Receptor Tyrosine Kinase Activity and Apoptosis in Gastrointestinal Stromal Tumors: a Pharmacodynamic Analysis of Response to Sunitinib Malate (SU11248) Therapy

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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

Split Kinase Domain
RTKs

VEGFR-1  PDGFR-α
VEGFR-2  PDGFR-β
VEGFR-3  CSF1R
Fms  KIT
     FLT-3

Enzymatic $K_i$ (µM)

<table>
<thead>
<tr>
<th></th>
<th>PDGFR-β</th>
<th>VEGFR-2</th>
<th>VEGFR-3</th>
<th>FGFR-1</th>
<th>EGFR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.008</td>
<td>0.009</td>
<td>0.017</td>
<td>0.83</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

*Cellular IC$_{50}$ (µM)

<table>
<thead>
<tr>
<th></th>
<th>PDGFR-β</th>
<th>VEGFR-2</th>
<th>KIT</th>
<th>FLT-3 (WT)</th>
<th>EGFR</th>
<th>MET</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.008</td>
<td>0.009</td>
<td>0.01</td>
<td>0.25</td>
<td>8.9</td>
<td>12.0</td>
</tr>
</tbody>
</table>

*Receptor phosphorylation

Hypothesis: SU11248 Inhibits RTKs on Tumor Cells, Pericytes and Endothelial Cells to Produce its Anti-cancer Efficacy

Anti-angiogenic effects

Pericyte, Endothelial Cell, Stromal and Tumor Cell RTKs ⇒ Tumor growth

Anti-tumor effects

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Phase I/II Trial of SU11248 in Imatinib-resistant GIST

SU11248 50 mg/day,
2–4 weeks on, 2 weeks off

Baseline (97 total) & post-treatment biopsies (20 patients)

PET scan

Pharmacodynamic Biomarker Analysis Plan¹,²

Tumor analysis

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

Blood-based markers

VEGF Circulating ECs
sVEGFR-2 sKIT

SU11248 Control of Imatinib-resistant GIST in a Patient with Primary Resistance to Imatinib

Baseline

Day 7 PET

CT after 2 months of SU11248

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Quantitative Analysis of RTK Activity and Apoptosis in Tumors

LSC = laser scanning cytometry

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1Davis 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>Heymach JV <em>Clin Cancer Res.</em> 2004 Sep</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 Jan</td>
</tr>
</tbody>
</table>

**VEGFR-2 Phosphorylation**

**PDGFR Phosphorylation**

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\textsuperscript{1}

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\textsuperscript{1}After 11 days of therapy (Scale x20)
Phosphorylated PDGFR-β Decreased in Responding Patients

After 11 days of therapy (Scale x20)

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1After 11 days of therapy (Scale x20)
Quantitative Analysis of p-PDGFR-β and p-VEGFR-2 Expression (% Change)

Patients (n=20)

PD = progressive disease; SD = stable disease; PR = partial response
## Change in p-PDGFR-β and p-VEGFR-2 Activity: Correlation with Clinical Benefit

<table>
<thead>
<tr>
<th>Clinical outcome</th>
<th>Number of patients</th>
<th>Δ p-PDGFR-β activity</th>
<th>Δ p-VEGFR-2 activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical benefit (PR or SD &gt;6 months)</td>
<td>8</td>
<td>18.2% ↓</td>
<td>26.67% ↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.006</td>
<td>p=0.02</td>
</tr>
<tr>
<td>Progressive disease (&lt;6 months)</td>
<td>12</td>
<td>9.9% ↑</td>
<td>9.62% ↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p=0.06</td>
<td>p=0.22</td>
</tr>
</tbody>
</table>

PR = partial response; SD = stable disease
Was Inhibition in p-PDGFR-β and p-VEGFR-2 Sufficient to Induce Apoptosis?

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SU11248 Increased Apoptosis in Patients with Clinical Benefit\(^1\)

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1\(^{After 11 days of therapy (Scale: x20)}\)
### 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

$^1$Compared to baseline

EC = endothelial cell; TC = tumor cell

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Summary

- PDGFR-β and p-VEGFR-2 phosphorylation decreased in tumors in patients with CB from SU11248
- EC and TC apoptosis increased during SU11248 treatment to a greater extent in the CB group than the PD group
- Suppression of PDGFR-β and VEGFR-2 activity implicates 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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www.ApoCell.com
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