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Pharmacology of imatinib (STI571)

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Abstract

Deregulation of protein kinase activity has been shown to play a central role in the pathogenesis of human cancer. The molecular pathogenesis of chronic myelogenous leukemia (CML) in particular, depends on formation of the *bcr-abl* oncogene, leading to constitutive expression of the tyrosine kinase fusion protein, Bcr-Abl. Based on these observations, imatinib was developed as a specific inhibitor for the Bcr-Abl protein tyrosine kinase. The expanding understanding of the basis of imatinib-mediated tyrosine kinase inhibition has revealed a spectrum of potential new antitumor applications beyond the powerful activity already reported in the treatment of CML. Imatinib has shown activity in vivo against PDGF-driven tumor models including glioblastoma, dermatofibrosarcoma protuberans and chronic myelomonocytic leukemia. Antiangiogenic effects have been demonstrated by inhibition of PDGF-, VEGF (vascular endothelial growth factor)- and bFGF- (basic fibroblast growth factor) induced angiogenesis in vivo, and by inhibition of angiogenesis and tumor growth in an experimental bone metastasis model. Imatinib has been shown to reduce interstitial fluid pressure in an experimental colonic carcinoma model by blocking PDGF-mediated effects on tumor-associated blood vessels and stromal tissue. It is also a potent inhibitor of the Kit receptor tyrosine kinase, and has demonstrated activity clinically against the Kit-driven gastrointestinal stromal tumor (GIST) and experimentally in small-cell lung cancer cell lines. The pharmacology of imatinib and its activity in various tumor models is discussed.

Keywords: Gastrointestinal stromal tumor; Chronic myelogenous leukemia; Imatinib; Bcr-Abl; Kit; Platelet-derived growth factor receptor

1. Introduction

The protein tyrosine kinases (PTKs) constitute a large and diverse family of homologous proteins that serve as important regulators of intracellular signal transduction pathways. Their activities control a range of fundamental cellular processes including growth, metabolism, differentiation, adhesion and apoptosis [1]. The deregulation of protein kinase activity has been shown to play a central role in the pathogenesis of human cancer [2,3]. In particular, the molecular pathogenesis of chronic myelogenous leukemia (CML) depends on formation of the *bcr-abl* oncogene, leading to constitutive expression of the tyrosine kinase fusion protein, Bcr-Abl [4].

Based on these observations, imatinib (Glivec®, formerly STI571, Novartis Pharma AG, Basel, Switzerland) was developed as a specific inhibitor of the Bcr-Abl protein tyrosine kinase [5,6]. Imatinib competes with ATP for its specific binding site in the kinase domain and has been shown to be highly active in the treatment of CML [7]. Recent clinical studies show that the drug might also be useful for the treatment of gastrointestinal stromal tumors (GISTs), which is driven by the deregulated tyrosine kinase activity of Kit [8–10].

As shown in Table 1, in addition to various oncogenic forms of the Bcr-Abl tyrosine kinase, imatinib selectively inhibits the ABL-related gene (ARG) protein [11], the platelet-derived growth factor (PDGF) receptor and Kit [12,13]; but does not inhibit other receptor or cytoplasmic tyrosine kinases [12]. With increasing understanding of the molecular, structural and biological aspects of imatinib-mediated tyrosine kinase inhibition, it has become possible to investigate what additional potential imatinib may have

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Table 1
Cellular selectivity of imatinib

Kinase	IC_{50} (μM)
v-Abl	0.1-0.3
$p210^{bcr\text{-}abl}$	0.25
$p190^{bcr\text{-}abl}$	0.25
TEL-Abl	0.35
TEL-Arg	0.5
PDGF receptor	0.1
TEL-PDGF receptor	0.15
Kit (SCF receptor)	0.15
Flt-3	>10
c-Fms and v-Fms	>10
KDR	>10
c-erbB1, c-erbB2	>100
Insulin receptor	>100
IGF-I receptor	>100
v-Src	>10
Jak-2	>100

Imatinib concentrations causing a 50% reduction in cellular kinase activity (IC_{50}) are given.

in the treatment of other solid tumors, and what effects it may have on aspects of cancer pathogenesis more generally.

1.1. Imatinib inhibition of the PDGF receptor

PDGF is a connective tissue cell mitogen that has also been shown to play an important role in tumorigenesis [14]. Four different isoforms of PDGF have been identified: PDGF chains A, B, C and D [14,15]. Active PDGF molecules are dimeric forms that include A, B, C and D chain homodimers and an AB heterodimer. The dimeric isoforms bind to two structurally similar tyrosine kinase receptors, PDGF-α and PDGF-β. Because the PDGF isoforms are dimeric molecules, their binding to the PDGF receptor causes dimerization, leading to autophosphorylation and activation of the kinase activity of the receptor. Ligand-induced receptor dimerization activates a number of intracellular signaling pathways that ultimately promote cell growth, changes in cell morphology and prevention of apoptosis [14].

1.1.1. Imatinib inhibits both the α and β receptors of PDGF

The fibroblast cell line Swiss 3T3 possesses significant numbers of both PDGF-α and PDGF-β receptors. PDGF-AA binds to and activates only PDGF receptor α, while PDGF-BB binds to and stimulates all PDGF receptors, including α and β homodimers and α-β heterodimers. Pretreatment of Swiss 3T3 cells with imatinib caused a concentration-dependent inhibition of PDGF-AA-induced PDGF-α receptor autophosphorylation with an IC_{50} value of approximately 0.1 μM; similar inhibition of autophosphorylation was observed after stimulation with PDGF-BB. These data indicate that imatinib inhibits both the PDGF-α and PDGF-β receptor [12].

1.1.2. Imatinib inhibition of PDGF-mediated cellular events

One of the early events that occurs after growth factor stimulation is the activation of so-called immediate early genes such as c-*fos*. As shown in Fig. 1, imatinib selectively inhibits PDGF-stimulated c-*fos* mRNA induction without affecting c-*fos* mRNA expression induced by epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), or phorbol ester [5]. Imatinib also inhibits the PDGF-mediated downstream cellular signaling event, inositol phosphate release, in rat A10 aortic smooth muscle cells (Fig. 2A) and selectively inhibits A10 cellular growth (Fig. 2B) [12].

1.2. Antitumor activity of imatinib in PDGF-driven tumors

1.2.1. v-sis transformed fibroblasts

The v-*sis* line is a NIH3T3 fibroblast line that has been stably transfected to express the viral homolog of PDGF, which renders this cell line tumorigenic. Imatinib appeared to be a well-tolerated and effective antitumor therapy for these tumors [16]. Treatment of nude mice bearing v-*sis* tumors derived from transplanted fragments with 50 mg/kg imatinib, p.o., q24 h produced weak antitumor effects (T/C 52%). At this dose, imatinib appeared rapidly in the circulation; at 30 min (the first time point measured) the mean plasma concentration was 7.6 ± 0.7

Fig. 1. Selective inhibition of c-*fos* mRNA induction [5]. Confluent, quiescent BALB/c 3T3 cells were incubated for 90 min with the indicated concentrations of imatinib. After stimulation with PDGF-BB (10 ng/ml) (lanes 2–7), EGF (20 ng/ml) (lanes 8–11), bFGF (100 ng/ml) (lanes 12–14), or PMA (25 ng/ml) (lanes 15–18), samples of total RNA were analyzed by Northern blotting using a v-*fos* probe.

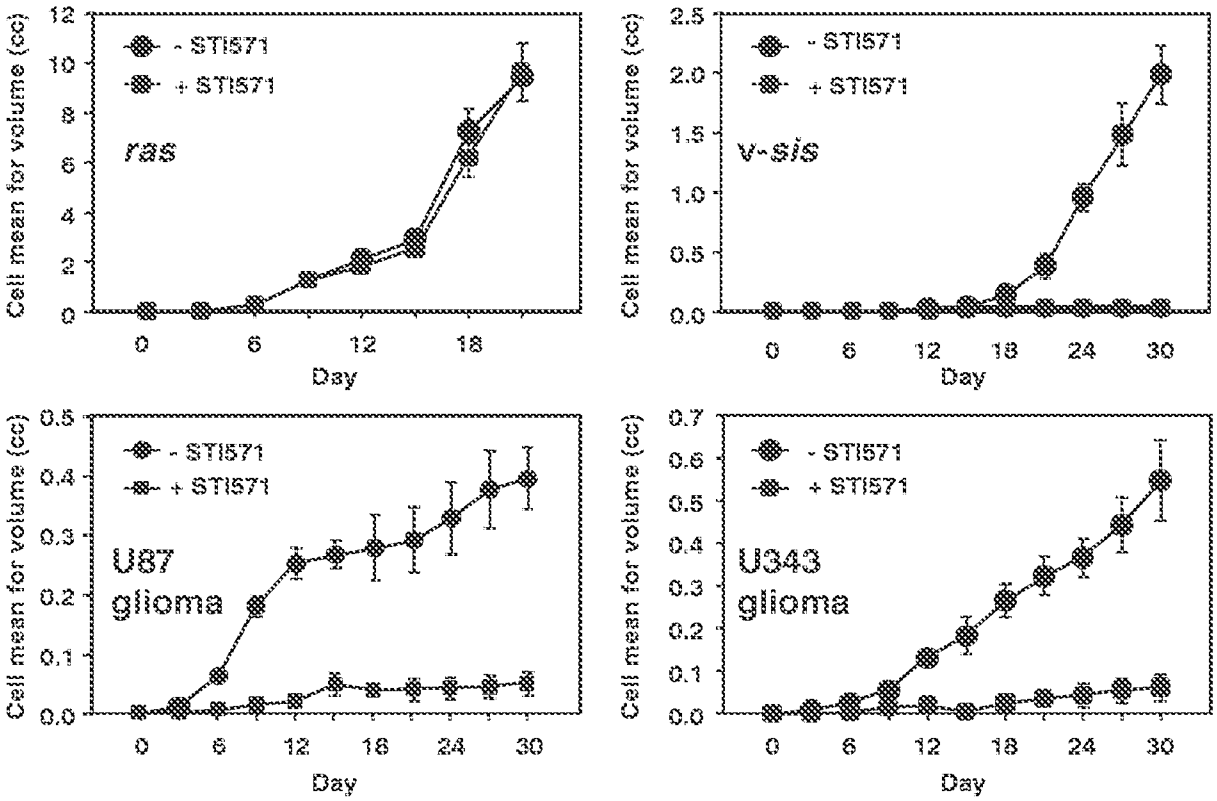

Fig. 2. Inhibition of PDGF-induced inositol phosphate formation and PDGF-induced proliferation [12]. A: [^{3}H]inositol-labeled A10 cells were preincubated with increasing concentrations of imatinib for 30 min and then stimulated with 10 ng/ml PDGF for 5 min at 37°C. Values shown are the mean ± SEM obtained from three experiments. Where no error bar is shown, the error is within the size of the symbol. B: Growth-arrested A10 cells were incubated with increasing concentrations of imatinib for 30 min before stimulation with 10 ng/ml PDGF or 10% serum at 37°C. The effects on proliferation were assessed using the colorimetric MTS reduction assay after 24 h of treatment. Values shown are the mean ± SEM obtained from three experiments.

μg/ml. The concentration declined thereafter, though even at 12 h a mean plasma concentration of 0.14 ± 0.02 μg/ml imatinib was found. At later time points, imatinib was detected at low levels around the limit of quantification (0.05 μg/ml) in some mice. Tumor concentrations peaked at lower concentrations (mean approximately 4.5 μg/g) than those in plasma but declined at a slower rate. Modeling of the pharmacokinetic data suggested that a once-daily p.o. administration of imatinib at 50 mg/kg is not sufficient to attain trough concentrations in tumors consistently above the IC_{50} for inhibition of cell growth. However, increasing either the dose to 150 mg/kg once per day or the frequency of administration to three times daily produces trough tumor concentrations that exceed 10× IC_{50} after the first day of treatment. 50 mg/kg imatinib, q8 h, improved effectiveness of imatanib (T/C 36%, $P < 0.05$ versus controls), and administration of 150 mg/kg, q24 h produced a T/C of 26% ($P < 0.05$ vs. controls) without evidence of regressions. All treatments were well tolerated, permitting increases in mouse body weight. In addition to demonstrating the activity of imatanib against PDGF-driven tumors, these data also indicate that continuous administration of imatinib improves effectiveness.

1.2.2. Glioma

There is extensive evidence that autocrine activation of the PDGF receptor plays a role in the pathogenesis of glioblastoma. The PDGF A and B ligands and PDGF-α receptor are coexpressed in virtually all glioma cell lines and in fresh surgical isolates of human malignant astrocytoma [17–19]. Evidence that the PDGF ligand receptor system plays an initiating role in glioblastoma is provided by studies showing that injection of a retroviral expression vector encoding PDGF B (the universal ligand, binding to all PDGF receptor types) into the brain of newborn mice induces astrocytomas [20]. Evidence for a maintenance role is derived from studies showing growth inhibition and reversion of the transformed phenotype using neutralizing antibodies to PDGF and dominant-negative mutations of either the PDGF ligand or PDGF receptor [21,22].

As shown in Fig. 3, imatinib inhibits the formation of tumors in nude mice by v-*sis*-transformed 3T3 cells and the two human glioblastoma cell lines U343 and U87, but does not inhibit tumor formation by *ras*-transformed 3T3 cells [16]. These results are consistent with selective inhibition by imatinib of tumor formation from cells that express PDGF receptor autocrine loops, and are in agree-

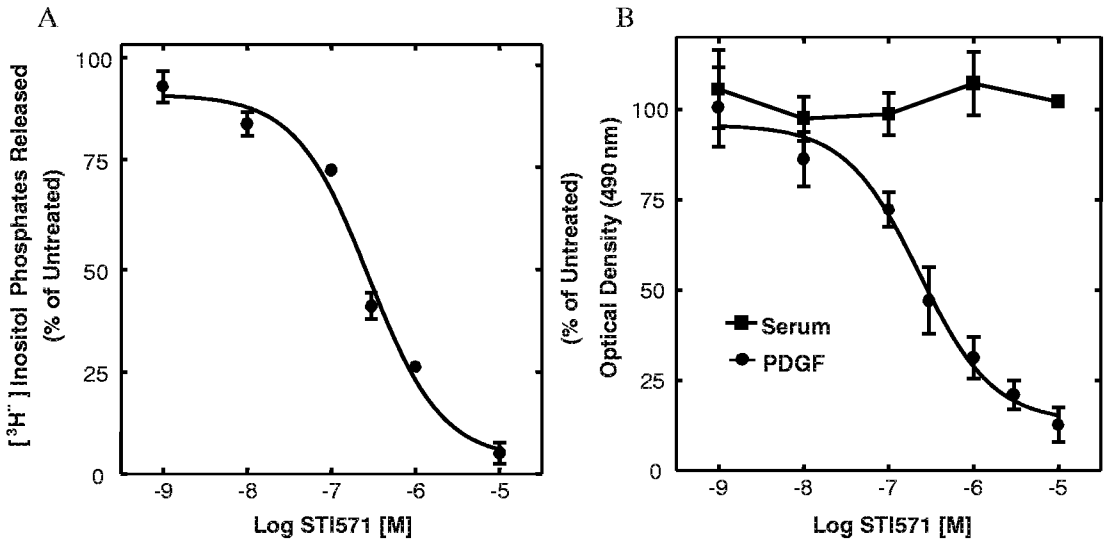

Fig. 3. Inhibition of PDGF-mediated tumor growth in nude mice [16]. Two independent lines of transformed Balb/c 3T3 cells (*ras* or v-*sis*) and two independent glioblastoma cell lines (U343 or U87) were inoculated into male nude mice. At 5 days after inoculation, the mice began receiving imatinib (50 mg/kg per day) or solvent control (DMSO) as indicated. The drug was injected into the peritoneal cavity in twice-daily doses of 25 mg/kg.

ment with the results obtained in vitro with more extensive panels of human tumor cell lines [16].

1.2.3. Dermatofibrosarcoma protuberans

The PDGF ligand receptor system has been implicated in human malignant soft tissue sarcoma [23]. Dermatofibrosarcoma protuberans (DFSP) is a highly recurrent, infiltrative skin tumor of intermediate malignancy that is locally aggressive and largely resistant to nonsurgical treatment. DFSP isolates display rearrangements involving chromosomes 17 and 22, resulting in fusion of the *COL1A1* and *PDGFB* genes. The fused gene is processed to functional PDGF-BB chain, triggering autocrine stimulation of the PDGF receptor [24].

In COL1A1/PDGF-transformed NIH3T3 cells, treatment with imatinib slows the growth rate in vitro and in vivo and reverses the malignant phenotype [25]. Similarly, in primary DFSP and giant cell fibrosarcoma (GCF) cell cultures, imatinib reduces PDGF receptor activation in vitro and in vivo and inhibits cellular growth [26]. The inhibitory effects are mediated predominantly through induction of tumor cell apoptosis [26]. Together, these data indicate that imatinib may be a candidate for pharmacologic treatment of DFSP and GCF.

1.2.4. Chronic myelomonocytic leukemia (CMML)

CMML is a subtype of myelodysplastic syndrome characterized by dysplastic monocytosis, bone marrow fibrosis and progression to AML [27]. It is associated with a recurring t(5;12) chromosomal translocation that results in formation of a TEL/PDGF receptor β fusion protein. Transformation of hematopoietic cells occurs through oligomerization of the TEL/PDGF-β receptor fusion protein, which causes constitutive activation of the PDGF-β receptor kinase domain. This unique activation occurs independently of ligand stimulation [28,29].

In transgenic mice expressing TEL/PDGF-β receptor, treatment with imatinib inhibited tumor formation and prolonged survival compared with phosphate-buffered saline (PBS)-treated controls. Imatinib showed therapeutic benefit both in animals treated before the development of overt clonal disease and in those transplanted with clonal tumor cells, suggesting that the compound is efficacious both early in the course of disease and after development of additional transforming mutations [27].

1.3. Inhibition of tumor angiogenesis

The PDGF-β receptor is expressed on vascular endothelial cells, and PDGF has been shown to have angiogenic activity in various models [14]. In addition, PDGF may not only act as a direct mitogen for endothelial cells, but has also been shown to induce expression of vascular endothelial growth factor (VEGF) in endothelial cells, which in turn causes an autocrine loop through stimulation of VEGF receptors [30]. Since PDGF-responsive stromal and perivascular cells are a major source of VEGF, PDGF may also support blood vessel formation indirectly through paracrine stimulation [31]. In addition, PDGF influences angiogenesis through recruitment of pericytes and stimulation of vascular smooth muscle cells [32].

Based on these observations, imatinib was investigated for possible antiangiogenic activity. As shown in Fig. 4, imatinib was found to inhibit potently serum-stimulated capillary sprouting from rat aorta. Furthermore, in an in vivo model for growth factor-induced angiogenesis, the drug inhibited PDGF- and — surprisingly — also VEGF- and bFGF-stimulated vascularization of a subcutaneous

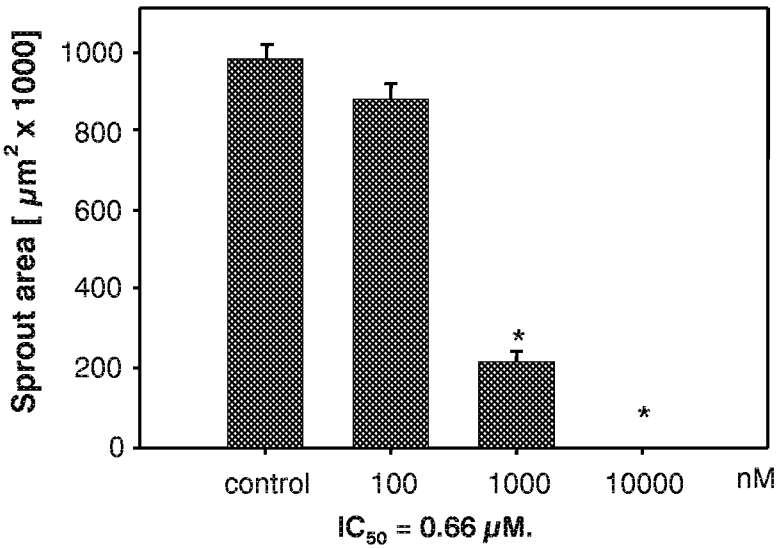

Fig. 4. Effects of imatinib on sprout formation induced by FCS in the rat aortic square assay. Pieces (1 × 1 mm) of rat aorta were grown in a fibrin gel in the presence of 10% fetal calf serum and increasing concentrations of imatinib (0.1–10 μM) or vehicle (control) (*x*-axis). Samples were then incubated for six days. Aminocaproic acid (300 μg/ml) was added on the first three days to prevent degradation of the gel. The media was changed on the fourth day, with fresh additions of all required factors and 50 μg/ml aminocaproic acid. The area of sprouts formed was measured from the image viewed under an inverse microscope using a KS-400 imaging system (*y*-axis). Imatinib induced concentration-dependent inhibition of the formation of sprouts. Two experiments were performed that showed the same results. Data presented in the graph are pooled from the two experiments. Results are presented as mean ± SEM, $n = 7–8$. $^*P < 0.001$ significance compared to control (Tukey test).

implant in mice (Table 2). These results raise the possibility that in vivo endothelial cell activation by VEGF or bFGF may result in local PDGF production, and imatinib may inhibit the response of smooth muscle cells and pericytes to endogenous, locally produced PDGF. This could prevent the stabilization of capillaries and larger vessels. Alternatively, the effects of imatinib might be due to another, as yet unidentified, target involved in the angiogenic response.

Imatinib has also been tested in an experimental bone metastasis model using PC-3MM human prostate cancer cells. Tumor cells were injected into the tibia of nude mice, where they grow by lysis of bone. Oral administration of imatinib — and, even more so, combined treatment with imatinib and taxol — resulted in significant inhibition of tumor growth and preservation of bone structure. Immunohistochemical studies showed that tumor cells growing in the bone (but not those in surrounding musculature) expressed high levels of PDGF-A, PDGF-B and PDGF-α and -β receptors. Tumor-associated endothelial cells within the bone also expressed PDGF-α and -β receptors. Furthermore, treatment with imatinib and taxol inhibited tumor cell proliferation and induced apoptosis both in tumor cells and tumor-associated endothelial cells. These data suggest that inhibition of the PDGF receptor, in combination with chemotherapy, may provide an approach to the therapy of bone metastasis, in part at least through inhibition of tumor-associated angiogenesis and perhaps a direct antitumor cell effect [33].

Table 2
Effects of imatinib on the angiogenic response induced by human angiogenic growth factors in normal mice

Response	Percent inhibition of response induced by growth factor-impregnated implants		
	VEGF	BFGF	PDGF
Increase in tissue weight	90 ± 8	74 ± 7	100 ± 4
Increase in blood content	94 ± 6	89 ± 4	100 ± 15

Porous chambers containing VEGF (2 μg/ml), bFGF (0.3 μg/ml) or PDGF (3 μg/ml) in 0.8% w/v agar containing heparin (20 U/ml) were implanted subcutaneously in the flank of Tiflbm : MAG mice. The growth factors induce the growth of vascularized tissue around the chamber. This response is quantified by measuring the weight and blood content of the tissue. Mice were treated with imatinib (50 mg/kg p.o. twice daily) starting one day before implantation of the chambers and the animals were sacrificed for measurement of the vascularized tissues after five days of treatment.
Values are mean ± SEM, $n = 11$, $^*P < 0.05$ (rank sum test) significant inhibition compared to the response in the group receiving growth factor alone.

1.4. Regulation of interstitial fluid pressure (IFP)

Several lines of evidence indicate a role for PDGF in the regulation of interstitial fluid pressure (IFP) [34,35]. The mechanism involves the PDGF-stimulated interactions between the connective tissue cells and extracellular matrix molecules, as well as effects of PDGF on the intracellular actin filament system causing a tensile strength. The exact mechanism involved in this effect of PDGF is not known, but is mediated by the action of phosphatidylinositol 3′-kinase (PI3-kinase), since transgenic mice carrying

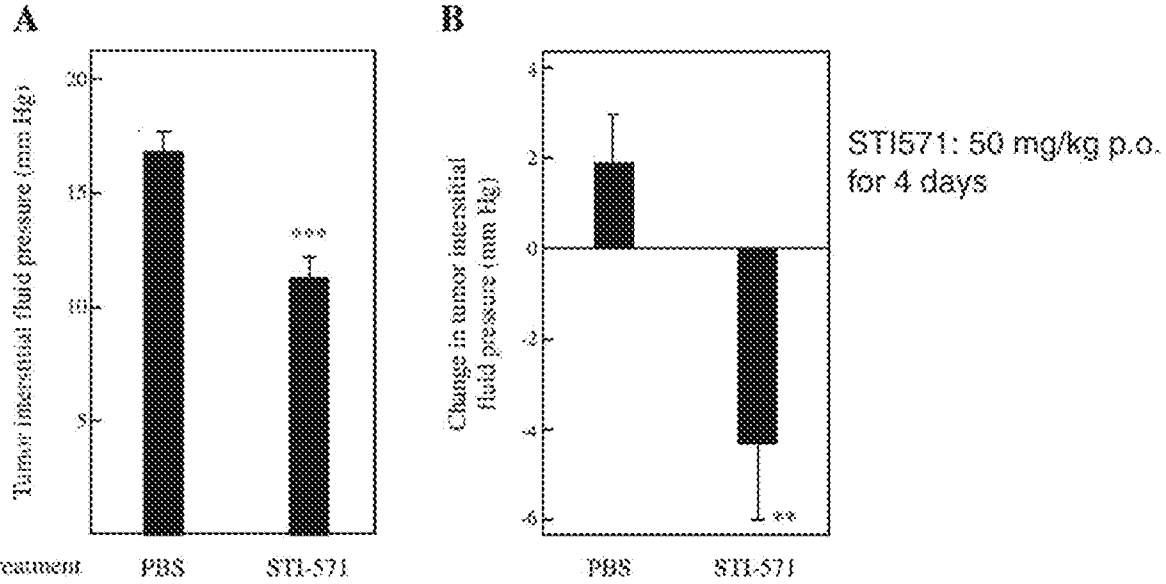

Fig. 5. Imatinib lowers tumor IFP in PROb tumors [42]. Tumor IFP was determined before and after a 4-day treatment with imatinib of BDIX rats bearing s.c. growing rat PROb colonic carcinomas. A: Average tumor IFP and B: change in tumor IFP induced by imatinib for each individual tumor. Reprinted with permission from Pietras *et al*, *Cancer Research*, Vol. 61, 2929–2934, 2001.

mutant PDGF-β receptor that is unable to bind and activate PI3-kinase cannot restore IFP upon PDGF stimulation following an allergic challenge [36].

The PDGF receptor is expressed in the stroma of a broad range of solid tumors, including colorectal adenocarcinomas [14,37,38]. Solid tumor stroma differs from normal loose connective tissue in several respects. The major physiological difference is increased IFP [39], resulting in a diminished hydrostatic gradient from capillary to interstitium with impaired exchange of solutes across the capillary membrane [40,41]. It has been proposed that pharmacological intervention to increase the transcapillary pressure gradient between capillary and tumor interstitium might provide a means to increase uptake of anticancer agents into tumors [39].

The effects of imatinib on tumor IFP and transcapillary transport were investigated in subcutaneously growing PROb rat colonic carcinomas [42]. As shown in Fig. 5, treatment with imatinib significantly reduced tumor IFP. Concomitantly, transcapillary transport of a radiolabeled tracer compound into the tumor interstitium was significantly increased [42]. These effects were mediated by inhibition of the PDGF receptors expressed on blood vessels and stromal cells, as tumor epithelial cells in this tumor model do not express PDGF receptors. These results indicate a previously unrecognized role of PDGF receptors in tumor cell biology. Since paracrine PDGF signaling in stromal cells occurs in various types of solid tumor, interference with PDGF receptors or their ligands may be a novel strategy to increase drug uptake and the therapeutic efficacy of cancer chemotherapy.

1.5. Activity of imatinib in kit-related tumors

As shown in Fig. 6, imatinib inhibits stem cell factor (SCF)-stimulated autophosphorylation of KIT, a member of the Type III group of receptor kinases that also includes the PDGF receptor. Imatinib additionally inhibits SCF-stimulated downstream MAP kinase activation [12]. The c-*kit* gene product is expressed in hematopoietic progenitor cells, mast cells and germ cells [43–45]. Mutations of c-*kit* resulting in ligand-independent activation of the receptor have been identified in a number of tumor types [46–48].

1.5.1. Gastrointestinal stromal tumor (GIST)

Gastrointestinal stromal tumors (GISTs) are a group of mesenchymal neoplasms that arise from precursors of gastrointestinal tract connective tissue. Sequencing of c-*kit* from GIST specimens has revealed activating mutations in-

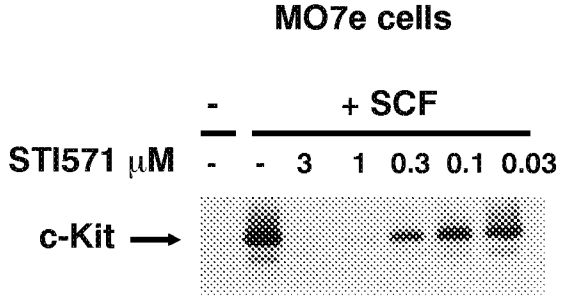

Fig. 6. Inhibition of Kit autophosphorylation by imatinib [12]. Serum-starved MO7e cells were incubated with the indicated concentrations of imatinib for 90 min prior to stimulation with SCF (50 ng/ml) for 10 min. Whole-cell lysates were corrected for protein content and analyzed by Western blotting using antiphosphotyrosine antibodies.

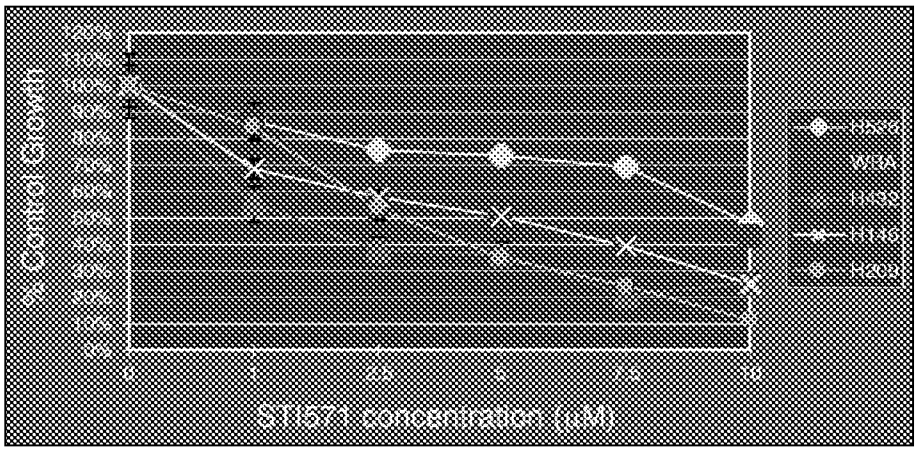

Fig. 7. Inhibition of serum-stimulated growth of SCLC cells [58]. Inhibition of cell proliferation in serum-containing medium (10% fetal calf serum) by imatinib was measured using the MTT colorimetric dye reduction method after 3 days of plating. Reprinted with permission from Krystal *et al*, *Clinical Cancer Research*, Vol. 6, 3319–3326, 2000.

volving exons 11, 9 and, less commonly, exon 13 [49,50]. Oncogenic c-*kit* mutations in GIST have been localized to the extracellular, juxtamembrane and kinase domains 1 and 2 of the c-Kit protein [49,50].

The human cell line GIST882 expresses an activating c-*kit* mutation in the first part of the cytoplasmic split tyrosine kinase domain. Treatment with imatinib rapidly and completely abolishes constitutive phosphorylation of GIST882 c-*kit*. Furthermore, prolonged incubation with imatinib induces decreased proliferation and the onset of apoptosis in GIST882 cells [51]. Similarly, a primary GIST cell culture expressing a c-*kit* exon 11 juxtamembrane mutation was also inhibited by imatinib [51]. GIST tumors historically have proven highly refractory to cytotoxic cancer chemotherapy, but clinical studies in patients with unresectable and/or metastatic GIST have shown that imatinib is an effective anticancer treatment for the majority of patients [8–10].

In human systemic mastocytosis, the majority of cases show a point mutation in codon 17 of c-*kit* resulting in an aspartic acid 816 to valine (D816V) amino acid substitution in the kinase 2 domain of Kit. Interestingly, this mutated Kit is resistant to inhibition by imatinib [52].

1.5.2. Small cell lung cancer (SCLC)

At least 70% of small cell lung cancers (SCLCs) express the Kit receptor tyrosine kinase and its ligand, SCF [53–55], suggesting that SCLC growth involves an autocrine loop. In support of this, inhibition of Kit activation by transfection of a dominant-negative c-*kit* gene results in loss of growth factor independence [56,57]. Furthermore, the Kit/PDGF receptor inhibitor AG1296 inhibits growth of SCLC cells in serum-containing medium [57].

In H526 SCLC cells, pretreatment with imatinib inhibited SCF-mediated Kit activation with an IC_{50} of 0.1 μM. Imatinib blocked downstream signal transduction, as evidenced by inhibition of SCF-mediated activation of MAP kinase and Akt, and potently inhibited SCF-mediated growth in serum-free medium, with marked increase in apoptosis [58]. Fig. 7 shows the growth of five SCLC cell lines in serum-containing medium in the presence of imatinib. All five lines coexpress Kit and SCF to some degree, with the exception of H146, which expresses high levels of SCF but not Kit. Imatinib inhibited the growth of all five cell lines in a dose-dependent fashion, with an average IC_{50} of approximately 5 μM. Surprisingly, even H146 showed intermediate sensitivity to the drug, suggesting that in addition to inhibiting Kit, imatinib may block other targets necessary for SCLC growth.

2. Conclusions

The expanding understanding of the structural and molecular bases of imatinib-mediated tyrosine kinase inhibition has revealed a spectrum of potential new antitumor applications, beyond the impressive activity already reported in the treatment of CML. Imatinib is a potent inhibitor of both the PDGF-α and -β receptors and has shown activity in vivo against PDGF-driven tumor models including glioblastoma, dermatofibrosarcoma protuberans and chronic myelomonocytic leukemia. Antiangiogenic effects have been demonstrated in the aortic sprout assay, in inhibition by imatinib of PDGF-, VEGF- and bFGF-induced angiogenesis in vivo, and by inhibition of angiogenesis and tumor growth in an experimental bone metastasis model. Imatinib has been shown to reduce the interstitial fluid pressure in the PROb rat colonic carcinoma model by blocking PDGF-mediated effects on tumor-associated blood vessels and stromal tissue. It is also a potent inhibitor of the Kit receptor tyrosine kinase, and has demonstrated activity clinically against the Kit-driven tumor GIST and experimentally in SCLC cell lines. Prospects for further clinical progress using the rational, molecularly designed

approach to cancer treatment exemplified by imatinib seem increasingly promising.

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