Abstract 57

Correlation of Receptor Tyrosine Kinase (RTK) Activity and Apoptosis with Response to Sunitinib Treatment in Patients with Gastrointestinal Stromal Tumor (GIST)

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Introduction

- Approximately 85% of gastrointestinal stromal tumors (GISTs) contain activating mutations in the gene encoding stem cell factor receptor (KIT), and another 5–7% contain activating mutations of *PDGFRA*. These mutations are generally considered to be the driving force in the pathogenesis of GIST.[1]
- Imatinib mesylate, a selective inhibitor of KIT and platelet-derived growth factor receptors (PDGFRs), has considerable activity as first-line treatment for GIST.[2] However, approximately 12–14%[2,3] of patients develop primary resistance to imatinib and more than 40% develop secondary resistance after a median of 25 months.[3,4]
- Sunitinib malate (SU11248; SUTENT®) is an oral, multitargeted receptor tyrosine kinase (RTK) inhibitor of 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).[5–9] Sunitinib was approved by the US Food and Drug Administration in January 2006 – and received conditional marketing authorization from the European Medicines Evaluation Agency in July 2006 – for the treatment of GIST after disease progression on or intolerance to imatinib mesylate therapy, and for advanced renal cell carcinoma refractory to cytokine therapy.
- Sunitinib demonstrated clinical benefit in patients with imatinib-resistant or -intolerant GIST in a recent phase III trial.[10] While sunitinib efficacy 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.
- This study investigated the ability of sunitinib to inhibit PDGFR-β and VEGFR-2 activity in patients with imatinib-resistant GIST, 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 on one of three schedules: 25, 50, or 75 mg/day for 2 weeks followed by 2 weeks off treatment (2/2 schedule); 50 mg/day for 4 weeks followed by 2 weeks off treatment (4/2 schedule); or 50 mg/day for 2 weeks followed by 1 week off treatment (2/1 schedule).
- Tumor biopsies were obtained from 20 patients at baseline and after ≥11 days of treatment during cycle 1.
- RTK expression and activity in tumor tissue and tumor-associated endothelial cells was analyzed 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.
- Apoptosis in tumor and endothelial cells was analyzed 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) in tumor tissue and tumor-associated endothelial cells decreased by approximately 18% and 42%, respectively, in patients for whom sunitinib therapy was associated with clinical benefit (Table 1 and Figure 1).
- Conversely, phosphorylation of PDGFR-β in tumor tissue and tumor-associated endothelial cells increased by approximately 10% and 23%, respectively, in patients progressing on sunitinib therapy (Table 1 and Figure 2).

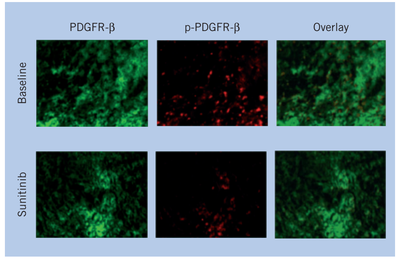

Figure 1. Clinical response was associated with substantial reduction in PDGFR-β phosphorylation. p = phosphorylated; scale: x20.

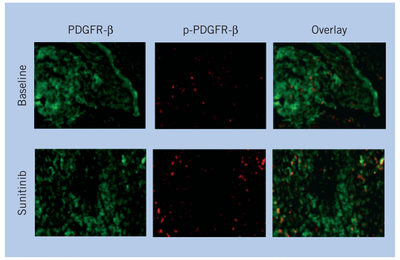

Figure 2. Disease progression was associated with increased phosphorylation of PDGFR-β. p = phosphorylated; scale: x20.

- Likewise, phosphorylated VEGFR-2 measured in tumor tissue decreased by 27% in patients who obtained clinical benefit from sunitinib treatment and increased by 10% in those who experienced disease progression (Table 1).
- Taken together and analyzed quantitatively, decreased 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).

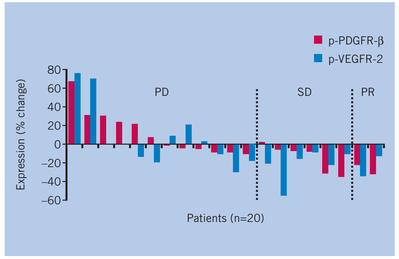

Figure 3. Quantitative analysis of phosphorylated PDGFR-β and VEGFR-2 expression. p = phosphorylated; PD = progressive disease; PR = partial response; SD = stable disease.

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

Clinical response	Number 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)
		(42% ↓ [P=0.008])†	
PR	2	26.1% ↓ (P=0.001)	–
SD >6 months	6	13.9% ↓ (P=0.04)	–
PD (SD <6 months)	12	9.9% ↑ (P=0.06)	9.6% ↑ (P=0.02)
		(23% ↑ [P=0.443])†	

†Change in p-PDGFR-β activity in tumor-associated endothelial cells.
p = phosphorylated; PD = progressive disease; PR = partial response; SD = stable disease.

- In summary:
  - PDGFR-β and VEGFR-2 phosphorylation decreased significantly from baseline in patients experiencing clinical benefit on sunitinib therapy.
  - Conversely, 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.
  - Changes in PDGFR-β phosphorylation were most pronounced in tumor-associated endothelial cells.

Correlation of Tumor Apoptosis with Clinical Response

- In general, apoptosis increased in patients exhibiting clinical benefit on sunitinib therapy (Figure 4).

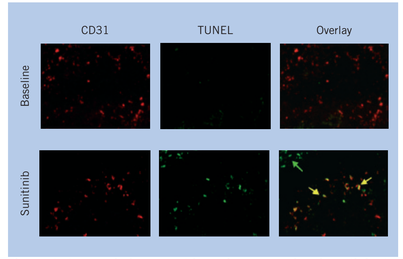

Figure 4. Apoptosis increased in patients experiencing clinical benefit. Scale: x20.

- Tumors from patients with clinical benefit displayed an overall 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 exhibited little or no change from baseline in endothelial and tumor cell apoptosis.

Conclusions

- PDGFR-β and VEGFR-2 inhibition (as assessed by decreased RTK phosphorylation) and induction of tumor and endothelial cell apoptosis appear to be biomarkers of clinical benefit in sunitinib-treated patients with imatinib-resistant GIST.
- Data from this study suggest that PDGFR-β and VEGFR-2 inhibition may play an important role in the antiangiogenic effects of sunitinib in patients with imatinib-resistant GIST. We hypothesize that the multitargeted nature of sunitinib results in the inhibition of RTKs on both tumor and vascular endothelial cells.
- Endothelial cell PDGFR-β phosphorylation may be a sensitive marker of sunitinib biological activity in patients with imatinib-resistant GIST. Additional work is required to better describe other potential biomarkers of sunitinib activity in this patient population. Other potential biomarkers include blood-borne endothelial cells, monocytes, soluble VEGFR-2, and VEGF.

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