Receptor Tyrosine Kinase Activity and Apoptosis as Biomarkers of Sunitinib Malate (SU11248) Activity in Patients with Gastrointestinal Stromal Tumor

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Introduction

- Most gastrointestinal stromal tumors (GISTs) (approximately 85%) contain activating mutations in the gene encoding stem cell factor receptor (KIT), and these mutations are generally considered to be the driving force in the pathogenesis of GISTs.[1]
- Imatinib mesylate, which selectively inhibits KIT and platelet-derived growth factor receptors (PDGFRs), has demonstrated efficacy as first-line therapy for GIST.[2] However, imatinib resistance has emerged as a problem in GIST therapy. Some GISTs are initially resistant to imatinib, and many others develop resistance with continued therapy.[3–5]
- Sunitinib malate (SU11248) is an oral, multitargeted receptor tyrosine kinase (RTK) inhibitor with direct antitumor and antiangiogenic properties related to its ability to inhibit the tyrosine kinase activities associated with KIT, vascular endothelial growth factor receptors (VEGFR-1, -2 and -3), PDGFRs (PDGFR-α and -β), glial cell-line derived neurotrophic factor (rearranged during transfection; RET) and Fms-like tyrosine kinase-3 receptor (FLT3).[6–9]
- In a recent phase III trial, sunitinib demonstrated clinical benefit in GIST patients for whom imatinib therapy had failed due to resistance or intolerance.[10] While the efficacy of sunitinib in imatinib-resistant GIST patients may be due to differential KIT binding, it is also possible that its activity in these patients is related to inhibitory effects on other RTKs, namely PDGFRs and VEGFRs.
- The present study evaluated the ability of sunitinib to inhibit PDGFR-β and VEGFR-2 activity in patients with imatinib-resistant GISTs and examined the relationship between this inhibitory activity and clinical benefit. The study also evaluated the ability of sunitinib to induce apoptosis of tumor cells and endothelial cells (presumably related to angiogenesis), and related this to clinical benefit.

Materials and Methods

- In this phase I/II trial, 97 adult male and female patients with metastatic imatinib-resistant GIST and ECOG status 0–2 received sunitinib orally once daily for 14 or 28 days followed by 14 days without treatment per cycle. Biopsies were obtained at baseline and 11 days after initiating therapy in 20 of the patients.
- Quantitative analysis of RTK expression and activity in tumors was performed using laser scanning cytometry (LSC) detection of fluorescently labeled total and phosphorylated RTKs, as described previously.[11] Active RTKs were measured using phosphorylation-site-specific antibodies.
- Quantitative analysis of apoptosis in tumor and endothelial cells was performed using LSC detection of CD31 immunofluorescence (endothelial cells) and TUNEL (terminal deoxynucleotidyl transferase dUTP nick-end labeling), as described previously.[11]
- Tumor responses were evaluated using radiographic measurements and RECIST, and correlated with changes in RTK activity and apoptosis following sunitinib treatment. Clinical benefit was defined as partial response or stable disease >6 months.

Results and Discussion

Correlation of RTK Activity with Clinical Response

- Phosphorylated PDGFR-β (reflecting PDGFR-β activity) decreased by 18% in patients in which sunitinib therapy was associated with clinical benefit (Figure 1).
- Conversely, phosphorylation of PDGFR-β increased by approximately 10 percentage points in patients progressing on sunitinib therapy (Figure 2).

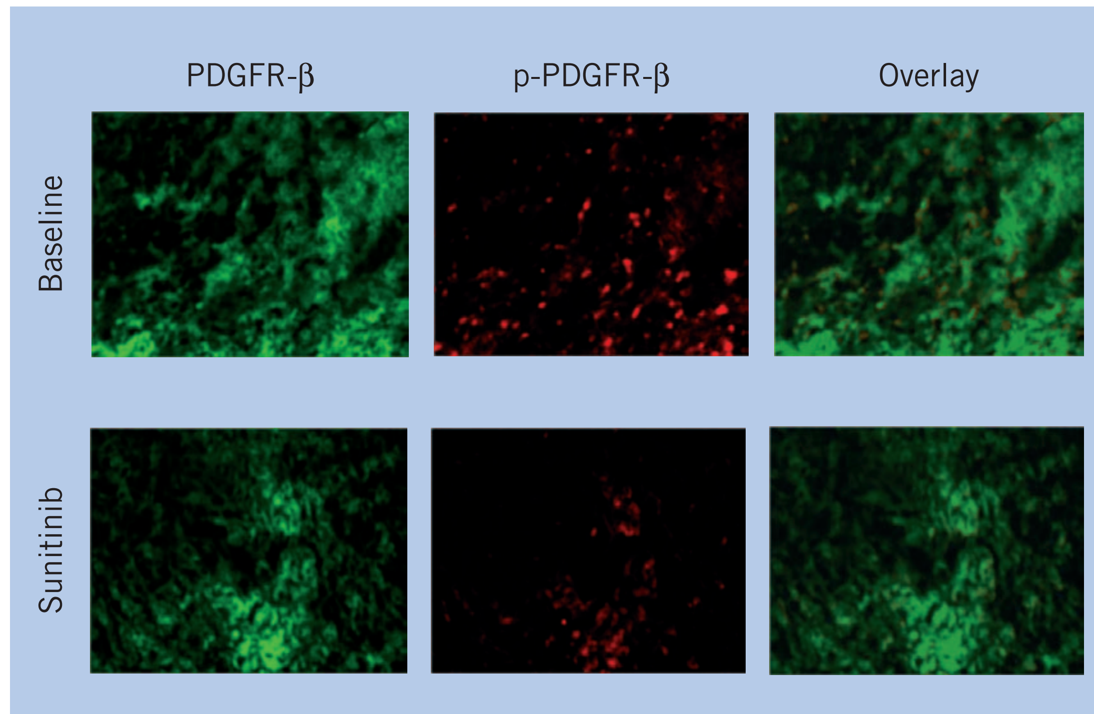

Figure 1. Clinical response was associated with substantial reduction in PDGFR-β phosphorylation. p = phosphorylated; scale: x20.

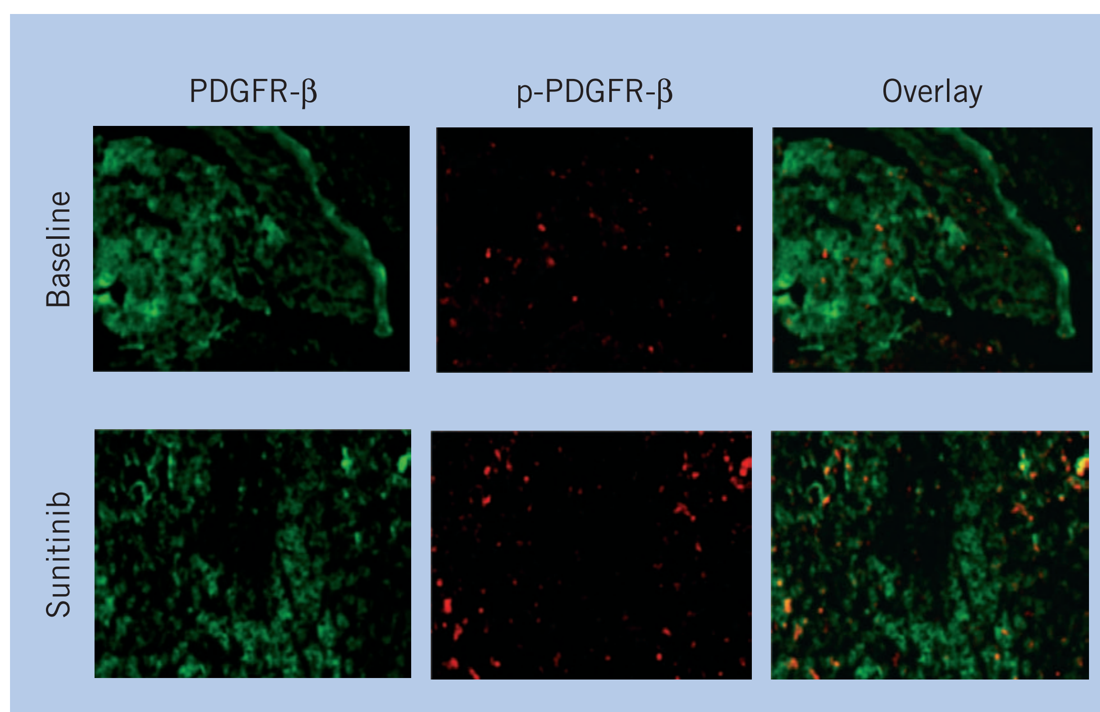

Figure 2. Disease progression was associated with increased phosphorylation of PDGFR-β. p = phosphorylated; scale: x20.

- Similar results were obtained with VEGFR-2 (Table 1).

Table 1. Change in RTK activity: correlation with clinical benefit.

Clinical response	No. of patients	Δ p-PDGFR-β	Δ p-VEGFR-2
Clinical benefit (PR or SD >6 months)	8	18.2% ↓ (P=0.006)	26.7% ↓ (P=0.02)
Progressive disease (SD <6 months)	12	9.9% ↑ (P=0.06)	9.6% ↑ (P=0.02)

p = phosphorylated; PR = partial response; SD = stable disease.

- When taken together and analyzed quantitatively (Figure 3), inhibition of PDGFR-β and VEGFR-2 phosphorylation appeared to be associated with clinical benefit, while increased RTK phosphorylation appeared to be associated with disease progression.

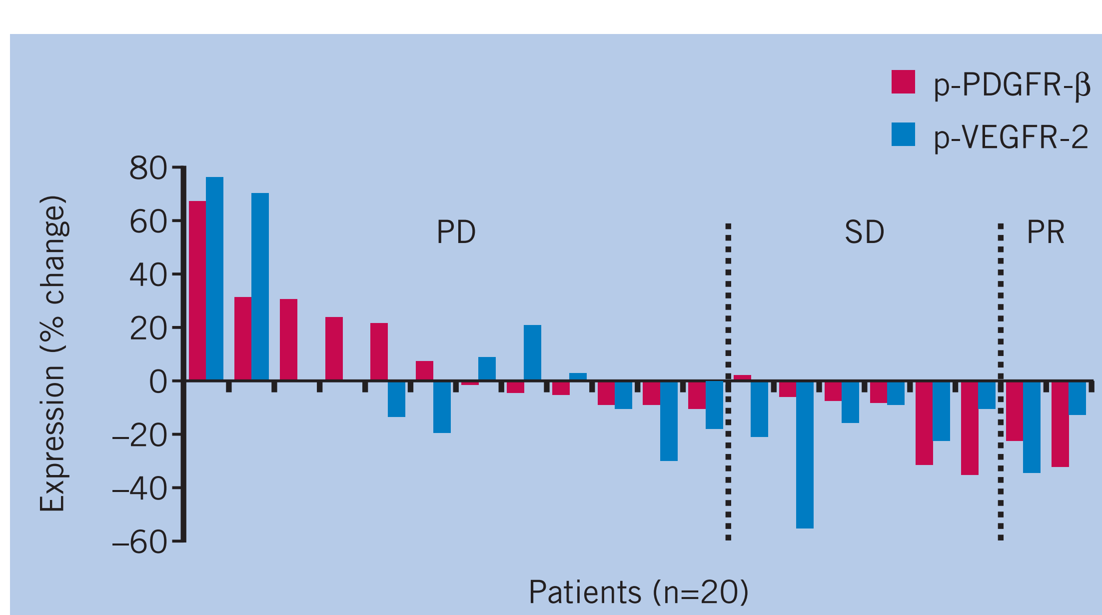

Figure 3. Quantitative analysis of phosphorylated PDGFR-β and VEGFR-2 expression. p = phosphorylated; PD = progressive disease; PR = partial response; SD = stable disease.

- Table 1 presents the correlation between change in RTK activity and clinical benefit. PDGFR-β and VEGFR-2 phosphorylation significantly decreased from baseline in patients experiencing clinical benefit on sunitinib therapy. The level of VEGFR-2 phosphorylation increased significantly in patients experiencing disease progression on sunitinib therapy, while there was a trend toward increased PDGFR-β phosphorylation compared with baseline in these patients.

Correlation of Tumor Apoptosis with Clinical Response

- Apoptosis generally increased in patients exhibiting clinical benefit on sunitinib therapy (Figure 4).

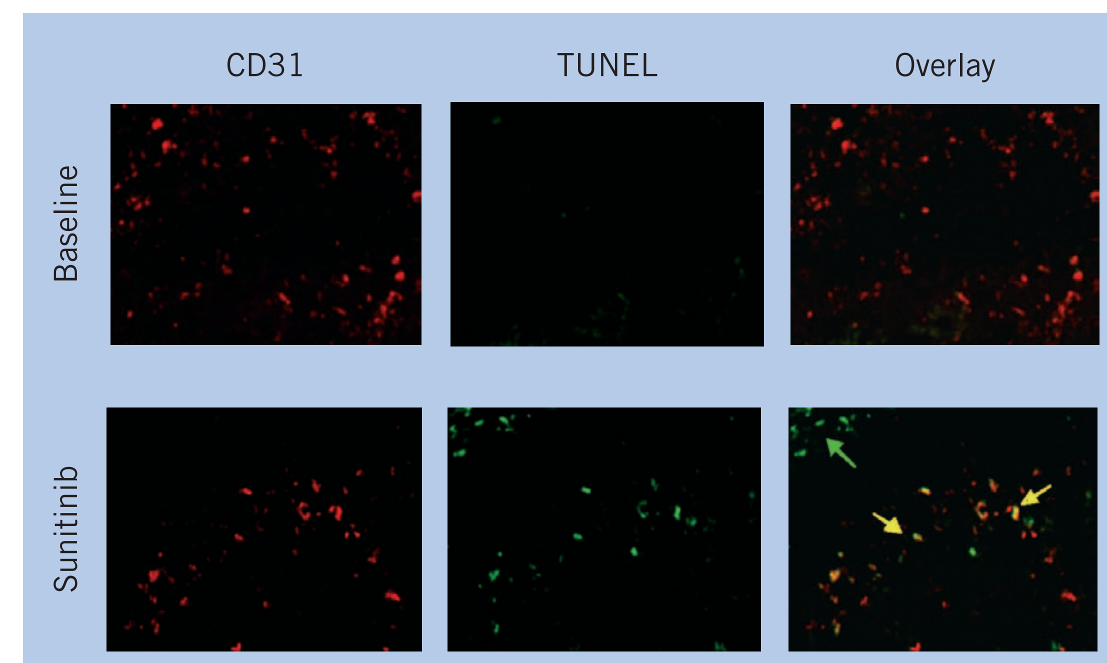

Figure 4. Apoptosis increased in patients experiencing clinical benefit. Scale: x20.

- Overall, tumors from patients with clinical benefit displayed a 10- and 6-fold (P<0.05) increase from baseline in endothelial and tumor cell apoptosis, respectively.
- In contrast, tumors from patients with progressive disease had little or no change from baseline in endothelial and tumor cell apoptosis.

Conclusions

- Inhibition of PDGFR-β and VEGFR-2 activities (as assessed by decreased RTK phosphorylation) or induction of tumor and endothelial cell apoptosis appear to be biomarkers of clinical benefit in patients with imatinib-resistant GIST treated with sunitinib.
- These data suggest that activity of sunitinib against PDGFR-β and VEGFR-2 may play an important role in the antitumor effects of sunitinib in patients with imatinib-resistant GIST. We hypothesize that the multi-targeted nature of sunitinib results in the inhibition of RTKs on both tumor and vascular endothelial cells.
- Additional work is required to better describe potential biomarkers of sunitinib activity in this patient population. Other potential biomarkers include blood-borne endothelial cells, monocytes, soluble VEGFR-2, and VEGF.

References

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