Synergy Between Two Mechanisms of Action Contributes to Species Differences in the Liver Safety Profile for PF-04895162

Vinal V. Lakhani1, Grant Generaux2, Yuching Yang3, Sashi Nadanaciva4, Leping Qiu5, Keith Riccardi6, Li Di6, Brett A. Howell1, Scott Q. Siler1, Paul B. Watkins5,6, Hugh A. Barton1, Michael D. Aleo7, Lisl K. M. Shoda1

1DILisym Services Inc., Research Triangle Park, NC; 2Compound Safety, Worldwide Medicinal Chemistry, Pfizer Inc., Groton, CT; 3Investigative Toxicology, Drug Safety Research and Development, Pfizer Inc., Groton, CT; 4Pharmacokinetics, Dynamics and Metabolism, Medicinal Sciences, Pfizer Inc., Groton, CT; 5UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC; 6UNC Institute for Drug Safety Sciences, University of North Carolina at Chapel Hill, Chapel Hill, NC; 7Translational Modeling and Simulation, Biomedical Design, Pfizer, Inc. Groton, CT

Contributed equally to this work

CONCLUSIONS

This investigative study shows the ability of DILisym to reproduce species differences in hepatotoxicity by integrating PK and in vitro data. Additionally, this study supports the contention that combined in vitro and in silico screening methods have the potential to identify latent hepatotoxic risks.

METHODS

We retrospectively analyzed PF-04895162 using a computational representation of drug induced liver injury, DILisym, which integrates in vitro data of hepatoxic mechanisms with in vivo predictions of liver exposure.

The in vitro data included bile acid transporter inhibition and measuring mitochondrial dysfunction. Specifically, IC50 values were measured using standard vesicular transport assays for human BSEP, NTCP, MRPI and MRP4, as well as, for rat Bsep, Mtp3 and Ntcp.

Mitochondrial dysfunction was determined by measuring the oxygen consumption rate in human and rat hepatocytes using the Seahorse XF Analyzer.

In vivo predictions of liver exposure are based on physiologically based pharmacokinetic (PBPK) models; these models were fit using pre-clinical and clinical measurements.

- The rat PBPK model included compartments for blood, liver, muscle, gut, and other tissue. This model was fit using rat plasma concentration measurements following IV and PO dosing.
- The human PBPK model included two compartments: blood and liver; the remaining tissues were aggregated into a general compartment representing systemic volume of distribution. This model was fit using clinical plasma concentration measurements following a single PO dose.

RESULTS

Simulation results reproduced lack of rat hepatotoxicity and presence of clinical hepatotoxicity.

Table 1. Sensitivity Analysis of Toxicity Mechanisms

<table>
<thead>
<tr>
<th>Simulations</th>
<th>Mechanisms On</th>
<th>Mechanisms Off</th>
<th>ALF Equations 23a ULN</th>
</tr>
</thead>
<tbody>
<tr>
<td>300 mg b.i.d for 14 days in MultiSim†</td>
<td>ETC, BA</td>
<td>-</td>
<td>8/16</td>
</tr>
<tr>
<td>14 days in MultiSim†</td>
<td>ETC</td>
<td>BA</td>
<td>0/16</td>
</tr>
<tr>
<td>300 mg b.i.d for 14 days in SimCohort</td>
<td>BA</td>
<td>ETC</td>
<td>1/16</td>
</tr>
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</table>

Although the IC50 for BSEP inhibition by PF-04895162 was higher (311 μM) than has been generally thought to contribute to hepatotoxicity, toxicity from the bile acid mechanism still occurred. Analysis of the modeling results thus indicated multiple contributors to the simulated species differences. Additionally, the simulated human liver exposure was greater than the simulated rat liver exposure, which allowed PF-04895162 to engage both mitochondrial toxicity and inhibition of bile acid transporters. Modeling even higher PF-04895162 liver exposure than were measured in the rat safety studies aggravated mitochondrial toxicity but did not result in rat hepatotoxicity due to insufficient accumulation of cytoxic bile acid species.

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