Tyrosine kinase receptors (RTKs) are a heterogeneous group of transmembrane proteins involved in signal transduction. These receptors are expressed in many different cells and regulate cellular growth, differentiation and angiogenesis. Overexpression and/or the structural alteration of different RTKs classes are generally associated to cancer and, when RTKs-mediated signal transduction pathways are abnormally activated, generate cancer growth, angiogenesis and metastatization. Therapeutic intervention targeting RTKs concerns antagonist drugs as little molecules or monoclonal antibodies. Sunitinib malate is a little molecule able to block intracellular tyrosine kinase domain of RTKs, which has both direct anticancer and antiangiogenetic activity. Sunitinib targets selectively vascular endothelial growth factor, KIT, Flt3 and platelet-derived growth factor receptors and the receptor encoded by the ret proto-oncogene. This drug is used in the treatment of gastrointestinal stromal cancer (GIST) resistant to imatinib and metastatic renal cell carcinoma (RCC). In this review, we report preclinical data of sunitinib, even about synergism with chemotherapy and radiotherapy, data relative to phase III trials of sunitinib in the treatment of GIST and RCC, and we try to plan what will be future applications of sunitinib in different types of cancer, even in association to chemotherapy, radiotherapy and monoclonal antibodies.

**Key words:** gastrointestinal stromal cancer, renal cell carcinoma, sunitinib, transduction pathways, Tyrosine kinase receptors

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**introduction**

Tyrosine kinase receptors (RTKs) are a heterogeneous group of transmembrane proteins involved in signal transduction. About 20 different classes of RTKs have been identified. These receptors have similar structure: a ligand-binding extracellular domain, a transmembrane portion and an intracellular tyrosine kinase domain.

These receptors are expressed in many different cells and regulate cellular growth, differentiation and angiogenesis. By the ligand-mediated activation, RTKs activate different intracellular pathways determining a great variety of cellular responses such as differentiation, proliferation, cell migration, angiogenesis and cell survival.

Overexpression and/or structural alteration of different RTKs classes are generally associated to cancer and, when RTKs-mediated signal transduction pathways are abnormally activated, generate cancer growth, angiogenesis and metastatization.

Platelet-derived growth factor (PDGF) stimulates the growth of fibrocytes, and PDGFR hyperexpression generates different diseases characterized by accelerated cellular growth, as fibrotic disorders and cancer.

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**preferential pathways**

Activated RTKs use the pathway Ras/Raf/mitogen-activated protein proteins, directly involved in the RTKs depending signal, as the catalytic subunit of phosphoinositide 3-kinase can work as onecogenes, determining mutations in gene transcription, cytoskeletal structure, cell mobility and in the apoptotic and anti-apoptotic signals. Even the interaction (cross talk) between RTKs and the oncostatin receptor can influence oncogenesis.

Therapeutic intervention targeting RTKs concerns antagonist drugs as little molecules or monoclonal antibodies [1, 2].
Sunitinib malate is a little molecule able to block intracellular tyrosine kinase domain of RTKs, which has both direct anticancer and antiangiogenetic activity.

Sunitinib targets selectively VEGF, KIT, Flt3 and PDGF receptors and the receptor encoded by the ret proto-oncogene (RET; Pfizer) (unpublished data, 2006). In an adenosine triphosphate site-dependent binding assay, sunitinib showed its high binding affinity for the primary targets (higher than sorafenib, which has a similar panel of targets) and the activity to bind 73 additional kinases, even serine–threonine kinases [3, 4].

Actually, sunitinib is used in the treatment of GIST resistant to imatinib and metastatic renal cell carcinoma (RCC), but many clinical ongoing trials are testing this drug in different types of cancer.

**preclinical trials**

In preclinical trials, sunitinib showed selectivity for epidermal growth factor receptor (EGFR), VEGFR2, PDGFRβ, PDGFRα and KIT, RET and Flt3 receptors. To identify biomolecular targets of sunitinib activity *in vivo*, different expression profiles have been conducted on epidermoidal cancer (A31), colorectal cancer (Colo 205 and HT–29), lung cancer (NCI-H226 and H460), breast cancer (MDA-MB-435), prostate cancer (PCB-3M-luc) and RCC (786-O).

**synergism with chemotherapy**

Sunitinib showed synergism with chemotherapeutic agents such as daunorubicin and citarabin, inhibiting AML cell lines with Flt3-ITD mutations [5]; this activity was present in the MV4-11 and MOLM14 cell lines, which have naturally this mutation, and in BaF3 cell lines, where mutation is induced.

Sunitinib showed synergism even in combination with docetaxel (Taxotere), 5-fluorouracil (5-FU) and doxorubicin in MX-1 breast cancer models [6]. After a 20-day treatment with sunitinib (40 mg/kg/die p.o.) or with docetaxel (10 mg/kg/week i.v. for 3 weeks), or with a combination of the two drugs, tumor volumes reduced, respectively, to 53%, 73% and 89%.

The association of 5-FU (100 mg/kg i.p.) and sunitinib (40 mg/kg/die p.o.) inhibited tumor growth of 74% and 79%, respectively, more respect to the two drugs alone.

The association of doxorubicin (4 mg/kg i.v. three times every week) and sunitinib obtained cancer inhibition of 60% and 81%, respectively, more than the two drugs alone [7].

In NCI-H526 SCLC xenograft cancer model, sunitinib showed synergism with cisplatin-based chemotherapy (1.5 mg/kg i.p.), respect to the two drugs alone.

**today**

**gastrointestinal stromal cancer**

GISTs are rare tumors that have constitutively activated KIT receptor in 90% of the cases or PDGFR in ~5%.

Imatinib, a selective inhibitor of these receptors, is effective in the treatment of metastatic or non-resectable GISTs. About 12%–14% of patients are resistant to imatinib, 40% develop resistance to the drug in 2 years and 5% have intolerance to imatinib [8]. This explains the need of new effective drugs for the treatment of these patients. Generally, resistance to imatinib is secondary to new mutations of KIT or PDGFRα, and sunitinib can bypass this resistance for the antiangiogenetic effects and for the activity on structural variants of mutated tyrosine kinases or on alternative pathways [9, 10].

This hypothesis was proved in a phase I/II trial enrolling 97 patients with metastatic GISTs resistant to imatinib. Of the 92 assessable patients, 88 progressed during therapy with imatinib and 4 had intolerance to the drug.

Partial response (PR), according to Response Evaluation Criteria in Solid Tumors (RECIST) criteria, was obtained in 7 (8%) and stable disease (SD) was obtained in 53 patients (58%), with clinical benefit of 65%.

The response of 39 patients was examined by 2-[fluorine-18]fluoro-2-deoxy-D-glucose (FDG–PET), evidencing a PR in 28 patients (72%). This trial showed that sunitinib is effective in patients with primary and secondary resistance to imatinib, and that FDG–PET is a method to have an early indication of response [18].

The encouraging results of this trial induced to start a randomized phase III trial of sunitinib versus placebo in patients with GIST resistant or intolerant to imatinib [16]. Patients were randomized in double blind 1:1 to receive sunitinib or placebo. The interim analysis of the first 13 weeks from randomization showed significant improvement in time to progression (TtP—primary objective of the trial), in sunitinib arm. Because of these results, all patients of the placebo arm were crossed over to sunitinib. TtP in placebo arm was 6.4 weeks versus 27.3 weeks in sunitinib arm (*P* < 0.00001).

PR was obtained in 7% and SD in 58%, with clinical benefit (SD + PR) in 65%. Even, if apparently low, 7% response rate (RR) was significantly higher than placebo (0%).

In this trial were enrolled 13 patients with intolerance to imatinib and 9 were in sunitinib arm. Between these nine patients, four obtained PR and four SD [11].

Fifty-nine patients were crossed over to sunitinib, obtaining PR in 10% and 31%. Even after only 13 weeks, 59% of the patients passed from placebo to sunitinib.

Is it important to consider the relationship between cancer mutations and efficacy to sunitinib, examined in a group of patients enrolled in a phase I/II trial? Clinical benefit, defined as PR and SD >6 months, was observed in all the more important molecular subtypes of GIST, with sunitinib. PR rate in GIST with primary mutations of exon 9 was 37% versus 5% in GIST with mutations of exon 11. TtP and overall survival (OS) rate were significantly higher in patients with KIT mutations on exon 9 or with native KIT/PDGFRα genes than in patients with KIT mutations on exon 11. Secondary KIT mutations were observed more frequently in GIST with primary mutations on exon 11 (62%) than GIST with primary mutations on exon 9 (16%). Secondary KIT mutations on exons 13 and 14 resulted sensible to sunitinib, much more than mutations on exons 17 and 18. As imatinib is more effective in GIST with KIT mutations on exon 11, sunitinib seems to be more active with mutations on exon 9, observable in 13% of patients [12].
renal cell carcinoma

Progresses of molecular biology opened new therapeutic prospectives for RCC treatment. There are different histological types of RCC, caused by different genes and with different prognosis. The most frequent is clear-cell carcinoma (75%). The hereditary and the sporadic forms of RCC are both related to mutations of von Hippel–Lindau (VHL) gene, an oncosuppressor gene, whose activation causes VHL syndrome. Hereditary RCC has an incidence of 40% in patients with VHL syndrome, for a germinal mutation of the VHL gene followed by a second somatic mutation, according to the Knudson hypothesis.

In patients with sporadic RCC, a biallelic inactivation for somatic mutations of VHL gene is detectable in 75%, while absence of gene expression for hypermethylation is detectable in ~20%.

The molecular therapy for RCC born after the explaining of working mechanism of VHL gene. VHL gene is located on chromosome 3p25–26, and has functions in the hypoxia-inducible pathway. VHL gene product is a multiprotein complex composed of ElonginB, ElonginC, Cul2 and Rbx1, and this complex ubiquitinates transcriptional hypoxia-inducible factor 1α (HIF-1α). The normal function of HIF, a heterodimer composed of α and β subunits, is to regulate expression of several genes in response to hypoxic stress. In normal conditions, as in presence of wild-type VHL and normal oxygen tension, HIF-1α is enzymatically hydroxylated on two proline residues located in the ‘oxygen-dependent degradation domain’. Then, HIF-1α is hydroxylated and this process allows for hydrogen bond-mediated complex formation between the complex and VHL. HIF-1α is subsequently ubiquinated by the VHL complex and ultimately degraded within proteasomes. Under hypoxic conditions, HIF-1α is not hydroxylated and thus cannot bind and be efficiently ubiquinated by the VHL protein complex. Biallelic inactivation of VHL would likewise prevent ubiquitination and ultimate degradation of HIF-1α. HIF-1α activity is regulated by growth factor that binding to a tyrosine kinase receptor increases HIF-1α levels by molecular pathways such as PI3K/AKT-mTOR and Ras/Raf/Map kinase. HIF-1α can increase by the integrin-mediated stimulation of PI3K/AKT-mTOR. Once stabilized, HIF-1α translocates into the nucleus where it complexes with the constitutively present HIF-1β to form the active transcriptional factor HIF 1 heterodimer. HIF 1 binds to different additional transcriptional cofactors, forming a pre-initiation complex of proteins that ultimately activate transcription of hypoxia-inducible genes, including VEGF, EGFR, PDGF, transforming growth factor α and erythropoietin. RCC is a highly vascular tumor, with increased VEGF level, and its growth can be stimulated by factors produced through the HIF 1 pathway [13].

As treatment of RCC, sunitinib was used for the first time at the dose of 50 mg/die for 28 days every 6 weeks. In a nonrandomized trial presented at the 2004 American Society of Clinical Oncology (ASCO) Congress and published on Journal of Clinical Oncology, were enrolled 63 patients with RCC pretreated with cytokine-based therapy [interferon (IFN), interleukin-2]. Primary objective was RR, and sunitinib obtained PR of 40% and SD of 27%. Median TTP was 8.7 months. Because of these results, a second nonrandomized phase II trial, enrolling 106 patients, confirmed the results of the first trial. On 105 assessable patients, RR was 43%, with 22% SD and progression-free survival (PFS) of 8.1 months [14, 15].

The combined analysis of these two phase II trials, on 168 enrolled patients, evidenced objective responses and SD for >3 months, respectively, in 42% and 24% of patients with PFS of 8.2 months. These results are incredibly important considering that the activity of the drugs in use in metastatic RCC obtains a 5% RR.

Then started an international randomized phase III trial of sunitinib versus IFNα in non-pretreated patients with metastatic RCC. On 750 patients, sunitinib arm RR was 31% versus 6% for IFNα. PFS was 11 months for sunitinib arm versus 5 months for IFNα, and the difference is statistically significant (P < 0.000001). Preliminary data were presented at the Plenary Session of 2006 ASCO Congress, where was presented the actuarial survival that will be significant and confirmed on the trial publication on January 2007, even if median OS is not still available [16, 17].

In another phase II trial of sunitinib in metastatic RCC resistant to bevacizumab was observed reduction in tumor size in 81%. This is because sunitinib could block some molecular pathways involved in resistance to bevacizumab [18].

future

Several trials are ongoing to test sunitinib in different types of cancer.

In the treatment of GIST, is in-course a trial of sunitinib versus imatinib 800 as first-line treatment, and will start a trial of sunitinib versus imatinib 400.

In the treatment of RCC, two trials are ongoing: the first is the Eastern Cooperative Oncology Group ASSURE trial of sunitinib versus sorafenib versus placebo, and the second is sunitinib versus placebo, both as adjuvant treatment of RCC [19].

In the treatment of NSCLC, after preliminary data from phase II trial in heavily pretreated patients, several trials are going to start: a randomized trial of sunitinib versus erlotinib, differentiating for the molecular typization, a trial of sunitinib as treatment of NSCLC brain metastases and some trials of sunitinib in association to standard chemotherapy as treatment of metastatic NSCLC.

Different trials are ongoing in metastatic breast cancer too. A trial of sunitinib as treatment of triple-negative patients, a trial of sunitinib versus capecitabine as second-line treatment and a trial of capecitabine with or without sunitinib. As first-line treatment, some trials are going to start using sunitinib in association to docetaxel ± trastuzumab.

After the encouraging results of a phase I trial presented recently at the ASCO GI about the association of sunitinib with Fluorouracil leucovorin oxaliplatin (FOLFOX) and Fluorouracil leucovorin irinotecan (FOLFIRI) as treatment of metastatic colorectal cancer, different trials will test the
association of sunitinib with standard chemotherapy as first-line treatment, will compare the association Fluorouracil leucovorin oxaliplatin irinotecan (FOLFOXIRI) bevacizumab versus FOLFOXIRI/sunitinib as neo-adjuvant treatment of liver metastases from colorectal cancer and will test the efficacy of the combination of sunitinib and radiotherapy as neo-adjuvant treatment of locally advanced rectal cancer.

Other trials are going to test the activity of sunitinib in the treatment of gastric cancer, sarcomas, NET, pancreas cancer, for example.

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