Pharmacodynamic Analysis of Target Receptor Tyrosine Kinase Activity and Apoptosis in GIST Responding to Therapy with SU11248

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Disclosure

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Introduction

- Most gastrointestinal stromal tumors (GIST) contain activating mutations in the *c-kit* gene
  - *KIT* is a key receptor tyrosine kinase (RTK) in GIST progression

- Imatinib mesylate, a potent inhibitor of KIT RTK activity, is currently first-line treatment for unresectable or metastatic GIST

- However, treatment effectiveness is hampered by imatinib resistance, with early resistance being noted in approximately 14% of GIST patients

**SU11248: Multitargeted Receptor Tyrosine Kinase Inhibitor**

<table>
<thead>
<tr>
<th></th>
<th>PDGFRβ</th>
<th>VEGFR-2</th>
<th>VEGFR-3</th>
<th>FGFR1</th>
<th>EGFR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enzymatic K_{i} (µM)</strong></td>
<td>0.008</td>
<td>0.009</td>
<td>0.017</td>
<td>0.83</td>
<td>&gt;10</td>
</tr>
<tr>
<td><strong>Cellular IC_{50} (µM)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDGFRβ</td>
<td>0.008</td>
<td>0.009</td>
<td>0.01</td>
<td>0.25</td>
<td>8.9</td>
</tr>
<tr>
<td>VEGFR-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.0</td>
</tr>
<tr>
<td>KIT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLT3 (WT)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Receptor phosphorylation

Hypothesis: SU11248 inhibits RTKs on tumor cells, pericytes and endothelial cells to produce its anticancer efficacy.

Pericyte, Endothelial Cell, Stromal and Tumor Cell RTKs ➞ Tumor growth
Phase I/II trial of SU11248 in imatinib-resistant GIST

Baseline (97 Total) & Post-treatment Biopsies (20 patients)

PET scan

Pharmacodynamic Biomarker Analysis Plan

Tumor analysis
- pPDGFRs/PDGFRs
- pKIT/KIT
- pVEGFRs/VEGFRs
  - Tumor Effects
  - Endothelial Cell Death
  - Microvessel Density

Blood-based markers
- VEGF
- sVEGFR-2
- sKIT
- Circulating ECs monocytes

(May 15, 2005, 1:00-5:00 pm Sarcoma)

SU11248 control of imatinib-resistant GIST in a patient with primary resistance to imatinib

Baseline

Day 7 PET

Normal heart

Normal kidneys

CT after 2 months of SU11248

Quantitative analysis of RTK activity and apoptosis in tumors

**LSC = laser scanning cytometry**

1. Davis DW et al. *Br J Cancer* 2003
## LSC-mediated analysis of biomarkers in clinical studies of RTK inhibitors

<table>
<thead>
<tr>
<th>Agent</th>
<th>Diagnosis</th>
<th>Key biomarkers</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SU5416</td>
<td>Sarcoma</td>
<td>Apoptosis &lt; 5%, 20% p-KDR Inhibition in 1 case</td>
<td>Davis DW <em>Clin Cancer Res</em> 2004</td>
</tr>
<tr>
<td>SU6668</td>
<td>Colon/ Liver Met.</td>
<td>Apoptosis &lt; 5%, 50% p-KDR and p-PDGFR Inhibition in 2 cases</td>
<td>Davis DW <em>Clin Cancer Res</em> 2005</td>
</tr>
<tr>
<td>Imatinib</td>
<td>Melanoma</td>
<td>Apoptosis in responder &gt; 10%</td>
<td>in press</td>
</tr>
<tr>
<td>Imatinib</td>
<td>GIST</td>
<td>Apoptosis 10%, 50% p-KIT Inhibition</td>
<td>Work in progress</td>
</tr>
<tr>
<td>SU11248</td>
<td>GIST</td>
<td>Apoptosis, p-PDGFR, p-KDR</td>
<td>Work in progress</td>
</tr>
</tbody>
</table>

LSC = laser scanning cytometry
Does SU11248 target only KIT or multiple RTKs in GIST?

To answer, assess effects of SU11248 on the activity of:

- PDGFR-β
- VEGFR-2
- KIT
**Phosphorylated-PDGFR-β levels increased in patients progressing on SU11248**

<table>
<thead>
<tr>
<th>Baseline</th>
<th>SU11248</th>
<th>Overlay</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDGFR-β</td>
<td>p-PDGFR-β</td>
<td>Overlay</td>
</tr>
</tbody>
</table>

*After 11 days of therapy (Scale x20)*
Phosphorylated PDGFR-β decreased by 31% in responding patients¹

¹After 11 days of therapy (Scale x20)
Quantitative analysis of pPDGFR-β Expression (% Change)

PD = Progressive Disease; SD = Stable Disease; PR = Partial Response
### Change in pPDGFR-β activity: Correlation with clinical benefit

<table>
<thead>
<tr>
<th>Clinical outcome</th>
<th>No. of patients</th>
<th>Δ p-PDGFR activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical benefit (PR or SD &gt;6 months)</td>
<td>8</td>
<td>18.2% ↓ (p=0.006)</td>
</tr>
<tr>
<td>- PR</td>
<td>2</td>
<td>26.1% ↓ (p=0.001)</td>
</tr>
<tr>
<td>- SD</td>
<td>6</td>
<td>13.9% ↓ (p=0.04)</td>
</tr>
<tr>
<td>Progressive disease (&lt; 6 months)</td>
<td>12</td>
<td>9.9% ↑ (p=0.06)</td>
</tr>
</tbody>
</table>

PR = partial response; SD = stable disease
Was inhibition in p-PDGFRβ sufficient to induce apoptosis?
SU11248 increased apoptosis in patients with clinical benefit\(^1\)

1After 11 days of therapy
(Scale: x20)
Effects of SU11248 on Endothelial and Tumor Cell Apoptosis

<table>
<thead>
<tr>
<th>Clinical Outcome</th>
<th>EC Apoptosis (Fold Change)(^1)</th>
<th>TC Apoptosis (Fold Change)(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Benefit</td>
<td>9.55 (p = 0.017)</td>
<td>5.80 (p = 0.002)</td>
</tr>
<tr>
<td>Progressive Disease</td>
<td>1.78 (p = 0.289)</td>
<td>1.15 (p = 0.406)</td>
</tr>
</tbody>
</table>

- Patients with CB displayed significantly higher levels of EC (p = 0.007) and TC (p = 0.006) apoptosis than patients with PD

EC = Endothelial Cell; TC = Tumor Cell

\(^1\) Compared to Baseline
Summary

- PDGFR-β phosphorylation decreased in tumors in patients with CB from SU11248
- EC & TC apoptosis increased during SU11248 treatment to a greater extent in the CB group than the PD group
- Suppression of PDGFR-β activity implicates other key RTKs in addition to KIT as targets for SU11248 in GIST
- We hypothesize that the multi-targeted nature of SU11248 inhibits RTKs on tumor and vascular cells producing anticancer efficacy

CB = Clinical Benefit; PD = Progressive Disease
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