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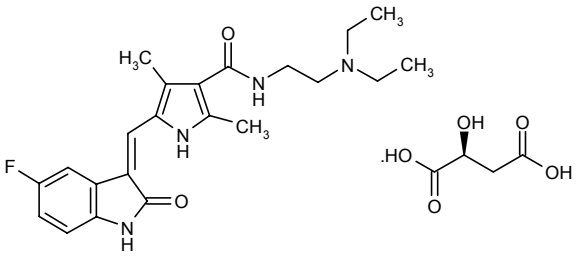
Sunitinib Malate

USAN; Prop INNM

Oncolytic Drug
Multitargeted Tyrosine Kinase Inhibitor

PNU-290940AD
SU-010398
SU-011248 L-malate salt
Sutent®

(Z)-*N*-[2-(Diethylamino)ethyl]-5-(5-fluoro-2-oxo-2,3-dihydro-1*H*-indol-3-ylidenemethyl)-2,4-dimethyl-1*H*-pyrrole-3-carboxamide 2(*S*)-hydroxybutanedioic acid (1:1)



$C_{22}H_{27}FN_4O_2.C_4H_6O_5$
Mol wt: 532.5614
CAS: 341031-54-7
CAS: 326914-13-0 (as free base)
EN: 309144

Abstract

Signal transduction via receptor tyrosine kinases (RTKs) is frequently dysregulated in malignant disease. Several type III split-kinase domain RTKs are expressed on tumor cells and directly affect tumor cell proliferation, as well as playing a prominent role in tumor angiogenesis. Inhibition of these RTKs represents an attractive therapeutic option in the treatment of human cancers. Sunitinib is an orally active, multitargeted TK inhibitor. In *in vitro* and *in vivo* assays, sunitinib demonstrated potent inhibition of vascular endothelial growth factor (VEGF)-dependent Flk-1, platelet-derived growth factor receptor-β (PDGFR-β), Flt3 and Kit TKs. In mouse xenograft models, daily treatment with sunitinib resulted in growth inhibition of a variety of established human tumors. Synergistic activity in combination with other chemotherapeutic agents and radiotherapy was demonstrated in murine tumor models. Phase I and II clinical studies in patients with advanced solid tumors, AML, renal cell carcinoma and gastrointestinal stromal tumors (GIST) showed early evidence of efficacy and an acceptable toxicity profile. A phase III study in imatinib-refractory GIST patients was terminated early due to demonstrated efficacy compared with placebo, and a phase III study in renal cell carcinoma is ongoing.

Synthesis

Nitrosation of *tert*-butyl acetoacetate (I) with $NaNO_2$/AcOH produces the oximino ester (II), which by reductive cyclization with ethyl acetoacetate (III) in the presence of Zn/AcOH leads to the pyrrole-dicarboxylate (IV). Selective decarboxylation of the *tert*-butyl ester of pyrrole (IV) under acidic conditions, followed by Vilsmeier formylation of the intermediate ethyl 2,4-dimethylpyrrole-3-carboxylate, furnishes the pyrrole-carboxaldehyde (V). Alternatively, compound (V) is directly obtained from (IV) upon treatment with trimethyl orthoformate and trifluoroacetic acid. Basic hydrolysis of the ethyl ester group of compound (V) yields the pyrrole-carboxylic acid (VI), which is coupled to 2-(diethylamino)ethylamine (VII) to afford the corresponding amide (VIII) (1, 2). Scheme 1.

Heating 5-fluoroisatin (IX) with neat hydrazine hydrate provides 2-amino-5-fluorophenylacetic hydrazide (X), which, by acidic treatment cyclizes to 5-fluorooxindole (XI). Finally, oxindole (XI) is condensed with the formylpyrrole-carboxamide (VIII) in the presence of piperidine or pyrrolidine in hot EtOH (1). In an alternative procedure, oxindole (XI) is condensed with the formylpyrrole, carboxylic acid (VI) to give compound (XII), which is further coupled with 2-(diethylamino)ethylamine (VII) in the presence of EDC/HOBt in DMF (2). Scheme 2.

Introduction

Signal transduction is a mechanism that controls diverse cellular processes, and signaling via receptor tyrosine kinases (RTKs) is frequently dysregulated in malignant disease. RTKs are transmembrane proteins containing extracellular ligand-binding domains and intracellular catalytic domains. Both receptors and ligands have defined patterns of expression resulting in specificity of signal transduction. Signaling molecules acting

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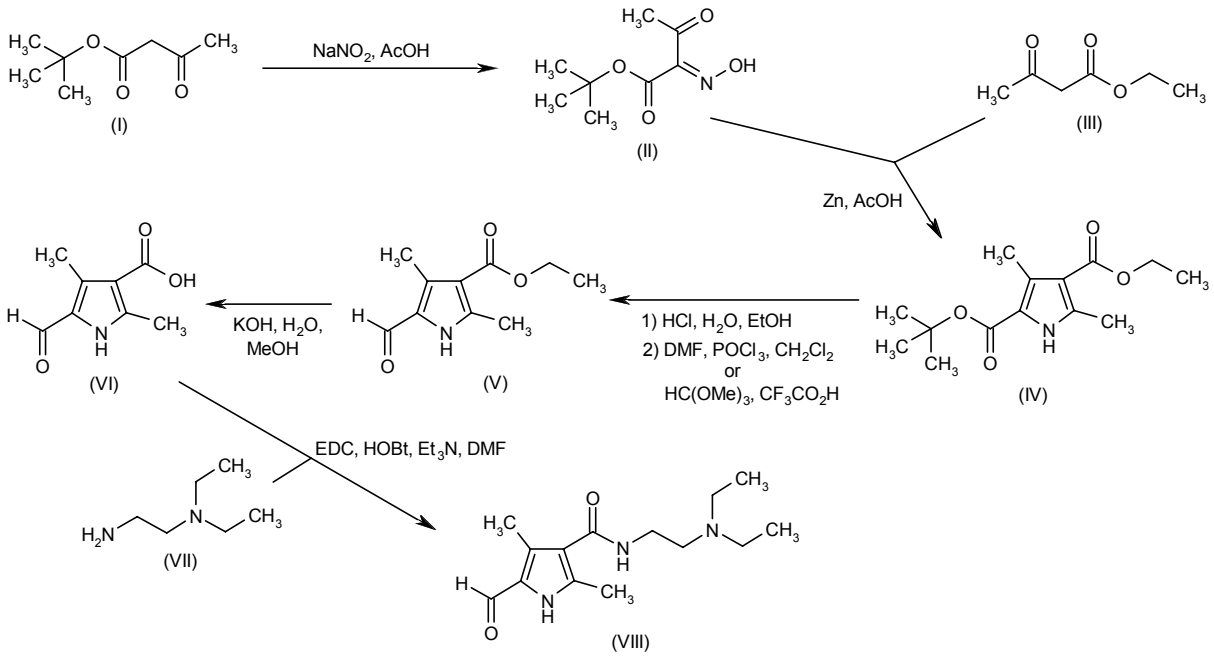
Scheme 1: Synthesis of Intermediate VIII



downstream of activated RTKs play a crucial role in tumor growth, progression and metastasis (3, 4).

Several RTKs from the superfamily of type III split-kinase domain RTKs are expressed on tumor cells and directly affect tumor cell proliferation, as well as playing a prominent role in tumor angiogenesis. Both vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) RTKs are involved in the transmission of proliferation, migration, differentiation and survival signals between tumor cells and endothelial cells (5-8). Other split-kinase domain RTKs implicated in tumorigenesis are Flt3 and Kit, and inhibition of these targets is an area of active investigation in the search for new chemotherapeutic agents (9).

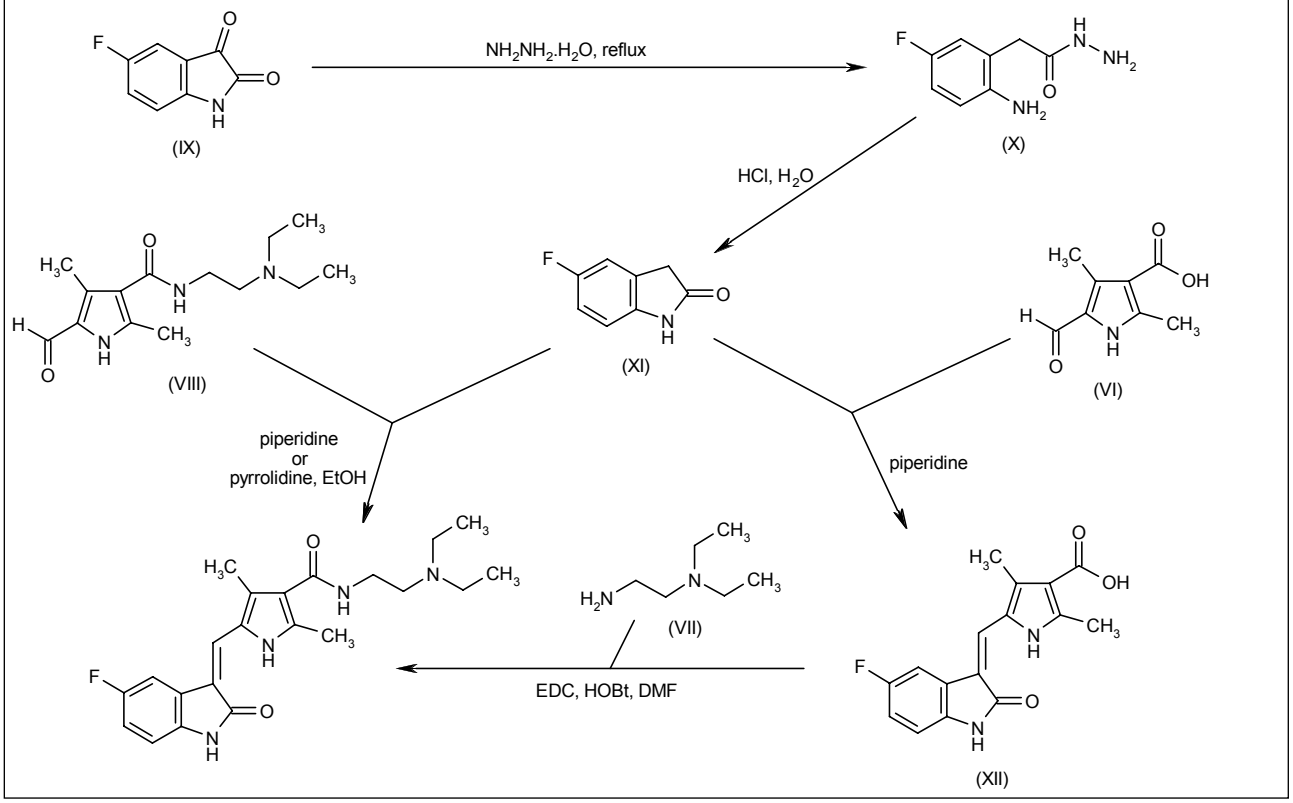
The indolinone-based compound sunitinib malate (SU-11248, Sutent®) represents the first in a class of orally active, multitargeted tyrosine kinase inhibitors. Sunitinib inhibits VEGFR and PDGFR, as well as Flt3 and Kit tyrosine kinases with nanomolar potency, and was advanced to clinical trials in cancer patients based on its favorable preclinical profile (2, 10, 11).

Pharmacological Actions

The *in vitro* activity of sunitinib was demonstrated in biochemical and cellular assays. In biochemical assays, the compound exhibited competitive inhibition (with regard to ATP) of Flk-1/KDR (VEGFR-2) and PDGFR-β, giving respective K_i values of 0.009 and 0.008 μM; its activity against other tyrosine or serine/threonine kinases was usually at least 10-fold lower. It inhibited ligand-dependent autophosphorylation of Flk-1/KDR and PDGFR-β RTK with IC_{50} values of approximately 0.01 μM, similar to the K_i values, confirming that sunitinib has comparable activity against both targets. Sunitinib also inhibited ligand-dependent mitogenic and proliferative activity in human endothelial cells with similar potency (12, 13).

Sunitinib displayed potent and broad-spectrum antitumor activity in mouse xenograft models. Oral treatment with sunitinib 40-80 mg/kg/day resulted in growth inhibition of established tumors from the human cell lines HT-29 and COLO 205 (colon), A-431 (epidermoid), NCI-H460 (non-small cell lung cancer), SF763T (glioma), A-375 (melanoma), MDA-MB-435 (breast), and rat glioma C6 tumor cells. Growth inhibition relative to vehicle-treated controls ranged from 64% to 93% at these doses of sunitinib, and it was also highly effective in the B16 melanoma lung metastasis model (12-19). The majority of these tumor cell lines, however, do not express the RTKs targeted by sunitinib, indicating that the antitumor activity in these models was primarily mediated through the antiangiogenic effect of sunitinib. Therefore, the activity of sunitinib against VEGF and PDGF receptors was investigated *in vivo*. Studies in athymic mice bearing A-375 or SF763T tumors demonstrated that a single oral dose of sunitinib resulted in dose- and time-dependent inhibition of Flk-1/KDR and PDGFR-β phosphorylation. Furthermore, in tumors derived from COLO 205 cells, which do not express PDGFR-β, sunitinib inhibited receptor phosphorylation on host cells that support developing vessels

Scheme 2: Synthesis of Sunitinib



within the tumor. Target modulation studies for inhibition of Flk-1/KDR and PDGFR-β phosphorylation in tumors and dose-finding studies for VEGF-mediated vascular permeability indicated a minimal active plasma concentration of 50-100 ng/ml and an effective dose of 40 mg/kg p.o. once daily. Complete target inhibition for 24 h thus appeared not to be necessary for potent antitumor activity (12-14).

Two classes of Flt3 mutations have been identified in patients with acute myelogenous leukemia (AML): the internal tandem duplication (ITD) mutation that is expressed in up to 30% of patients with AML, and point mutations in the activation loop of the kinase domain, found in approximately 7% of patients. In phosphorylation assays, sunitinib had potent, concentration-dependent activity against wild-type Flt3 and both classes of mutations (IC_{50} = 10-300 nM). This inhibition resulted in a corresponding inhibition of cellular proliferation and subsequent apoptosis, as indicated by poly(ADP-ribose) polymerase (PARP; NAD^+ ADP-ribosyltransferase) cleavage and caspase-3 levels. Sunitinib inhibited the transforming ability and transcriptional activity of Flt3-ITD proliferation-related and differentiation-related genes in AML 32D cells and primary AML blasts. In tissue culture supernatents from cell lines, sunitinib also inhibited VEGF production induced by downstream signaling of Flt3 (20-25).

The *in vivo* efficacy of sunitinib was demonstrated in two models of the Flt3-ITD mutation. In a subcutaneous xenograft model, treatment with sunitinib 40 mg/kg/day for 4 days resulted in a marked reduction in tumor volume and visual disappearance of tumors of 300-500 mm^3 in athymic mice. A dose of 20 mg/kg/day also resulted in complete, although less rapid tumor regression. In a bone marrow engraftment model representing a more physiologically relevant leukemia model, prolonged dose-dependent survival was observed in mice treated with 5, 10 and 20 mg/kg/day of sunitinib (46, 56 and at least 83 days, respectively, compared with a mean survival time of 41 days in mice treated with vehicle). In addition to the increased survival time, animals treated with sunitinib lacked the hindlimb paralysis, ruffled fur and decreased spontaneous activity observed in the vehicle-treated animals (20-23).

The combination of sunitinib and the standard AML chemotherapy drugs cytarabine and daunorubicin was evaluated in cell lines expressing either mutant or wild-type Flt3. Sunitinib increased the antiproliferative effects of the drugs on mutant (ITD) cell lines, reducing the concentration of cytotoxic agent necessary to produce a given antiproliferative effect. Synergistic effects of the agents were also observed for the induction of apoptosis. Moreover, sunitinib significantly inhibited the proliferation of primary AML myeloblasts expressing mutant Flt3-ITD,

and had a synergistic effect in combination with cytarabine. No significant effect was observed in myeloblasts expressing wild-type Flt3 (26, 27).

Activation of the Kit tyrosine kinase by somatic mutation has been documented in a number of human malignancies, including gastrointestinal stromal tumors (GIST) and small cell lung cancer (SCLC). Coexpression of Kit and its ligand stem cell factor (SCF) occurs in up to 70% of SCLC cell lines and tumor specimens. The ability of sunitinib to inhibit Kit phosphorylation was evaluated in the Kit-positive SCLC cell line NCI-H526. Treatment with sunitinib resulted in a concentration-dependent inhibition of SCF-stimulated phosphorylation, with an IC_{50} value of approximately 10 nM. The growth of established subcutaneous NCI-H526 tumors in athymic mice was also inhibited by sunitinib treatment (28, 29; see also 30).

The target RTKs of sunitinib are all expressed in human breast cancer or its supporting tissues. Thus, preclinical evaluation of sunitinib as monotherapy or in combination with other agents was performed to support its potential use in the treatment of breast cancer. In a transgenic model of mutant Ras-driven breast cancer, treatment with sunitinib 40 mg/kg/day for 20 days resulted in tumor regression. In athymic mice bearing established MX-1 human breast tumors, sunitinib treatment resulted in 52% tumor inhibition compared with controls. In the MX-1 model, combination therapy with docetaxel was well tolerated and resulted in enhanced antitumor activity compared with either therapy alone (89% inhibition of tumor volume compared with controls). Sunitinib also showed greater efficacy against MX-1 tumor growth when combined with 5-fluorouracil than when either agent was used alone (31, 32).

In addition to its target RTKs, sunitinib has also been shown to inhibit the phosphorylation of the receptor for macrophage colony-stimulating factor (M-CSF) CSF-1R expressed on NIH/3T3 cells (IC_{50} = 50-100 nM). The receptor has structural similarity to the other targets within class III RTKs and its stimulation enhances the osteolytic activity of osteoclasts. Production of M-CSF by tumor cells causes bone destruction in patients with metastatic bone disease. The activity of sunitinib has been evaluated in an experimental breast cancer bone metastasis model. Bioluminescent imaging of firefly luciferase (Luc)-expressing MDA-MB-435/HAL tumor cells showed that sunitinib had dual inhibitory effects on both breast cancer metastases and osteolysis. In mice inoculated with tumor cells into the left ventricle of the heart, metastatic tumors in bone were detected in approximately 70% of animals. In those animals treated with sunitinib 40 or 80 mg/kg/day for 21 days, tumor growth in bone was inhibited by 64% and 89%, respectively. The effect of sunitinib on osteolytic activity was demonstrated by a 30% reduction in serum levels of the collagen breakdown product pyridinoline compared with vehicle-treated mice (32, 33).

In another study evaluating the effects of sunitinib in a breast cancer xenograft model, groups of athymic mice were implanted with MCF7 cells and MCF7 cells transfected with VEGF. Treatment of established tumors with sunitinib 40 mg/kg/day for 4 weeks resulted in regression of VEGF-transfected MCF7 tumors. Tumor growth inhibition was more pronounced in tumors expressing VEGF (73% *versus* 59% inhibition in tumors not expressing VEGF compared with controls) (34).

The ability of sunitinib to improve growth delay in tumors treated with radiotherapy was evaluated in murine tumor models. Mice implanted with Lewis lung carcinoma or glioblastoma multiforme (GL261) to develop hindlimb tumors were treated with sunitinib 40 mg/kg/day i.p. during 7 days of radiation treatment (21 Gy). The combination resulted in a significant reduction in tumor volume compared with either treatment alone. This was associated with a greater reduction in tumor vasculature. Administration of sunitinib 20 mg/kg twice daily for 7 days beyond the completion of radiotherapy also resulted in a prolongation of tumor control. Pharmacodynamic assessment of the tumor response to combined sunitinib and radiation therapy showed a reduction in tumor blood flow and subsequent depletion of ATP. Tumors devoid of ATP showed an increased radiosensitivity correlating with the increased tumor growth delay. An increase in endothelial cell apoptosis was observed following treatment with combined sunitinib and radiation (35-37).

Pharmacokinetics and Metabolism

A randomized, crossover, single-dose study was conducted to investigate the potential pharmacokinetic interaction between sunitinib and ketoconazole, a potent cytochrome P-450 (CYP3A4) inhibitor. Twenty-six healthy male Caucasian and Asian subjects received either sunitinib 10 mg or ketoconazole 400 mg once daily for 7 days with sunitinib 10 mg administered on day 3. Following a 4-week washout period, the alternative treatment was administered. Concurrent administration of sunitinib and ketoconazole resulted in statistically significant increases in the mean peak plasma concentration (C_{max}) and the area under the concentration-time curve (AUC) of sunitinib, and small but significant decreases in the values for the active metabolite SU-12662. However, the half-lives of sunitinib and its metabolite were not significantly altered by coadministration of ketoconazole (38).

Clinical Studies

A phase I dose-escalating study was performed in patients with advanced solid tumors not amenable to conventional therapy. Twenty-eight patients received sunitinib 30 mg/m^2 every other day, 30 mg/m^2/day, 42 mg/m^2/day or 59 mg/m^2/day for 4 weeks, followed by 2 weeks without therapy. Dose-limiting toxicity (DLT) of fatigue and hypertension occurred at the highest dose and the maximum tolerated dose (MTD) was defined as 42 mg/m^2/day. At this dose, grade 2 and 3 toxicities

(fatigue, hypertension, skin toxicity) were observed which were reversible upon treatment discontinuation; thus, the recommended phase II dose was defined as 50 mg/day. Responses were observed in 6 of 23 evaluable patients with renal cell cancer and neuroendocrine tumors. Pharmacokinetic evaluation showed good oral bioavailability of sunitinib, with modest inter- or intrapatient variability. VEGF plasma levels increased more than 3-fold in approximately 70% of patients (39, 40).

In another phase I study in patients with a broad range of advanced solid tumors, sunitinib was administered for either 2 or 4 weeks, followed by a 2-week rest period, at the following doses: 25, 50 and 75 mg/day and 50 mg every other day. In 33 patients initially recruited, the most frequently reported adverse events were fatigue, gastrointestinal and hematological events. Of 13 patients on the 2-week schedule who were evaluable for response, 1 patient with thyroid cancer had a partial response and the other 12 patients had stable disease. Pharmacokinetic analyses demonstrated a prolonged half-life for sunitinib and SU-12662 of approximately 40 and 80 h, respectively. Linear kinetics were observed at the doses administered, and an increase in plasma VEGF levels was also observed in 70% of patients tested. Plasma concentrations of sunitinib of at least 50 ng/ml, sufficient to inhibit PDGFR and VEGFR preclinically, were achieved with 50 mg/day, the recommended phase II dose (41).

In an open, multicenter, exploratory study, positron emission tomography (PET) imaging and corresponding pharmacodynamic biomarkers were evaluated in patients with advanced malignancies refractory to standard therapies. Sunitinib was administered in 6-week cycles at a dose of 50 mg/day for 4 weeks followed by a 2-week rest period. Of 41 patients recruited, 20 patients had grade 3 or 4 toxicities. Of 31 patients evaluable at 4 weeks, 45% had at least a 20% reduction in fluorodeoxyglucose (FDG) uptake on serial PET scans, the primary endpoint. Reduced thymidine uptake and rapid tumor shrinkage were observed in some patients, with central tumor necrosis evident in many patients. Of 25 patients evaluable at 12 weeks, there was 1 radiologically confirmed partial response (sarcoma) and 5 patients had stable disease (42, 43). Radiolabeled [^{18}F]-sunitinib has also been synthesized for evaluation as a new potential PET tracer for imaging cancer tyrosine kinase (44).

A multicenter, single-dose phase I study was conducted in 29 AML patients to demonstrate inhibition of Flt3 phosphorylation by sunitinib. The dose was escalated in 50-mg increments from 50 mg to 350 mg in cohorts of 3-6 patients. The primary endpoint of > 50% inhibition of Flt3 phosphorylation in 50% of patients was achieved in the dose cohorts of 200 mg and above. Genotype analysis indicated mutant Flt3 in 5 patients and inhibition of Flt3 phosphorylation was observed in all of these patients and in 50% of patients bearing wild-type Flt3. Downstream signaling pathways were also inhibited. Evaluation of the pharmacokinetic/pharmacodynamic relationship indicated that a target plasma concentration of 100 ng/ml was required for potent (> 50%) inhibition of wild-type Flt3, whereas less was required for inhibition of ITD mutants. Achievement of target plasma concentrations and maintenance of these levels for 8-12 h in a 24-h period were consistent with preclinical predictions of efficacy. An indication of biological activity was demonstrated in 5 patients by large decreases in peripheral blood blast counts 24 or 48 h after administration of a single dose of sunitinib. Study drug-related adverse events were reported in 31% patients and were generally mild in severity (45, 46).

A preliminary demonstration of the safety and clinical activity of sunitinib was obtained in patients with AML who had failed or were not eligible for conventional chemotherapy. Doses of sunitinib of 25, 50, 75 or 100 mg/day were administered to 32 patients for 2 weeks, followed by a 2-week rest period. In 22 patients evaluable for safety and response in the first cycle only (25, 50 and 75 mg), dose-limiting toxicity (grade 3 fatigue) was observed in 1 patient in each of the higher dose groups. Other drug-related adverse events were generally mild. In 13 of 16 patients with detectable blasts in the peripheral blood at baseline, a greater than 50% decrease in absolute blast count was observed as their best response (47).

A further study was conducted in patients with refractory or resistant AML or those not amenable to conventional therapy. In this study assessing the safety and tolerability of sunitinib and its biological and molecular activity in relation to the Flt3 genotype, 15 patients received 50 or 75 mg/day for 4 weeks, followed by either a 1- or 2-week rest period. No DLT was observed at the dose of 50 mg (n=13); however, both patients treated with the higher dose experienced treatment-related adverse events that were dose-limiting. Fatigue was observed in almost all patients at the end of each cycle, and a relationship with sunitinib treatment was considered likely. Four patients had Flt3 mutations at baseline, and all of these patients had morphological or partial responses, compared with only 2 of 10 evaluable patients with wild-type Flt3. The responses were of short duration, although patients with Flt3 mutations remained longer on therapy. Immunohistological studies of bone marrow biopsies showed clear evidence of molecular activity consistent with inhibition of Flt3 (48, 49).

Sunitinib was administered as second-line therapy in a phase II study in patients with metastatic renal cell cancer. A total of 63 patients received repeat cycles of sunitinib at a dose of 50 mg/day for 4 weeks, followed by a 2-week rest period. The best responses were a partial response in 15 (24%) patients and stable disease in 29 (46%) patients. Of the patients who achieved a partial response, 1 patient progressed at 5 months and 14 had a median duration of response of 6+ months. Sunitinib demonstrated an acceptable toxicity profile, with the most common grade 1 or 2 events being fatigue, nausea, diarrhea and stomatitis. Grade 3 or 4 lymphopenia occurred in 30% of patients. Plasma samples were collected on days 1 and 28 of each cycle to evaluate a panel of solu-

ble proteins as biomarkers of pharmacodynamic activity. At the end of the first cycle, relative to baseline, levels of VEGF increased more than 3-fold in 45% of patients, and a greater than proportional increase in VEGF levels was observed in patients who had a partial response compared with those who had stable disease or rapid progression. Levels of the biomarkers tended to return to near baseline levels at the end of the 14-day rest period (50, 51).

GIST represents a molecularly diverse family of mesenchymal neoplasms characterized by expression of mutant Kit RTK in more than 85% of patients. A phase I dose-escalating pharmacokinetic and pharmacodynamic evaluation of sunitinib was performed in patients with imatinib-resistant GIST. In 32 patients administered sunitinib 25, 50 or 75 mg/day for 14 days, followed by a 14-day rest period, good oral absorption was observed. Ten of 12 patients treated with a dose of 50 mg/day demonstrated a decrease in tumor uptake of FDG by PET scan in cycle 1, indicating a decrease in tumor-associated metabolic activity. Five of these patients had measurable decreases in tumor area by CT scan, all of whom had plasma concentrations sufficient to inhibit RTK phosphorylation. Plasma levels of soluble Kit decreased dose-dependently during sunitinib treatment, but decreases of 20% or more relative to baseline were only observed in patients with at least an 8% reduction in unidimensional tumor size (52-54).

In another study, 75 patients with metastatic GIST refractory or intolerant to imatinib were treated with sunitinib 50 mg/day according to the following dose schedules: 2 weeks of treatment followed by a 2-week rest period (n=33), 4 weeks of treatment followed by a 2-week rest period (n=35) or 2 weeks of treatment followed by 1-week rest period (n=7). Following administration of a total of 389 cycles and in patients treated for at least 6 months, evidence of clinical benefit was demonstrated in 54% of patients; this was defined as an objective response (6 of 41 patients, or 15%) or prolonged progression-free survival (16 patients, or 39%). Transient grade 3 or 4 adverse events occurred infrequently in patients treated over multiple cycles, and 3 patients developed intratumoral bleeding during cycle 1, considered to be temporally associated with biopsy of the affected tumor mass. The study demonstrated promising clinical activity in a disease setting where no other effective therapy exists (55).

A placebo-controlled phase III trial of sunitinib in patients with imatinib-resistant GIST demonstrated early efficacy and safety, and an independent panel of experts recommended stopping the trial 7 months ahead of schedule. Another phase III trial in renal cell carcinoma is ongoing, as are phase II trials in metastatic breast cancer, NSCLC, neuroendocrine cancer and metastatic renal cell carcinoma in patients with bevacizumab-refractory disease (56).

The Committee for Orphan Medicinal Products of the European Medicines Evaluation Agency (EMEA) has granted orphan drug designation to sunitinib for the treatment of GIST and renal cell carcinoma (57). In the U.S., Pfizer has submitted an NDA for sunitinib as a treatment for malignant GIST and metastatic renal cell carcinoma among patients whose tumors do not respond to or do not tolerate standard treatment options (58).

Source

Pfizer, Inc. (US).

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