Metabolism and Excretion in Humans of the Pharmacoenhancer GS-9350

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Abstract # 18
Introduction

♦ RTV boosting is a cornerstone of PI-based ARV regimens

♦ Key Considerations:
  ♦ Specificity as an inhibitor
  ♦ Induction liability (CYP, UGT, Pgp, etc.)
  ♦ GI tolerability and lipid disorders
  ♦ Non-PI -based Regimens
    – Might RTV 100mg select protease inhibitor resistance?

Develop a new booster without anti-HIV activity
GS-9350 (Cobicistat)

- Potent, mechanism-based inhibition of human CYP3A

- Greater CYP450 enzyme inhibition specificity
  - IC$_{50}$ values (µM)

- Less induction of drug metabolizing enzymes and transporters

<table>
<thead>
<tr>
<th>CYP450 enzyme</th>
<th>2B6</th>
<th>2C8</th>
<th>2C9</th>
<th>2D6</th>
<th>3A</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS-9350</td>
<td>2.8</td>
<td>30</td>
<td>&gt;25</td>
<td>9.2</td>
<td>0.2</td>
</tr>
<tr>
<td>RTV</td>
<td>2.9</td>
<td>5.5</td>
<td>4.4</td>
<td>2.8</td>
<td>0.2</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GS-9350</th>
<th>RTV</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{\text{inact}}$ (min$^{-1}$)</td>
<td>0.44</td>
<td>0.23</td>
</tr>
<tr>
<td>$K_i$ (µM)</td>
<td>0.94</td>
<td>0.26</td>
</tr>
</tbody>
</table>

hPXR Activation

Response (%$E_{\max}$) vs. Concentration (µM)

Mean ± SD, n=3
Introduction

Metabolite profiles for GS-9350 have been analyzed

- *In vitro* in hepatic microsomal fractions and hepatocytes from 5 species (CD1 mouse, Sprague Dawley rat, beagle dog, cynomolgus monkey and human)
- *In vivo* in rat and mouse following dosing of $[^{14}\text{C}]\text{GS-9350}$

- Major metabolites have also been quantified in the rat and dog *in vivo*
- There is no evidence for phase II metabolism of GS-9350
- In all species the predominant metabolites (E1, E3 and E5) are due to oxidation
GS-9350 exhibited non-linear increases in exposure with dose and time
- Time- and dose-dependent PK consistent with mechanism-based inhibition

GS-9350 achieved potent inhibition of CYP3A activity
- Near-maximal inhibition achieved at ≥ 100mg
Objectives

Primary Objective
♦ To determine the mass balance of GS-9350 at steady-state using a tracer dose of radiolabeled $[^{14}\text{C}]\text{GS-9350}$

Secondary Objectives
♦ To determine the metabolite profile of GS-9350 in humans at steady-state using a tracer dose of radiolabeled $[^{14}\text{C}]\text{GS-9350}$
♦ To determine the safety of administration of multiple doses of GS-9350
Male subjects 150 mg once-daily, dosed with food
  − Unlabeled GS-9350 tablets: Days 1-6
  − \([^{14}\text{C}]\text{GS-9350 (100 } \mu\text{Ci)}\) combined with unlabeled material (total dose 150 mg) in capsule administered on Day 7

PK sampling performed for at least 96 hours
  − Serial whole blood samples, cumulative voided urine and all stool were collected
Methods

♦ Radioactivity in whole blood, plasma, urine, and feces analyzed using LSC with rapid turnaround
  - Post 96-hr whole blood and plasma collected until radioactivity in 2 consecutive samples were ≤ 2-times background OR urine & feces collection was discontinued, whichever occurred first
  - Post 96-hr urine and feces collected until radioactivity in 2 consecutive samples were ≤ 1% AND cumulative [14C] recovered in urine/feces is > 90% of administered dose

♦ Samples were analyzed for total radioactivity and subject to HPLC- radioprofiling and HPLC-MS/MS analyses
Results

Demographics

♦ 8 male subjects enrolled and completed study
  – Mean age: 32 yrs (range: 23 - 40)
  – Mean weight: 82.6 kg (range: 73.9 – 92.9)
  – Ethnicity: 8 White

Safety

♦ GS-9350 was well tolerated
♦ There were no study drug discontinuations or Grade 2 or higher laboratory abnormalities, or drug-related adverse events (AE)
Results-Safety

<table>
<thead>
<tr>
<th>Treatment-Emergent Adverse Events</th>
<th>GS-9350 (N = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Musculoskeletal and Connective Tissue Disorders</strong></td>
<td>1 (12.5%)</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>1 (12.5%)</td>
</tr>
<tr>
<td><strong>Renal and Urinary Disorders</strong></td>
<td>1 (12.5%)</td>
</tr>
<tr>
<td>Glycosuria</td>
<td>1 (12.5%)</td>
</tr>
</tbody>
</table>

- Laboratory abnormalities:
  - Three subjects (37.5%) had 4 treatment-emergent; glucose in urine, elevated serum potassium and uric acid, and elevated serum sodium (Grade 1)
Results

Excretion of GS-9350 (Mean ± SD)

Excellent total recovery of radioactivity in excreta
GS-9350 is eliminated primarily in the feces
Results

No quantifiable metabolites in the plasma
In most subjects, plasma radioactivity was undetectable beyond 28 to 32 hours post dose.
Whole blood to plasma concentration ratio of GS-9350 was approximately 0.5 indicating that GS-9350 was excluded from erythrocytes.
Results

♦ GS-9350 was the major species in the feces (27%) followed by oxidative metabolites E3 (14%) and E1 (5.5%)
  - Recombinant CYP3A4 can catalyze the formation of all of these metabolites, while CYP2D6 can also generate E3
  - Metabolites were previously identified by LC-MS/MS and/or NMR with comparison to authentic standards

♦ All other metabolites detected in the feces were in trace amounts, with no values exceeding 3% of the dose, and all had previously been detected in the rat

♦ E1 and E5 are very weak inhibitors of CYP3A activity and do not have any increased liabilities compared to GS-9350.
  - Mechanism-based inhibition of CYP3A by E3 can be detected (significantly weaker than GS-9350) - but exposure is too low for it to contribute to the clinical effect

♦ No metabolites display anti-HIV activity
Conclusion

♦ GS-9350 is extensively metabolized and primarily eliminated in the feces in humans

♦ Following administration of GS-9350, systemic exposure is almost exclusively parent drug

♦ The human data are consistent with the established preclinical profile of GS-9350