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

Vinal V. Lakanhi1, Grant Generaux1, Yuching Yang1, Sashi Nandanaciva2, Luping Qiu3, Keith Riccardi4, Li Di4, Brett A. Howell3, Scott Q. Siler1, Paul B. Watkins5,6, Hugh A. Barton7, Michael D. Aleo3, Lisl K. M. Shoda1

1 DILysim Services Inc., 6 Davis Drive, Research Triangle Park, North Carolina
2 Investigative Toxicology, Drug Safety Research and Development, Pfizer Inc., Groton, Connecticut
3 UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina
4 Translational Modeling and Simulation, Biomedical Design, Pfizer, Inc. Groton, Connecticut
6 Pharmacokinetics, Dynamic and Metabolism, Medicinal Sciences, Pfizer Inc., Groton, Connecticut
7 Contributed equally to this work

PF-04895162 (ICA-105665), a drug in development for the treatment of epilepsy, was terminated after transaminase elevations (up to grade 3) were observed in healthy volunteers (NCT01691274). The human hepatotoxicity was unexpected because liver safety concerns had not been raised in preclinical safety studies (Aleo et al. 2019).

We retrospectively analyzed PF-04895162 using a computational representation of drug induced liver injury. DILysim, which integrates in vitro data of hepatotoxic 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, MRp3 and MRp4, as well as for rat Bsep, Mrp3 and Ntcp.
• Mitochondrial dysfunction was determined by measuring the oxygen consumption rate in human and rat hepatocytes using the Seahorse XF Analyzer.

RESULTS

Liver cytotoxic bile acid levels and liver ATP suggest species-specific differences in both mechanisms of toxicity.

CONCLUSION

This investigative study shows the ability of DILysim 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.

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 representation of systemic volume of distribution. This model was fit using clinical plasma concentration measurements following a single PO dose.

Although the IC50 for BSEP inhibition by PF-04895162 was higher (311 µM) than ETcI, BAi - ETcI concomitant inhibition of both mechanisms of toxicity with inhibition of bile acid transporters. Modeling even higher PF-04895162 liver exposures than were measured in the rat safety studies aggravated these exposures than were measured in the rat safety studies.

The simulated human hepatotoxicity was demonstrated to be due to synergistic interaction between these two mechanisms; elimination of either mechanism from the model abrogated injury (Table 2).

Table 2. Sensitivity Analysis of Toxicity Mechanisms

<table>
<thead>
<tr>
<th>Simulations</th>
<th>Mechanisms On</th>
<th>Mechanisms Off</th>
<th>ALT Elevations ≥2xULN</th>
</tr>
</thead>
<tbody>
<tr>
<td>300 mg po BID for 14 days in Multi16</td>
<td>ETO, BAi</td>
<td>ETO, BAi</td>
<td>8/16</td>
</tr>
<tr>
<td>300 mg po BID for 14 days in Multi16</td>
<td>BAi</td>
<td>BAi</td>
<td>0/16</td>
</tr>
<tr>
<td>300 mg po BID for 14 days in Multi16</td>
<td>ETO</td>
<td>ETO</td>
<td>0/16</td>
</tr>
</tbody>
</table>

*Multi16 is a human Sim Cohort (n = 160), which includes individuals sensitive to different mechanisms of toxicity; ETCi = electron transport chain inhibition; BAi = bile acid transporter inhibition.

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 exposures than were measured in the rat safety studies aggrated mitochondrial toxicity but did not result in rat hepatotoxicity due to insufficient accumulation of cytotoxic bile acid species.