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ORIGINAL ARTICLE

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Pediatric phase I trial and pharmacokinetic study of the platelet-derived growth factor (PDGF) receptor pathway inhibitor SU101

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Abstract *Purpose*: To determine the maximum tolerated dose and the toxicity profile of the PDGF receptor pathway inhibitor SU101 in pediatric patients with refractory solid tumors, and to define the plasma pharmacokinetics of SU101 and its active metabolite SU0020 in children. *Experimental design*: Patients between 3 and 21 years of age with CNS malignancy, neuroblastoma, or sarcoma refractory to standard therapy were eligible. The starting dose of SU101 was 230 mg/m^2 per day administered as a 96-h continuous infusion every 21 days. Blood for pharmacokinetic analysis was obtained during the first cycle. *Results*: Entered into the trial were 27 patients, and 24 were fully evaluable for toxicity. Dose-limiting central nervous system toxicity was observed in two patients at the 440 mg/m^2 per day dose level. Non-dose-limiting toxicities included nausea, vomiting, headache, fatigue, abdominal discomfort, diarrhea, pruritus, anorexia, constipation, and paresthesias. There were no complete or partial responses. One patient with rapidly progressive desmoplastic small round-cell tumor experienced symptomatic improvement and prolonged stable disease. Steady-state concentrations of SU101 were rapidly achieved and proportional to dose. The concentration of SU0020 was 100- to 1000-fold greater than that of SU101. The median clearance of SU0020 was 0.19 l/day per m^2 and its terminal elimination half-life was 14 days. *Conclusions*: SU101 administered on this schedule was generally well tolerated. The maximum tolerated dose of SU101 is 390 mg/m^2 per day for 4 days repeated every 3 weeks. The neurotoxicity observed at the 440 mg/m^2 per day dose level suggests that patients receiving repetitive cycles must be monitored closely, as SU0020 may accumulate over time.

Keywords Signal transduction · Pharmacokinetics · Pediatric · SU101 · Leflunomide

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Introduction

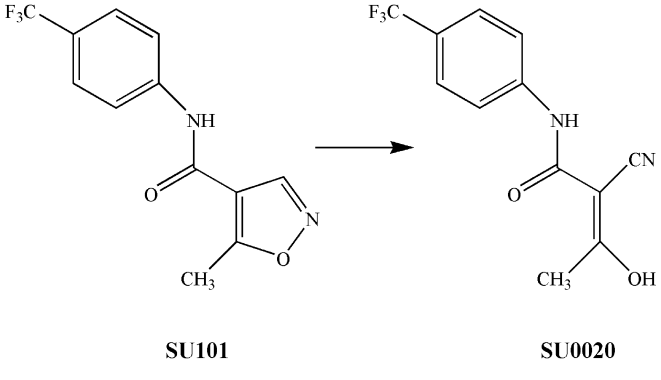
Signal transduction inhibitors represent an important new class of molecularly targeted agents for the treatment of cancer. Recently, two small molecule signal transduction inhibitors, imatinib mesylate (Gleevec, STI-571), which targets the bcr-abl kinase, and gefitinib (Iressa, ZD1839), which targets the EGF-R pathway, have received FDA approval. SU101 (leflunomide, *N*-[4-(trifluoromethyl)phenyl] 5-methylisoxazole-4-carboxamide) is one of the first signal transduction inhibitors developed for clinical study in adult cancer patients. SU101 is a small molecule that inhibits platelet-derived growth factor (PDGF) receptor-mediated cell signaling. In preclinical studies, SU101 has been shown to inhibit PDGF-stimulated receptor phosphorylation, DNA synthesis and cell cycle progression in a dose-dependent manner [1].

PDGF has been implicated in the aberrant proliferation of a variety of cancers [2]. The binding of PDGF

ligands to the PDGF receptor results in a cascade of events that induce many intracellular events including the increased expression of proteins involved in the regulation of cell growth and differentiation. The v-*sis* oncogene of the simian sarcoma virus (SSV) is a retroviral homolog of the B-chain of PDGF [3]. SSV induces malignant glioma in experimental animals, suggesting that autocrine activation of PDGF receptors may play a role in tumorigenesis [4, 5]. The PDGF ligands and receptors have been detected in various tumor cell lines such as gliomas, melanomas, sarcomas, neuroblastomas, osteosarcomas, and several types of carcinomas [3, 6, 7, 8, 9, 10]. These findings have lent support to the hypothesis of autocrine stimulation in these tumor types. For some other tumor types in which stromal expression of PDGF or its receptors has been detected, paracrine stimulation of neoplastic growth has been suggested to occur [11, 12, 13]. The degree of expression of PDGF and its receptors has also been associated with the progression of some tumors from a benign to a malignant phenotype [14, 15] and in other tumor types with increasing tumor grade [16, 17, 18]. The expression of PDGF and its receptor has been found to have prognostic significance in some patients with advanced breast cancer or ovarian carcinomas [19, 20].

In vivo, SU101 undergoes rapid and complete conversion to its major metabolite SU0020 (*N*-[4-(trifluoromethyl)phenyl] 2-cyano-3-hydroxyl-2-butenamide; Fig. 1). SU0020 interferes with de novo pyrimidine biosynthesis by inhibiting dihydro-orotate dehydrogenase. Thus, administration of SU101 could result in both PDGF receptor-dependent and -independent antiproliferative effects.

Several phase I trials of SU101 in adult patients have been completed [21, 22, 23]. Activity observed during phase I trials in adult patients with malignant glioma led to phase II single-agent and combination studies in anaplastic astrocytomas and glioblastoma multiforme [24, 25]. In addition, phase II trials in adult patients with prostate, ovarian, and non-small-cell lung cancer have been performed [25, 26, 27, 28, 29, 30]. Although not published, a randomized phase III trial of SU101 versus procarbazine in adult recurrent primary brain cancer did not support SU101 efficacy in this indication, and the clinical development of the agent was suspended in the year 2000 (A.L. Hannah, personal communication).



SU101 **SU0020**

Fig. 1 Structure of SU101 and its primary metabolite SU0020

PDGF ligand and receptor have been found in pediatric tumor cell lines including glioma, osteosarcoma [6, 31], desmoplastic small round cell tumor [32, 33, 34], neuroblastoma [35, 36, 37, 38] and synovial cell sarcoma [39]. Based on these findings and the initial adult experience, we performed a pediatric phase I trial and pharmacokinetic study in children with refractory sarcomas and neural cancers.

Several schedules of administration have been used in adult trials, the most common of which is a 4-day continuous infusion loading dose of 400 to 440 mg/m^2 per day followed by the same dose administered weekly or biweekly. Considering that the elimination half-life of SU0020 is greater than 14 days in adults, a more rational and convenient 4-day continuous infusion schedule of SU101 administered every 21 days was selected for the pediatric trial. The starting dose for this trial was approximately 50% of the adult maximum tolerated dose (MTD), with 30% increments between dose levels until achievement of the adult MTD.

Patients and methods

Patient eligibility

Patients between 3 and 21 years of age with histologically confirmed primary CNS tumors, neuroblastoma, or sarcoma refractory to standard therapy were eligible for this trial. The requirement for histologic verification was waived for patients with brainstem gliomas. Patients must have had measurable or evaluable disease at study entry. Patients must have recovered from the toxic effects of prior therapy. All patients had adequate hepatic and renal function as defined by a serum bilirubin ≤ 1.5 mg/dl, serum transaminases less than three times the upper limit of normal, and a serum creatinine less then 1.5 times normal for age. Patients evaluable for hematologic toxicity were required to have a granulocyte count of $>1500/\text{mm}^3$ and a platelet count of $>100{,}000/\text{mm}^3$.

Trial design

SU101 was supplied by Sugen (San Francisco, Calif.) as a liquid formulation in 50-ml vials containing 400 mg of study drug in 40 ml vehicle. The starting dose of SU101 was 230 mg/m^2 per day administered daily for four consecutive days (total dose 920 mg/m^2 per course) as a 96-h continuous infusion. Planned dose escalations were to 300, 390 and 440 mg/m^2 per day. Courses of SU101 were repeated every 21 days. Patients were evaluated for response prior to the second cycle, and then every other cycle, using either CT or MRI scans. The sum of the product of the two longest perpendicular diameters of all measurable tumors was used to define response, with a partial response defined as at least a 50% decrease in the sum, and stable disease as a $<50\%$ reduction or $<25\%$ increase in the sum on two evaluations at least 3 weeks apart.

The MTD of SU101 was defined as the highest dose level at which fewer than two of a cohort of six patients experienced a dose-limiting toxicity (DLT). At least three patients within a cohort had to be evaluable for toxicity before escalating to the next higher dose level. If one of the first three patients entered at a dose level experienced DLT, an additional three patients were entered at that dose level. There was no intrapatient dose escalation. At the MTD, an additional six patients could be studied to better define drug

disposition. Toxicities were graded according to the NCI/CTEP Common Toxicity Criteria v.1 [40]. Hematologic DLT was defined as grade 4 neutropenia of more than 7 days duration or grade 4 thrombocytopenia. Nonhematologic DLT was defined as any grade 3 or 4 nonhematologic toxicity with the specific exclusions of grade 3 nausea and vomiting, grade 3 fever, or grade 3 hepatotoxicity that returned to grade 1 prior to the next cycle of therapy.

Patients who experienced a reversible DLT while receiving SU101 could receive additional cycles of drug at the next lower dose level; DLT occurring after one dose reduction resulted in discontinuation of treatment. Any patient with a plasma SU0020 concentration at the end of the first 96-h infusion greater than 1400 μ*M* had the dose of SU101 reduced to the next lower dose level for subsequent infusions, as pharmacokinetic data from adult trials have suggested an increased risk of severe neurotoxicity with plasma SU0020 concentrations exceeding 1600 μ*M*.

Pharmacokinetics

Blood samples for pharmacokinetic analysis of SU101 and its active metabolite SU0020 were obtained during the first cycle of administration prior to the infusion, at 24, 48, 72 h after the start of the infusion, at the end of the infusion, and at 30, 60, 120, 240 min and 24 and (if possible) 48 h after the end of the infusion. Whenever possible, samples were also collected on approximately days 7 and 14 of the first cycle. A sample was obtained immediately prior to subsequent cycles of therapy. Plasma was separated from blood cells by centrifugation and stored at −70°C until assayed.

The plasma concentrations of SU101 and SU0020 were measured in our laboratory by a specific HPLC assay developed by Sugen. Plasma samples were thawed on ice, and a 100-μl aliquot was spiked with 50 μl of a 0.2 mg/ml internal standard (SU0070, 5-methylpyrazole-4-carboxylic acid-4-trifluoromethylanilide), and acidified with 7.5 μl 1 *M* HCl. Samples were extracted with 1.5 ml acetonitrile, centrifuged, and the supernatant was removed and evaporated to dryness under dry nitrogen using a Zymark Turbo-Vap LV evaporator (Hopkinton, Mass.). Samples were reconstituted in 100 μl methanol/mobile phase (75:25, v/v).

The HPLC system consisted of a Waters 2690 Separation Module (Alliance HPLC system) with column heater and sample cooling chamber (Milford, Mass.) and a Hewlett-Packard ODS Hypersil 5 μm 100×4.6 mm column maintained at 40°C. The mobile phase consisted of 35 m *M* KH_2PO_4/methanol (45:55, v/v) containing 4 m *M* triethylamine with a flow rate of 1.2 ml/min. The autosampler was maintained at a temperature of 4°C. Eluent was monitored with a Waters 996 photo diode array detector at 254 nm. These conditions provided clear separation of SU0020, SU0070 and SU101 with retention times of approximately 2.0, 4.4, and 6.7 min, respectively.

A two-compartment model, with unidirectional conversion of SU101 to SU0020, and first-order elimination of SU0020 was fitted to the plasma concentration-time data using MLAB (Civilized Software, Bethesda, Md.). The model has V_{SU101} and V_{SU0020} as the volumes of the SU101 and SU0020 compartments, k_{12} as the first-order rate constant for the conversion of SU101 to SU002, and k_{el} as the first order elimination rate constant for SU0020. The model assumes that SU101 is completely converted to SU0020. Clearance and volume of distribution were derived from the fitted model parameters. The terminal half-life was determined by regression analysis.

Results

Toxicity

Entered into the trial were 27 patients (Table 1), and 24 were fully evaluable for toxicity (Table 2). Patients who were not fully evaluable for toxicity included one patient who died of progressive disease prior to completion of the first 21-day cycle and one patient who received concomitant alternative therapy consisting of i.v. hydrogen peroxide from another practitioner after completing the SU101 infusion. A third patient withdrew after receiving 24 h of SU101. The toxicity profile in these inevaluable patients did not differ from that in the evaluable patients, and none experienced DLT.

Table 1 Patient characteristics. Values are number of patients, except age in years

Male/female	19/8
Age (years)	
Median	14
Range	3–21
Number of prior regimens	
Median	3
Range	1–6
Diagnosis	
Ewing's sarcoma	6
Medulloblastoma	4
Ependymoma	3
Glioma	3
Osteosarcoma	2
Brain stem glioma	2
Desmoplastic small round-cell tumor	2
Neuroblastoma	1
Alveolar soft-part sarcoma	1
Esthesioneuroblastoma	1
Glioblastoma multiforme	1
Melanoma of the soft parts	1

Table 2 Toxicity

Dose level	Dose (mg/m^2/day)	No. of patients entered	No. evaluable	No. of DLTs	DLT
1	230	6	4	0	
2	300	3	3	0	
3	390	11	10	1	SIADH (cycle 1)
4	440	7	7	2*	Neurotoxicity (cycle 2)[a], neurotoxicity (cycle 3)

[a]Neurotoxicity vs progressive disease (see text)

At the 390 mg/m^2 per day dose level, a 12-year-old male with glioblastoma multiforme developed SIADH during cycle 1 of drug administration necessitating discontinuation of drug. The patient's serum sodium decreased from 135 to 125 mEq/l within the initial 24 h of drug administration and returned to baseline within 24 h of discontinuation of SU101, suggesting a probable association with drug administration. The subsequent five patients at this dose level tolerated the drug without DLT (Table 2).

Dose-limiting neurotoxicity was observed at the 440 mg/m^2 per day dose level. A 15-year-old female patient with recurrent refractory metastatic osteosarcoma developed a reversible period of confusion and disorientation 8 days following her third infusion of SU101. An MRI of the brain was normal. Symptoms

resolved fully within 1 day of hospital admission. A second patient with recurrent refractory medulloblastoma developed signs of progressive midbrain dysfunction following his second cycle of SU101. An MRI demonstrated progressive disease. The patient died 2 days later. Given the proximity of the event to the infusion, neurotoxicity secondary to SU101 could not be excluded. Of note, to gain additional experience at this dose level, which was the adult phase II recommended dose, following the initial cycle 1 tolerability in three patients, the cohort was expanded. The expansion occurred prior to the development of DLTs in later cycles. Additional patients were then entered at the MTD of 390 mg/m^2 per day to better define drug tolerability and pharmacokinetic parameters.

Non-dose-limiting toxicities possibly attributed to SU101 and observed in more than one patient included nausea or vomiting ($n=16$), headache ($n=15$), fatigue ($n=11$), abdominal discomfort ($n=9$), diarrhea ($n=7$), pruritus ($n=6$), anorexia ($n=4$), constipation ($n=3$), paresthesias ($n=3$), reversible minor change in mental status ($n=5$), asymptomatic hypotension ($n=2$), urinary frequency ($n=2$) and anemia grade 3 or less ($n=6$), thrombocytopenia ($n=4$) and neutropenia ($n=2$). These toxicities did not appear to be dose-related.

Responses

Of the 27 patients entered, 26 were considered evaluable for response. The one patient who withdrew from the study after having received only one-fourth of the initial dose was considered inevaluable. There were no complete or partial responses observed. One patient with rapidly progressive desmoplastic small round-cell tumor experienced marked symptomatic improvement enabling discontinuation of all narcotic pain medications. This patient had diffuse peritoneal disease at study entry and received 19 cycles of SU101 before developing symptomatic and radiographic progression. One patient with alveolar soft-part sarcoma received nine cycles of SU101 and one patient with medulloblastoma received six cycles (Table 3).

Pharmacokinetics

Of the 27 patients entered, 23 had pharmacokinetic sampling performed. Steady-state concentrations of SU101 were rapidly achieved and appeared to increase in proportion to dose, with mean ($\pm$SD) steady-state plasma concentrations of 1.3 ± 0.3, 1.4 ± 0.4, 2.4 ± 0.6 and 3.0 ± 1.3 μM at the 230, 300, 390 and 440 mg/m^2 per day dose levels, respectively. The concentrations of the SU0020 metabolite were 100 to 1000 times greater than those of SU101, with a high degree of interpatient variability across all dose levels (Fig. 2). Average ($\pm$SD) SU0020 end of infusion plasma concentrations were 813 ± 145, 1010 ± 208, 1150 ± 131 and 1380 ± 280 μM at the 230, 300, 390 and 440 mg/m^2 per day dose levels, respectively.

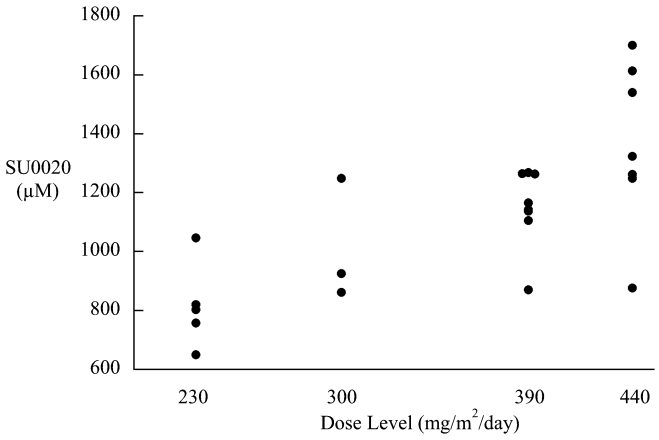


Fig. 2 Peak (end of infusion) SU0020 plasma concentrations observed during the initial 96-h infusion of SU101 by dose level

The two-compartment model, with unidirectional conversion of SU101 to SU0020 and first-order elimination of SU0020 was successfully fitted to the plasma concentration-time data from 20 patients. Model-derived parameters are shown in Table 4. SU0020 was cleared slowly from the plasma (Fig. 3), with a median clearance of 0.19 l/day per m^2 (range 0.05 to 0.73 l/day per m^2). The median terminal elimination half-life of SU0020 was 14 days (range 4 to >21 days). The volume of distribution of SU0020 approximated blood volume with a median value of 4.6 l (range 3.2 to 6.3 l).

Discussion

The potential of receptor tyrosine kinases as therapeutic targets in cancer has recently been established with the demonstrated activity of trastuzumab (Herceptin), a monoclonal antibody against the Her-2/neu receptor tyrosine kinase, imatinib mesylate (STI-571, Gleevec), a

Table 3 Cycles of SU101 administered

No. of cycles	No. of patients
1	16
2	4
3	4
6	1
9	1
19	1

Table 4 Model and model-derived parameters

Parameter	Mean $\pm$ SD
V_{SU101} (l/m^2)	18.5 ± 42.4
k_{12} (per hour)	8.1 ± 5.3
V_{SU0020} (l/m^2)	4.6 ± 0.9
k_{el} (per hour)	0.0018 ± 0.0009
Cl_{SU0020} (l/day/m^2)	0.19 ± 0.09

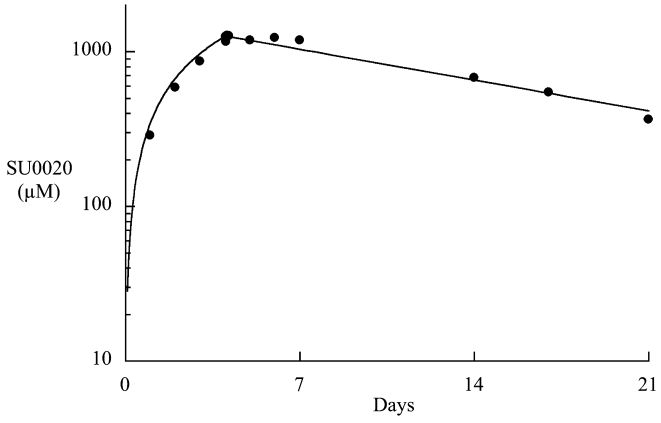


Fig. 3 The concentration time profile of SU0020 from a representative patient studied at the dose level of 390 mg/m^2 per day for 4 days. SU0020 is cleared slowly from plasma, with a median half-life of 14 days

small molecule inhibitor of the bcr-abl, c-kit and PDGF receptor tyrosine kinases, and gefitinib (Iressa, ZD1839), a small molecule inhibitor of the EGF-R tyrosine kinase. Based upon its activity in preclinical models of tumors in which the PDGF receptor pathway has a contributing role in the malignant process, SU101 was the first tyrosine kinase inhibitor brought into clinical trials in adult patients with cancer. Our pediatric phase I study reported here was designed to determine the tolerability of a 96-h continuous infusion of SU101 administered every 3 weeks, and to define the pharmacokinetics of SU101 and its principal metabolite SU0020, in children with refractory cancer.

Overall, SU101 doses up to and including the MTD were well tolerated. Most of the toxicities observed were minor in nature and tended to occur during the 4-day infusion. When it occurred, the mild nausea was readily controlled with antiemetics. The toxicity profile observed in children with non-dose-limiting nausea, diarrhea, fatigue and, infrequently, paresthesias was similar to the profile observed in adult patients [21, 23, 26, 30]. Myelosuppression was not a prominent toxicity.

Two patients enrolled at the highest dose level of 440 mg/m^2 per day experienced probable neurotoxicity. As these events occurred beyond the first cycle, it is likely that SU0020 had accumulated to concentrations higher than those observed during the initial cycle of therapy. In adult trials, dose-limiting neurotoxicity in the form of fatal cerebral edema occurred in two of three patients receiving 735 mg/m^2 after five doses. The 440 mg/m^2 per day dose was therefore considered to have exceeded the MTD, with the DLT being neurotoxicity. The recommended phase II dose of SU101 for pediatric patients with cancer is therefore 390 mg/m^2 per day for 4 days repeated every 3 weeks. Of note, given the high degree of interpatient variability in SU0020 drug disposition, and the occurrence of severe neurotoxicity observed in adult patients, significant caution must be exercised if consideration is given to utilizing this agent in future trials.

The pharmacokinetics of SU101 and SU0020 in children were similar to those observed in adults. Peak plasma concentration of both SU101 and SU0020 increased in proportion to the dose, but there was wide interpatient variability. There was no correlation between peak (end of infusion) SU0020 concentration and toxicity. Clearance in pediatric patients (0.19 l/day per m^2) was modestly slower than in adults (0.26 l/day per m^2), but the wide interpatient variability observed in both children (range 0.05 to 0.73 l/day per m^2) and adults (range 0.04–1.35 l/day per m^2) makes it difficult to reach definitive conclusions regarding age-related differences in drug disposition. The mechanism of elimination of SU0020 involves both renal clearance and metabolism to an oxalinic acid derivative, including possible involvement of CYP2C9 [41], but the basis for the wide degree of interpatient variability in SU0020 disposition has not been well studied. The correlation between age and clearance in patients enrolled on our pediatric study ($r = 0.28$, data not shown), however, suggests that younger children should be monitored closely because of the potential for slower clearances.

SU101 does not have a favorable pharmacokinetic profile. The drug is rapidly metabolized to a compound, SU0020, that is cleared much more slowly, accumulates in patients treated with multiple cycles, and is thought to be responsible for the DLT. The exposure to SU101 over the 21-day cycle is minimal and SU0020 is not an inhibitor of the PDGF receptor.

Although no complete or partial responses were observed, seven patients with stable disease received three or more cycles of therapy. The prolonged stable disease and symptomatic improvement in the patient with desmoplastic small round-cell tumor, a tumor that is known to express high levels of the PDGF receptor [32, 33, 34], suggests that targeting the PDGF receptor pathway for this rare tumor may be a potential therapeutic strategy for future PDGF receptor inhibitors. As repeat biopsies were not required for study entry, no information is available on individual patients' PDGF receptor expression. Attributing clinical activity to SU101, however, is complicated by the fact that the predominant circulating metabolite, SU0020, is an inhibitor of dihydro-orotate dehydrogenase that may also exert an anticancer effect [42] and is present in significantly higher concentrations than the parent drug for extended periods of time.

Defining the importance of a diversity of signal transduction pathways, including PDGF receptor, in childhood cancers remains an important area of ongoing research, and such knowledge will help guide future development of receptor tyrosine kinase inhibitors for children with cancer. Currently, imatinib mesylate, which can also inhibit the PDGF receptor pathway, is in phase II study in children with select solid tumors. The experience of SU101 presented here, combined with the results of ongoing related inhibitors, will help determine the utility of this therapeutic strategy for select childhood cancers.

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