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 the stem cell factor receptor (KIT), and another 5–7% contain activating mutations of PDGFRα. These mutations are generally considered to be the driving force in the pathogenesis of GIST.

- Imatinib mesylate, a selective inhibitor of KIT and platelet-derived growth factor receptors (PDGFRs), has been considered as first-line treatment for GIST. However, approximately 12–14% (P = 0.008) of patients develop primary resistance to imatinib and more than 40% develop secondary resistance after a median of 25 months.

- Sunitinib malate (SU11248; SUTENT®), an oral, multitargeted receptor tyrosine kinase (RTK) inhibitor of KIT, vascular endothelial growth factor receptors (VEGFR-1, -2 and -3), PDGFRs (PDGFRα and β), and platelet-derived growth factor receptors (PDGFRs), has been 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.

- Imatinib demonstrated clinical benefit in patients with imatinib-resistant or -refractory GIST in a recent phase III trial. While imatinib efficacy in imatinib-naive or -naïve GIST patients may be due to differential KIT binding, it is also possible that its activity in these patients is related to inhibition of other RTKs, namely PDGFRs and VEGFRs.

- This study investigated the ability of sunitinib to inhibit PDGFR and VEGFR-2 activity in patients with imatinib-resistant GIST, and examined the relationship between this inhibition activity and clinical benefit. The study also evaluated the ability of sunitinib to induce apoptosis of tumor and endothelial cells (previously related to angiogenesis), and related this to clinical benefit.

Materials and Methods
- In this phase III trial, 97 adult male and female patients with metastatic imatinib-resistant GIST and ECOC status 0–2 received sunitinib orally (420 mg orally once daily on one of three schedules: 25, 50, or 75 mg/day for 2 weeks of treatment/2 weeks off; 42 schedule 2; 50 mg/day for 4 weeks followed by 2 weeks off treatment; 42 schedule 2; or 50 or 75 mg/day for 3 weeks followed by 1 week off treatment/2 weeks off; 42 schedule 2).

- 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 automated immunofluorescence microscopy (LSI detection of fluorescently-labeled total and phosphorylated RTKs, as described previously). 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 nick-end labeling), as described previously.

- 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 40% 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 responding to sunitinib therapy (Table 1 and Figure 2).

- 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 (Figure 3).

- Tumors from patients exhibiting clinical benefit on sunitinib therapy appeared to be associated with clinical benefit, while increased disease progression appeared to be associated with disease progression (Figure 3).

- Changes in PDGFR-β phosphorylation were more pronounced in tumor-associated endothelial cells.

- In summary:
  - PDGFR-β and VEGFR-2 phosphorylation decreased significantly in patients experiencing clinical benefit on sunitinib therapy.
  - Conversely, PDGFR-2 phosphorylation increased significantly in patients experiencing disease progression on sunitinib therapy, while there was a trend toward increased PDGFR-β phosphorylation compared with bases in these patients.

- Changes in PDGFR-β phosphorylation were more pronounced in tumor-associated endothelial cells.

Conclusions
- PDGFR 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 biomarker of clinical 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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References

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

<table>
<thead>
<tr>
<th>Clinical response</th>
<th>Number of patients</th>
<th>PDGFR-β</th>
<th>VEGFR-2</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD (SD &lt;6 months)</td>
<td>12</td>
<td>9.9%</td>
<td>10.2%</td>
<td>0.98</td>
</tr>
<tr>
<td>PR</td>
<td>10.9%</td>
<td>10.2%</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>21%</td>
<td>21%</td>
<td>0.98</td>
<td></td>
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Table 2. Change in RTK activity: correlates with clinical benefit

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Figure 1. Clinical response was associated with substantial reduction in PDGFR-2 phosphorylation, p = phosphorylated; scale: x20.

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

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