling pathways (37). Thus, antitumor efficacy of a single-target agent may be limited. Compounds with broader target specificity may not only be more effective than agents with narrower selectivity, they may also decrease the likelihood of development of resistance. This concept has been shown both in preclinical and clinical studies using sunitinib (SU11248), a small-molecule inhibitor closely related to SU14813 (16, 20, 22, 23). In this article, we describe a new indolinone compound, SU14813, selected from the same chemical library used to identify sunitinib (38). Like sunitinib, SU14813 was also designed to directly target both tumor survival and proliferation of tumors expressing VEGFRs, PDGFRs, KIT, or FLT3, as well as to inhibit angiogenesis (via inhibition of VEGFRs and PDGFR-β expressed on endothelial cells and pericytes, respectively). We report that SU14813 has potent and selective *in vitro* biochemical and cellular activity. SU14813 effectively inhibited VEGFR-2, FLT3-ITD, and PDGFR-β phosphorylation and biological activity *in vivo* with a target plasma concentration of 100 to 200 ng/mL, and exhibited strong and broad efficacy in multiple xenograft tumor models. When SU14813 was

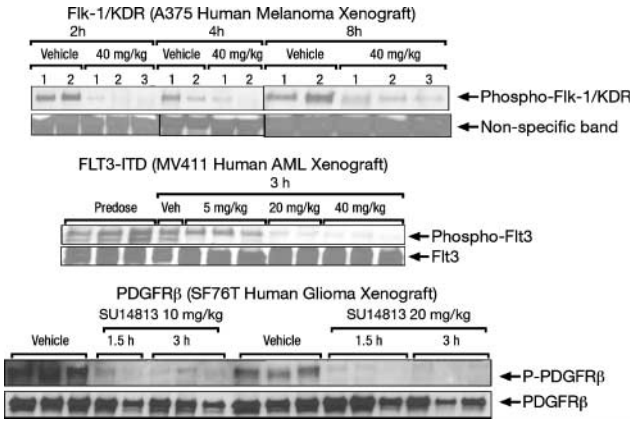


Figure 5. SU14813 inhibits VEGFR-2, FLT3-ITD, and PDGFR-β phosphorylation in human xenograft tumors. Mice with established (300 – 400 mm^3) xenograft tumors were treated with SU14813 or vehicle as indicated, euthanized at the indicated time points after treatment, and their tumors were harvested. *Top,* A375 human melanoma tumors were homogenized and lysed, and phosphotyrosine-containing proteins were immunoprecipitated and immunoblotted using an antibody recognizing total VEGFR-2 as previously described (18). A nonspecific band that was not regulated by SU14813 is included for comparison. *Middle,* MV4;11 human AML tumors were homogenized and lysed, and total FLT3-ITD was immunoprecipitated and immunoblotted for phosphotyrosine content as previously described (19). The blot was subsequently stripped and reprobed for total FLT3-ITD. *Bottom,* SF767T human glioma tumors were homogenized and lysed, and total PDGFR-β was immunoprecipitated and immunoblotted for phosphotyrosine (*P-PDGFR-β*), then stripped and reprobed for total PDGFRβ as previously described (18). Each lane represents results from an independent animal.

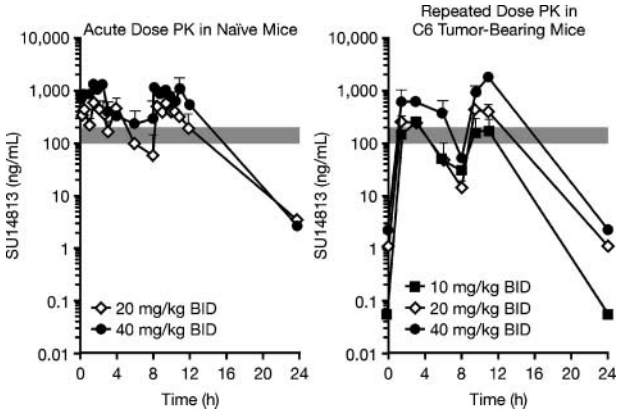


Figure 6. Plasma concentration-time profile of SU14813 in mice. *Left,* pharmacokinetic profile in naïve nude mice dosed with SU14813 (single day, p.o., BID, 8 h apart, $n = 4$ mice per time point). *Right,* pharmacokinetic profile of SU14813 after repeated dosing in C6 rat glioma tumor-bearing athymic mice (17 d dosing, p.o., BID, $n = 2-3$ mice per time point). In this study, 20 and 40 mg/kg SU14813 produced similar tumor growth inhibition of 57% and 64%, respectively. Mouse plasma samples were stored at −80°C until they could be analyzed for drug concentration using liquid chromatography with tandem mass spectrometric detection. *Shaded band,* range of *in vivo* C_{eff}, 100 – 200 ng/mL, derived from pharmacokinetic/pharmacodynamic studies.

administered in combination with docetaxel chemotherapy, tumor growth inhibition was enhanced compared with inhibition produced by SU14813 or docetaxel administered alone.

The role of KIT in the regulation of hair pigmentation is well documented. Depigmentation of hair can serve as a dose-dependent, dynamic, biological readout of KIT inhibition that can be tested *in vivo* (39). In addition to its observed activity in tumor models, SU14813, administered p.o. in repeat doses, was active against wild-type KIT, as assessed by its ability to inhibit pigmentation of newly regrown hair in depilated black C57BL/6 mice; dose-dependent partial inhibition was shown at the highest dose evaluated, 80 mg/kg BID (data not shown).

Results of preclinical studies with sunitinib and SU14813 are similar and encouraging. Both agents exhibited broad and potent antitumor activity, due presumably to their broad target profiles, which include target RTKs that drive tumor cell growth plus endothelium RTKs that mediate tumor neoangiogenesis. Like sunitinib, agents such as SU14813 that target and inhibit multiple RTKs are expected to generate enhanced antitumor efficacy and reduced resistance to treatment when used as single agents, especially against tumor types with complex signaling pathways and cellular heterogeneity (40).

Additionally, SU14813 exhibited enhanced preclinical efficacy when administered with cytotoxic chemotherapy, i.e., in a docetaxel-resistant preclinical model. Several single-receptor RTK inhibitors have been combined with standard cytotoxic chemotherapy in preclinical studies; results have suggested additive to synergistic antitumor effects of the combination compared with either agent given as monotherapy (41). In addition, bevacizumab, a monoclonal antibody directed against VEGFA, showed promising clinical outcome recently in colorectal cancer when combined with cytotoxic agents (42).

Small-molecule inhibitors of multiple RTKs implicated in tumor growth and survival, such as SU14813 or sunitinib, which has shown potent single-agent antitumor efficacy, may successfully augment treatment with conventional cytotoxic chemotherapeutic agents. In addition, there may be specific attributes of SU14813 that are advantageous compared with sunitinib, such as a shorter plasma kinetics and a lack of major detectable circulating metabolites. SU14813 exhibited moderate clearance comparable with sunitinib in preclinical species, but had a relatively shorter half-life than sunitinib. Although the maximum plasma concentrations of SU14813 and sunitinib were similar following a 40 mg/kg p.o. dose to mice, BID doses for SU14813 are required to achieve plasma concentrations at or above the target concentration of 100 to 200 ng/mL for ~12 hours (Fig. 6), whereas a single daily dose of sunitinib was sufficient to achieve plasma concentrations above the target concentration (50–100 ng/mL) for 12 hours (18). The half-life of SU14813 in rats and monkeys enabled QD dosing in repeated-dose nonclinical safety studies, without significant drug accumulation after repeated p.o. dosing (data not shown). In addition, in multiple preclinical species, SU14813 exhibited linear pharmacokinetics with no active metabolites (data not shown). These pharmacokinetic characteristics of SU14813 observed across preclinical species, coupled with potent pharmacodynamic activity and antitumor efficacy in mice, constituted the initial rationale for developing this compound to capitalize and expand upon the success of sunitinib.

In a recent phase I, dose-escalation study of several tumor types ($n = 49$ patients), Fiedler et al. (43) showed that QD doses of SU14813 at 100 to 150 mg produced persistent plasma levels at or above the target value of 100 to 200 ng/mL, resulting in objective tumor responses [one patient with complete response (renal cell carcinoma) and seven patients with partial responses (biliary cancer, thyroid cancer, renal cell carcinoma, soft tissue sarcoma, thymus cancer, colon carcinoma, and non–small cell lung carcinoma)] and prolonged disease stabilization (nine patients on therapy for at least five cycles). This study also showed that SU14813 exhibits the moderate pharmacokinetic properties predicted from preclinical data that will likely enable patients to receive continuous daily doses by allowing plasma concentrations to reach their target values, but, subsequently, rapidly decrease within a 24-hour period. These findings are consistent with a conclusion reached by Mendel et al. (18) in a key *in vivo* pharmacokinetic/pharmacodynamic study of sunitinib that complete and continuous (24 hours) inhibition may not be required to achieve complete or near-complete efficacy for agents that target VEGF and PDGF receptor pathways. Specifically, it was observed that, at an efficacious dose, phosphorylation of VEGFR and PDGFR was substantially inhibited for at least 12 hours, but recovered by the 24-hour time point, before the next dosing, confirming previous findings for another RTK inhibitor, SU6668 (25), which targets VEGFR, PDGFR, and FGF receptors.

In conclusion, given the encouraging preclinical data reported here for SU14813 and the clinical implications for its use, and based on a similar potency to sunitinib but distinct pharmacokinetic profile, which may favorably effect tolerability and allow greater dosing flexibility, SU14813 warrants further clinical evaluation in human malignancies, both alone and in combination with cytotoxic chemotherapy.

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References

1. Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell 2000; 103:211 – 25.

2. Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. MET, metastasis, motility and more. Nat Rev Mol Cell Biol 2003;4:915 – 25.

3. Laird AD, Cherrington JM. Small molecule tyrosine kinase inhibitors: clinical development of anticancer agents. Expert Opin Investig Drugs 2003;12:51 – 64.

4. Druker BJ. Imatinib as a paradigm of targeted therapies. Adv Cancer Res 2004;91:1 – 30.

5. Herbst RS, Fukuoka M, Baselga J. Gefitinib—a novel targeted approach to treating cancer. Nat Rev Cancer 2004;4:956 – 65.

6. Slamon D, Pegram M. Rationale for trastuzumab (Herceptin) in adjuvant breast cancer trials. Semin Oncol 2001;28:13 – 9.

7. Muhsin M, Graham J, Kirkpatrick P. Bevacizumab. Nat Rev Drug Discov 2004;3:995 – 6.

8. Masood R, Cai J, Zheng T, Smith DL, Hinton DR, Gill PS. Vascular endothelial growth factor (VEGF) is an autocrine growth factor for VEGF receptor-positive human tumors. Blood 2001;98:1904 – 13.

9. Lokker N, Sullivan CM, Hollenbach SJ, Israel MA, Giese NA. Platelet-derived growth factor (PDGF) autocrine signaling regulates survival and mitogenic pathways in glioblastoma cells: evidence that the novel PDGF-C and PDGF-D ligands may play a role in the development of brain tumors. Cancer Res 2002;62:3729 – 35.

10. Abrams TJ, Lee LB, Murray LJ, Pryer NK, Cherrington JM. SU11248 inhibits KIT and platelet-derived growth factor receptor β in preclinical models of human small cell lung cancer. Mol Cancer Ther 2003;2:471 – 8.

11. Tamborini E, Bonadiman L, Negri T, et al. Detection of overexpressed and phosphorylated wild-type kit receptor in surgical specimens of small cell lung cancer. Clin Cancer Res 2004;10:8214 – 9.

12. Gilliland DG, Griffin JD. Role of FLT3 in leukemia. Curr Opin Hematol 2002;9:274 – 81.

13. Beghini A, Larizza L, Cairoli R, Morra E. c-kit activating mutations and mast cell proliferation in human leukemia. Blood 1998;92:701 – 2.

14. Reilly JT. Class III receptor tyrosine kinases: role in leukaemogenesis. Br J Haematol 2002;116:744 – 57.

15. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med 2003;9:669 – 76.

16. Bergers G, Song S, Meyer-Morse N, Bergsland E, Hanahan D. Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. J Clin Invest 2003;111:1287 – 95.

17. Potapova O, Laird AD, Nannini MA, et al. Contribution of individual targets to the antitumor efficacy of the multitargeted receptor tyrosine kinase inhibitor SU11248. Mol Cancer Ther 2006;5:1280 – 9.

18. Mendel DB, Laird AD, Xin X, et al. *In vivo* antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. Clin Cancer Res 2003;9:327 – 37.

19. O'Farrell AM, Abrams TJ, Yuen HA, et al. SU11248 is a novel FLT3 tyrosine kinase inhibitor with potent activity *in vitro* and *in vivo*. Blood 2003;101:3597 – 605.

20. Demetri GD, Desai J, Fletcher JA, et al. SU11248, a multi-targeted tyrosine kinase inhibitor, can overcome imatinib (IM) resistance caused by diverse genomic mechanisms in patients (pts) with metastatic gastrointestinal stromal tumor (GIST) [abstract 3001]. J Clin Oncol 2004;22:3001.

21. Maki RG, Fletcher JA, Heinrich MC, et al. Results from a continuation trial of SU11248 in patients (pts) with imatinib (IM)-resistant gastrointestinal stromal tumor (GIST). American Society of Clinical Oncology Meeting, Orlando Florida; 2005. Abstract 9011.

22. Motzer RJ, Rini BI, Michaelson MD, et al. Phase 2 trials of SU11248 show antitumor activity in second-line therapy for patients with metastatic renal cell carcinoma (RCC). American Society of Clinical Oncology Meeting, Orlando Florida; 2005. Abstract 4508.

23. Miller KD, Burstein HJ, Elias AD, et al. Phase II study of SU11248, a multitargeted receptor tyrosine kinase inhibitor (TKI), in patients (pts) with previously treated metastatic breast cancer (MBC). American Society of Clinical Oncology Meeting, Orlando Florida; 2005. Abstract 563.

24. SUTENT (sunitinib malate) prescribing information. New York (NY): Pfizer. Inc.; 2006.

25. Laird AD, Christensen JG, Li G, et al. SU6668 inhibits Flk-1/KDR and PDGFRβ *in vivo*, resulting in rapid apoptosis of tumor vasculature and tumor regression in mice. FASEB J 2002;16:681 – 90.

26. Mendel DB, Laird AD, Smolich B, et al. Development of SU5416, a selective small molecule inhibitor of VEGF receptor tyrosine kinase activity, as an anti-angiogenesis agent. Anticancer Drug Des 2000;15:29 – 41.

27. Christensen JG, Schreck RE, Chan E, et al. High levels of HER-2 expression alter the ability of epidermal growth factor receptor (EGFR) family tyrosine kinase inhibitors to inhibit EGFR phosphorylation *in vivo*. Clin Cancer Res 2001;7:4230 – 8.

28. Laird AD, Vajkoczy P, Shawver LK, et al. SU6668 is a potent antiangiogenic and antitumor agent that induces regression of established tumors. Cancer Res 2000;60:4152 – 60.

29. Murray LJ, Abrams TJ, Long KR, et al. SU11248 inhibits tumor growth and CSF-1R-dependent osteolysis in an experimental breast cancer bone metastasis model. Clin Exp Metastasis 2003;20:757 – 66.

30. Waltenberger J, Claesson-Welsh L, Siegbahn A, Shibuya M, Heldin CH. Different signal transduction properties of KDR and Flt1, two receptors for vascular endothelial growth factor. J Biol Chem 1994;269:26988 – 95.

31. Roberts WG, Whalen PM, Soderstrom E, et al. Antiangiogenic and antitumor activity of a selective PDGFR tyrosone kinase inhibitor, CP-673,451. Cancer Res 2005;65:957 – 66.

32. Rubinstein LV, Shoemaker RH, Paull KD, et al. Comparison of *in vitro* anticancer-drug-screening data generated with a tetrazolium assay versus a protein assay against a diverse panel of human tumor cell lines. J Natl Cancer Inst (Bethesda) 1990;82:1113 – 8.

33. Institute of Laboratory Animal Resources Commission on Life Sciences. Guide for the care and use of laboratory animals. Washington (District of Columbia): National Academy Press; 1996.

34. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100: 57 – 70.

35. Iliopoulos O, Kibel A, Gray S, Kaelin WG, Jr. Tumour suppression by the human von Hippel-Lindau gene product. Nat Med 1995;1:822 – 6.

36. Kim WY, Kaelin WG. Role of *VHL* gene mutation in human cancer. J Clin Oncol 2004;22:4991 – 5004.

37. Kim JA. Targeted therapies for the treatment of cancer. Am J Surg 2003;186:264 – 8.

38. Sun L, Tran N, Liang C, et al. Identification of substituted 3-[(4,5,6,7-tetrahydro-1*H*-indol-2-yl)methylene]-1,3-dihydroindol-2-ones as growth factor receptor inhibitors for VEGF-R2 (Flk-1/KDR), FGF-R1, and PDGF-Rβ tyrosine kinases. J Med Chem 2000;43:2655 – 63.

39. Moss KG, Toner GC, Cherrington JM, Mendel DB, Laird AD. Hair depigmentation is a biological readout for pharmacological inhibition of KIT in mice and humans. J Pharmacol Exp Ther 2003;307:476 – 80.

40. Baselga J, Arribas J. Treating cancer's kinase ''addiction.'' Nat Med 2004;10:786 – 7.

41. Arteaga CL. The epidermal growth factor receptor: from mutant oncogene in nonhuman cancers to therapeutic target in human neoplasia. J Clin Oncol 2001;19:32 – 40S.

42. Hurwitz HI, Fehrenbacher L, Hainsworth JD, et al. Bevacizumab in combination with fluorouracil and leucovorin: an active regimen for first-line metastatic colorectal cancer. J Clin Oncol 2005;23:3502 – 8.

43. Fiedler W, Giaccone G, Lasch P, et al. Phase I Study of SU-014813, a novel oral multi-targeted receptor tyrosine kinase (RTK) inhibitor. AACR-NCI-EORTC International Conference, Molecular Targets and Cancer Therapeutics, Philadelphia, Pennsylvania; 2005. Abstract B119.

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