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

Vinal V. Lakhani[1†], Grant Generaux[1†], Yuching Yang[1], Sashi Nadanaciva[2], Luping Qiu[3], Keith Riccardi[4], Li Di[4], Brett A. Howell[1], Scott Q. Siler[1], Paul B. Watkins[5,6], Hugh A. Barton[7], Michael D. Aleo[3], Lisl K. M. Shoda[1]

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

[†]Contributed equally to this work

CONTACT INFORMATION: VLakhani@DILIsym.com

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PURPOSE

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).

The Purpose is to better understand the mechanisms underlying the apparent species differences, between rat and human, when evaluating the liver safety of compound PF-04895162. This case study of PF-04895162 fits into the broader, pharmacology goal of improving the detection of potential liver liabilities prior to the introduction of compounds to the clinic.

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.

Reference:

Aleo MD, Aubrecht J, D Bonin P, et al. Phase I study of PF-04895162, a Kv7 channel opener, reveals unexpected hepatotoxicity in healthy subjects, but not rats or monkeys: clinical evidence of disrupted bile acid homeostasis. Pharmacol Res Perspect. 2019.

METHODS

We retrospectively analyzed PF-04895162 using a computational representation of drug induced liver injury, DILIsym, 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, IC_{50} 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.

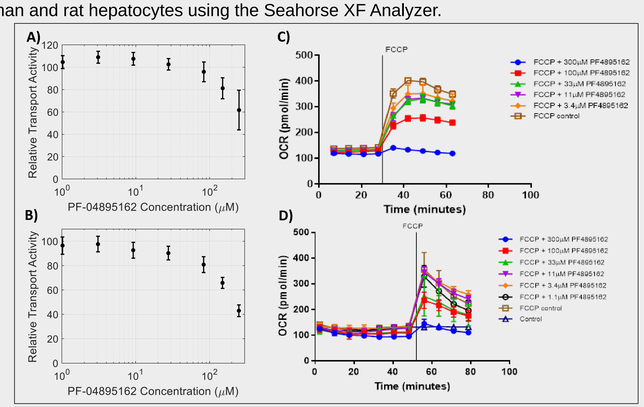

FIGURE 1. Examples of *in vitro* data collected. (A – B) Assessment of PF-04895162 inhibiting (A) human BSEP and (B) rat Bsep. (C – D) Oxygen consumption rate (OCR) of primary (C) human and (D) rat hepatocytes after incubation with PF-04895162, followed by addition of FCCP.

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.

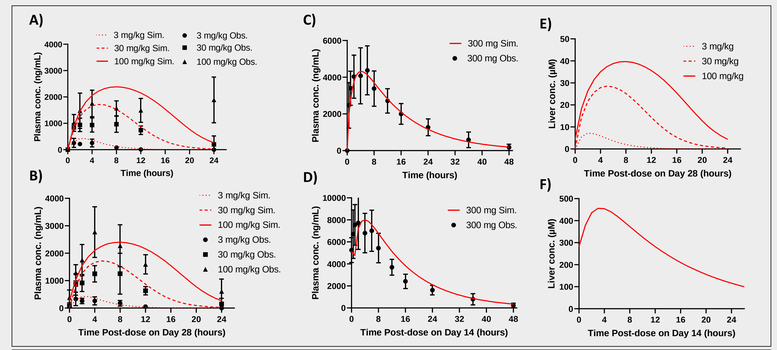

FIGURE 2. PBPK Models and Measured Data. (A – B) Rat plasma PF-04895162 (ng/mL) for (A) a single dose, and (B) on day 28, following daily dosing. (C – D) Human plasma PF-04895162 (ng/mL) for (C) a single dose, and (D) on day 14, following 300 mg BID dosing. (E – F) Liver concentrations in (E) rat on day 28 following daily dosing, and (F) human on day 14 following 300 mg BID.

These *in vitro* data (Fig 1) and PBPK models (based on *in vivo* data) (Fig 2) were integrated in DILIsym to predict hepatotoxicity in both species. Furthermore, simulations were conducted in SimPops, a simulated population where sensitivity to hepatotoxic mechanisms varies with variability in physiologic parameters.

RESULTS

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

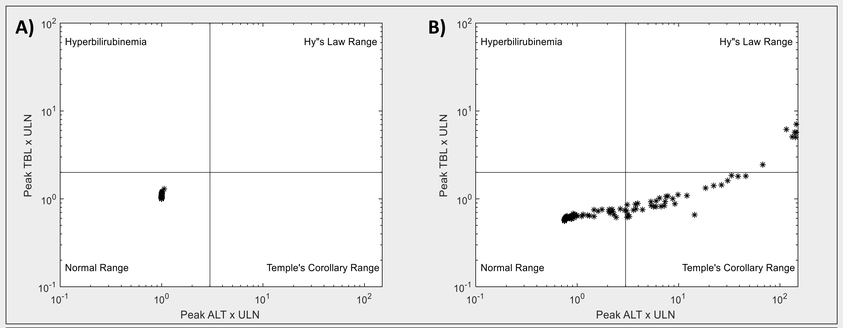

FIGURE 3. Evaluation of Drug-Induced Serious Hepatotoxicity (eDISH) Plots. Simulation of PF-04895162 (A) 100 mg/kg/day for 28 days in rat SimPops (n=294). (B) 300 mg BID for 14 days and 14 day follow-up in human SimPops (n=285).

While simulations reproduce species differences, ALT elevations in simulated humans were less frequent than observed clinically, 59/285 (21%) *vs*. 6/8 (75%) respectively.

Simulated human liver injury was sometimes more severe than observed clinically, ALT >10x ULN 18/285 (6%) vs. 0/8 (0%), respectively.

The simulated human hepatotoxicity was demonstrated to be due to interaction between these two mechanisms; elimination of either mechanism from the model abrogated injury (Table 1).

Table 1. Sensitivity Analysis of Toxicity Mechanisms

Simulations	Mechanisms On	Mechanisms Off	ALT Elevations ≥3x ULN
300 mg po BID for 14 days in Multi16‡	ETCi, BAi	-	8/16
	ETCi	BAi	0/16
	BAi	ETCi	0/16

‡ Multi16 is a Human SimCohort (n = 16), which includes individuals sensitive to different mechanisms of toxicity. ETCi = electron transport chain inhibition. BAi = bile acid transporter inhibition.

Although the IC_{50} 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 aggravated mitochondrial toxicity but did not result in rat hepatotoxicity due to insufficient accumulation of cytotoxic bile acid species.

Simulated individuals' time to peak ALT is similar to clinical observations.

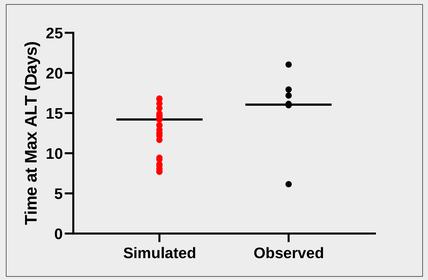

FIGURE 4. Comparison of time to peak ALT in human SimPops versus measured clinical data. Simulated individuals are represented in red. Clinical measurements for subjects in the Phase I study are represented in black. The median value is indicated by a line.

300 mg BID dosing of PF-04895162 for 14d was associated with delayed ALT elevations. Delayed timing is often considered indicative of a putative adaptive immune response. Simulations demonstrate that delayed ALT elevations due to intrinsic mechanisms, where the more gradual bile acid accumulation accounts for the delayed response.