Elevations of liver enzymes have been observed in clinical trials with BAL30072, a novel antibiotic. In vitro assays have identified potential mechanisms for the observed hepatotoxicity, including electron transport chain (ETC) inhibition and reactive oxygen species (ROS) generation. DILIsym, a quantitative systems pharmacology (QSP) model of drug-induced liver injury, has been used to predict the likelihood that each mechanism explains the observed toxicity. DILIsym was also used to predict the safety margin for a novel BAL30072 dosing scheme; it was predicted to be low. DILIsym was then used to recommend potential modifications to this dosing scheme; weight-adjusted dosing and a requirement to assay plasma alanine aminotransferase (ALT) daily and stop dosing as soon as ALT increases were observed improved the predicted safety margin of BAL30072 and decreased the predicted likelihood of severe injury. This research demonstrates a potential application for QSP modeling in improving the safety profile of candidate drugs.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☒ Mechanisms have been implicated in observed BAL30072 hepatotoxicity but the relative importance of these mechanisms is unclear, as is the potential safety of alternate BAL30072 dosing protocols.

WHAT QUESTION DID THIS STUDY ADDRESS?

☒ What toxicity mechanisms are most likely to be the main contributors to BAL30072-mediated hepatotoxicity? Would a lower dose provide an acceptable safety margin? Are there alternate dosing methods that could improve BAL30072’s safety profile?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☒ Oxidative stress and ETC inhibition both contribute to BAL30072 toxicity. A novel dosing protocol provides a narrow safety margin; however, dosing on a weight-adjusted basis and with stringent monitoring for ALT elevations could avoid the development of severe liver injury.

HOW THIS MIGHT CHANGE DRUG CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE

☒ This study demonstrates how quantitative systems toxicology modeling can contextualize in vitro results and explain mechanisms behind toxicity. It also demonstrates how quantitative systems toxicology can be used to explore alternate dosing protocols and methods to improve the safety profile of candidate therapeutics.
pharmacokinetic (PBPK) modeling with a mechanistic model of several liver processes with the intention of predicting the likelihood of the drug disrupting these liver processes and thus causing DILI. Several in vitro assays can be used as inputs for DILIsym; thus, it is a useful tool for in vitro to in vivo extrapolation (IVIVE) and contextualizing these potentially predictive or explanatory in vitro assays. DILIsym has previously been used to predict and explain observed liver injury from various drugs using in vitro data inputs. In this study, DILIsym version 3A was used in combination with previously reported in vitro toxicity data to predict the likelihood that oxidative stress generation and ETC inhibition observed in vitro can account for the observed elevations in serum ALT in the clinic. DILIsym version 3A was also used to predict the likely safety margin for BAL30072 dosing and whether that safety margin was improved by protocol modifications, such as using weight-adjusted dosing or strict guidelines to stop dosing after the appearance of elevations of serum ALT.

METHODS
Simulation platform
DILIsym version 3A was used for the simulations conducted in this article. DILIsym is available to the member companies of the DILI-sim Initiative and to researchers in an academic setting via an academic licensing program. The full set of equations for DILIsym version 3A (and indeed for all versions of DILIsym past and present) is fully available to the members of the Initiative. Importantly, key equations from DILIsym have been published in the literature, including equations related to oxidative stress, bile acid homeostasis and transporter disruption, mitochondrial dysfunction, and the dynamics of the innate immune system. These previous publications also provide significant insight into the scientific theory and knowledge behind the development of DILIsym.

Reactive oxygen species representation
In DILIsym v3A, reactive nitrogen species/reactive oxygen species (RNS/ROS) are generated and cleared as an equilibrium process in healthy simulated individuals. Additional RNS/ROS can be generated by the parent drug or any of its metabolites according to a first-order relationship. The generation of RNS/ROS disrupts the oxidative stress equilibrium resulting in RNS/ROS buildup in the hepatocytes. The RNS/ROS buildup leads to cellular apoptosis and necrosis; whether apoptosis or necrosis occurs depends on the extent of the RNS/ROS buildup and the presence of sufficient ATP inside the cell to complete the apoptotic process.

Cells that undergo necrosis in DILIsym version 3A release ALT upon their death. In addition, apoptotic cells release cleaved cytokeratin 18 (cK18), a specific biomarker of apoptosis. Apoptotic cells do not release ALT in DILIsym version 3A.

The submodel of mitochondrial toxicity in DILIsym has been reported elsewhere. The DILIsym mitochondrial submodel includes the production of ATP from glycolysis and from the respiratory chain. Mitochondrial homeostasis can be disrupted through inhibition of the ETC enzymes, through uncoupling of the proton gradient, or through inhibition of fatty acid oxidation; ATP production can further be disrupted through inhibition of glycolysis. Any of these processes will lead to a disruption of ATP production, leading to loss of cellular ATP and subsequent hepatocyte death through necrosis. The effect of each mechanism on ATP production is determined by the strength of the effect and on known mitochondrial bioenergetics and glycolysis.

PBPK modeling
In order to produce an accurate DILIsym prediction of a drug’s DILI risk, it is necessary to reasonably estimate the drug’s concentration within the liver, and specifically within the hepatocyte. To that end, a PBPK model of BAL30072 was developed within DILIsym in order to describe the disposition of intravenously dosed BAL30072 and its major metabolite, the ring-opened product BAL104936, in human liver and plasma. The model was constructed with the goal of representing the range of exposures observed in the single-dose clinical study SFM-CP-001 and the multiple-dose clinical studies SFM-CP-002 and SFM-CP-004.

The basic DILIsym PBPK model framework consists of a compartmental model of the body with compartments for blood, gut, liver, muscle, and other tissues and has been described in previous publications. Because BAL30072 has been shown to be a substrate of organic anion transporter (OAT)1, OAT3, organic anion-transporting polypeptide (OATP)1B1, and OATP1B3, the saturable liver uptake model was used for BAL30072. Protein binding in the plasma and tissues is represented by fraction unbound parameters; only free compound is available for transport and metabolism, whereas toxicity is based on total concentration. Metabolite distribution is described by a partition coefficient between liver and blood and a volume of distribution that describes partitioning into other organs.

Although only the human was considered for this project, rat whole-body autoradiography studies and in vitro experimental data were used as part of the parameter optimization process. In vitro experiments showed that BAL30072 was between 48.8% and 57.2% bound to protein and that the blood:plasma ratio for BAL30072 was between 0.771 and 0.851 (internal data). Further in vitro experiments showed that BAL30072 was not efficiently taken up by HepaRG or HepG2 cells; the intracellular/extracellular concentration ratio was between 0.15 and 0.194; protocols for these experiments are provided in the Supplementary Material A. The rat whole-body autoradiography, conversely, showed that liver concentration was between 0.9-fold and 1.5-fold that of plasma, and that the muscle tissue concentration was between 0.098-fold and 0.165-fold that of plasma (internal data). The average of the unbound fraction assays, the blood:plasma ratio assays, and the muscle: blood concentration ratio were used to parameterize DILIsym. A value of 0.9 was used for the liver: blood partition coefficient; this value was used to model only the passive permeability portion of the saturable liver uptake model.

Further description of the PBPK modeling process is available in Supplementary Material B.

Representation of exposure variability
In modeling potential liver toxicity due to BAL30072, it was necessary to ensure that the full range of exposures observed in the clinical studies for each dosing cohort was simulated.
In order to do this, the simulated DILIsym dose of BAL30072 was modulated to replicate the highest, lowest, and median exposure levels observed for each dosing cohort; the parameters from the baseline model from the optimization were not changed. Toxicity was simulated within DILIsym at each of the exposure levels for each dose in order to determine the variability in toxicity that could have been attributable to pharmacokinetic variation among the volunteers.

For dose levels that had not been previously given in the clinic, such as 750 mg t.i.d., it was assumed that the spread of exposures for the proposed 750 mg 4-hour infusion would be similar to that for the 1,000 mg 4-hour infusion but shifted downward by 25%. This rationale was carried forward for all prospective dose levels in the dose escalation simulations as well.

**Toxicity parameter determination from in vitro data**

The DILIsym model for BAL30072 uses previously reported in vitro experimental data from assays measuring oxidative stress generation and induced mitochondrial dysfunction. These assays implicated three primary mechanisms that could have led to the hepatotoxic effects observed: ETC inhibition, glycolysis inhibition, and ROS generation. The ETC inhibition and ROS generation data were previously reported. Extracellular acidification rate data from in vitro respiration studies with BAL30072 were examined to confirm the direct inhibition of glycolysis (see Supplementary Figure S1). DILIsym parameter values were first derived from in vitro data for each mechanism; these parameter values were used to generate a preliminary prediction of BAL30072 toxicity. The parameter values were then refined based on the BAL30072 clinical study results to ensure that the final DILIsym parameter values led to simulations as consistent as possible with results from the clinical studies of BAL30072.

The optimization process for the initial estimate of the ETC inhibition parameter for DILIsym started with simulations in MITOsym. The MITOsym is a companion software to DILIsym that allows the user to simulate in vitro hepatocellular respiration experiments and optimize mitochondria toxicity parameters that can then be translated over to DILIsym. BAL30072 was modeled in MITOsym as an ETC inhibitor; simulated intracellular BAL30072 exposure in the MITOsym simulations was calibrated to represent measured intracellular exposure levels in HepG2 cells, which were 15–20% of the extracellular exposure levels (data not shown). A parameter value was identified that gave simulation results consistent with the measured hepatocellular oxygen consumption exposure-response relationship.

Lactate production in the 3D human liver microtissues (InSphero AG, Schlieren, Switzerland) was investigated with BAL30072; these data were previously reported and confirmed direct inhibition of glycolysis by BAL30072. An inhibitor of glycolysis within DILIsym was simulated at constant liver exposure levels that matched the measured intracellular concentrations of BAL30072. Parameter values were identified that gave simulation results consistent with the in vitro dose response.

Determining the RNS/ROS toxicity parameter requires setting up DILIsym to mimic the in vitro conditions in the assay. A model with constant intracellular liver exposure was set up in DILIsym, and an RNS/ROS producer within DILIsym was simulated at these constant liver exposure levels that matched the measured intracellular concentrations of BAL30072. Using these simulations, a parameter value was identified that gave simulation results consistent with the in vitro dose response.

**Toxicity parameter optimization to clinical data**

The initial stage of optimization for the appropriate DILIsym toxicity parameters for BAL30072 involved the use of the in vitro data, as described above, to determine which mechanisms of DILI were involved and to find the initial parameter estimates. At the conclusion of this process, the clinical study results for BAL30072 were simulated. The parameters for RNS/ROS production and ETC inhibition were further refined manually until the DILIsym simulation results were as consistent with the BAL30072 clinical data as possible. This process of iterative optimization is represented graphically in Figure 1.

**Toxicity simulations**

The dosing protocols in each of the clinical trials were recapitulated for a duration of 2 weeks with the toxicity parameters in place. The first set of toxicity parameters tested were those calculated directly from the in vitro experimental data; these results were used to further optimize the toxicity parameters, and a second set of toxicity simulations was performed with these parameters.

Toxicity was simulated using SimPops version 3B-1, a simulated population included in DILIsym version 3B that includes variability in parameters related to apoptosis, oxidative stress, and mitochondrial dysfunction. A list of parameters varied for SimPops version 3B-1 is presented in Supplementary Table S5. For each of the toxicity simulations, SimPops version 3B-1 was simulated with three different doses, corresponding to the maximum, average, and minimum exposure levels for each dose, as described above. The results of the simulation were reported in terms of the number of individuals that have plasma levels of ALT and cK18 above clinically significant cutoff levels. For ALT, the cutoff level is the standard threefold above the upper limit of normal (ULN); in DILIsym version 3B, the baseline ALT is normalized to 30 U/L, so this corresponds to an ALT concentration of 90 U/L. For cK18, no clinical standard for significant elevation exists; however, the number of simulated cells undergoing necrosis that produces a 60 U/L increase in ALT is equivalent to the number of simulated cells undergoing apoptosis that produces a 70 U/L increase in cK18 concentrations. Because the baseline cK18 concentration in DILIsym version 3B is 140 U/L, a “clinically significant” cK18 increase was determined to be any cK18 concentration above 210 U/L. Plasma bilirubin was also calculated as a result of the simulations; any individual with concurrent increases of plasma bilirubin above 2× > ULN (corresponding in DILIsym to a concentration of 1.1 mg/dL) and ALT concentration higher than threefold above the ULN was recorded as a “Hy’s Law” case.

Each of the toxicity simulations was performed with a simulated stop protocol in place. At the beginning of the first dose for each day, the ALT levels in the blood were...
calculated. If these levels were above 90 U/L, the simulation would stop the dosing of the drug 8 hours after the ALT calculation occurred; the 8-hour time lag represents the length of time estimated to perform the assay in the laboratory and return the results to the site during an actual clinical trial.

The novel dosing protocol simulated for this study was a 750 mg intravenous infusion given over 4 hours three times a day. This protocol was investigated at increasing doses in order to determine the safety margin. Two key modifications to this protocol were investigated: (i) the “stop protocol” was turned off and on for these increasing-dose simulations; this would determine how likely severe hepatotoxicity (as defined by “Hy’s Law” cases) would be if ALT monitoring were not included in the clinical dosing regimen; and (ii) in addition to the standard protocol, dosing was given on a weight-adjusted basis to each patient in the SimPops. The dosing was based on the DILIsym baseline human body mass of 70 kg; as a result, the 750 mg dose was given as a 10.7 mg/kg dose to each individual in the SimPops.

RESULTS

Results from the simulations used to determine DILIsym toxicity parameters are shown in Supplementary Material C, along with the list of final parameters. Results from the toxicity simulations using the directly calculated parameters are also described in Supplementary Material C.

RNS/ROS parameter optimization from the in vitro data

Supplementary Figure S2 shows the data and simulation results for the RNS/ROS toxicity parameter determination. The DILIsym parameter value that was consistent with the in vitro data was then directly translatable to initial BAL30072 toxicity simulations. The value of the relevant DILIsym parameter, “RNS_ROS_prod_const,” was $3.5 \times 10^6$ mL/mol/hour. The final DILIsym parameter value chosen for RNS/ROS generation differed from the initial estimate described here due to the iterative optimization process involving the clinical data described below.

Mitochondrial parameter optimization from in vitro data

Supplementary Figure S3 shows the data and simulation results for ETC inhibition parameter determination. The MITOsym parameter value was then translated to DILIsym by comparing the MITOsym to DILIsym factor of translation for rotenone, which is also an ETC inhibitor. The value of the relevant MITOsym parameter, “MitoS_ETC_Inhib_1,” was found to be $1.05 \times 10^{-2}$ mM. The value of the DILIsym parameter, “MitoS_ETC_Inhib,” was found to be $5.73 \times 10^{-8}$ mol/mL (5.73 $\times 10^{-2}$ mM). The final DILIsym parameter value chosen for ETC inhibition differed from the initial estimate described here due to the iterative optimization process involving the clinical data described below.

Supplementary Figure S3 shows the data and simulation results for the glycolysis inhibition parameter determination. The resulting DILIsym parameter values were then directly translatable to initial BAL30072 toxicity simulations. The values of the relevant DILIsym parameters were $2.1 \times 10^{-8}$ mol/mL for “MitoS_glycolysis_Inhib” and 5 for “MitoS_glycolysis_Inhib_Hill” (dimensionless units for the Hill coefficient). The final DILIsym parameter values chosen for glycolysis inhibition matched the initial estimates described here due to a lack of sensitivity to glycolysis inhibition within DILIsym when BAL30072 hepatotoxic effects were simulated.
Toxicity simulation results

As shown in Supplementary Table S1, the direct predictions of BAL30072 hepatotoxic effects using only IVIVE were fairly good. The prediction of hepatotoxicity compared well qualitatively to the clinical data; that is, hepatotoxicity was observed at increasing doses, and the dose response was maintained. The simulation results suggested that the margin of safety for BAL30072 was not very high for certain protocol designs, which was substantiated by the clinical study results. However, the predicted dose response hepatotoxicity effects were less than observed for the longer infusion protocols, with minimal toxicity being predicted; hepatotoxicity was also predicted at lower doses for the shorter infusion protocols than was clinically observed.

Toxicity parameter optimization using clinical data

Supplementary Table S2 shows the resulting final parameter values relevant to the DILI mechanisms activated within DILisym as compared with the values calculated directly from the in vitro experiments. The primary outcomes of the second phase of optimization involving the BAL30072 clinical data were as follows:

- ETC inhibition potency was reduced;
- RNS/ROS generation potency was increased;
- Glycolysis inhibition was a minor factor, and was not adjusted.

The results for the toxicity simulations on the dosing protocols from the clinical trials can be found in Figure 2. The results for these simulations compare favorably to those from the IVIVE parameter set; less toxicity is predicted for higher single-dose protocols and more toxicity is predicted for the longer-duration doses, consistent with clinical observation.

Overall, the adjustments led to a higher dependency on cumulative (area under the curve (AUC)) liver exposure of BAL30072 within DILisym for hepatotoxic effects, compared with peak plasma concentration (Cmax) BAL30072 liver exposure. Importantly, prospective BAL30072 clinical studies fell within the clinical dosing range used for the optimization process (i.e., interpolation), making the use of the resulting DILisym setup for BAL30072 reasonable for prospective clinical studies.

Safety margin prediction for novel dosing protocol

The optimized DILisym toxicity parameter set for BAL30072 was used to simulate a novel proposed dosing regimen of 750 mg dosed intravenously over 4 hours t.i.d. The 1,000 mg dosing protocol from the existing study was also simulated here for completeness.

Figure 3 shows the simulation results for both doses in the SimPops version 3B-1. The dose-stopping ALT criterion was included in the simulations. Some key observations from the simulated protocols were: zero Hy’s Law
cases were predicted to occur, necrosis was fairly insignificant, and apoptosis was predicted to be important and likely to occur at therapeutic doses. Although necrosis and ALT elevations were not overwhelmingly observed for the 1,000 mg dose, 26 simulated humans did reach or surpass three times the baseline ALT level. This suggested that the margin of safety for BAL30072 is low. To predict the margin of safety with respect to a significant DILI event, dose escalation simulations were performed at various BAL30072 exposure levels. The results are described below.

To estimate the average or overall margin of safety for BAL30072, dose escalations were done in the baseline human within DILIsym. The baseline human was used, rather than the SimPops version 3B-1, because discussing the margins of safety for 300 different simulated humans is conceptually difficult. The term or metric “safety margin” is often meant as a general guide as to how safe the drug is overall, and is, therefore, best estimated with a representative human. Figure 4 shows the DILIsym simulations of BAL30072 in the baseline human administered t.i.d. over 4 hours at escalating doses. BAL30072 doses were simulated at low, median, and high exposure levels, and the dose stopping criterion (ALT monitoring) was included.

The margin of safety regarding necrosis was suggested as twofold or less by DILIsym at the median exposure level. Apoptosis was suggested as likely to occur at therapeutic doses. The margin of safety regarding potential Hy’s Law cases was suggested as at least sixfold over the potential therapeutic dose of 750 mg t.i.d at all exposure levels. Overall, the margin of safety for BAL30072 was predicted as low, but severe liver injury was suggested as unlikely if stringent daily monitoring of ALT was implemented.

Safety margin for weight-adjusted dosing
The results for the weight-adjusted 4-hour t.i.d. dosing protocol are presented in (Figure 5). The safety margin is predicted to increase significantly as a result of dosing by weight; the dose at which Hy’s Law cases begin to appear is higher in comparison to the proposed dose than was the case for the non-weight-adjusted dosing.

DISCUSSION
In this study, QSP liver toxicity modeling using DILIsym was used to contextualize in vitro toxicity data for BAL30072. The initial DILIsym modeling (i.e., the parameters taken directly from the in vitro data regarding ROS generation and mitochondrial dysfunction) demonstrated that both ROS generation and ETC inhibition contributed somewhat to the observed toxicity. However, comparison to the existing clinical toxicity data suggested that oxidative stress was more important than was suggested by the in vitro experiments, whereas ETC inhibition was less important. In this way, DILIsym was used to combine knowledge gathered from both in vitro assays and clinical trials in order to make more effective safety predictions.

The final toxicity parameter results incorporating both in vitro and clinical data were used to predict the safety margin of a proposed dosing protocol for BAL30072 involving three 4-hour infusions of 750 mg BAL30072 per day. The simulation results demonstrated that although the safety margin was predicted to be small, there were steps that could be taken in order to improve the safety profile of the molecule and prevent severe liver injury from occurring. First, a strict monitoring regime could be implemented in which ALT levels were checked every day and dosing was halted if ALT...
levels above $3 \times$ the ULN were detected. Second, safety would be improved if dosing were given on a weight-adjusted basis. This implies that the observed toxicity is somewhat exposure-dependent, and that a more controlled approach to compound exposure could mitigate the observed toxicity. Adjusting dosage by weight would prevent smaller individuals from receiving a larger concentration of drug than is necessary and, thus, would prevent exposing smaller individuals to undue risk. Both of these strategies are available for a drug that would be given in an inpatient setting.

There are two implications to these conclusions that bear mentioning. First, the results show the promise of modifying dosing protocols for interindividual variability in body mass and of stringent liver function test monitoring for improving QSP Prediction of Toxicity for Novel Antibiotic Woodhead et al. [504] Clinical and Translational Science
safety outcomes of various therapies. It seems likely, given the predicted improvement in the BAL30072 safety profile of BAL30072, that there are many other therapies whose safety profile could be improved by using some form of weight-adjusted dosing. This could potentially apply to outpatient therapies as well. Although stringent liver function test monitoring could only be applied to inpatient therapies, many of those could likely benefit from monitoring.

The second implication of this work is that DILIsym, and QSP modeling in general, can be used to investigate and predict the safety margin of novel clinical protocols and the improvement in those safety margins due to potential therapeutic interventions. Testing the safety of numerous potential dosing protocols and interventions in those protocols would be impractical in a clinical setting; computer modeling, however, makes this process easier and can leverage data produced from earlier clinical trials and in vitro assays to make predictions with a reasonable level of confidence. This is a novel application for QSP modeling and one that deserves to be explored more in future drug development situations.

QSP modeling, furthermore, provides advantages over less mechanistic approaches, such as pharmacokinetic/pharmacodynamic modeling that are apparent in this work. First, QSP modeling allows the incorporation of mechanistic information from in vitro assays into the toxicity predictions, providing insight into whether putative mechanisms of toxicity suggested by experiments could plausibly contribute to the observed toxicity. Second, mechanisms of toxicity (especially off-target toxicity, such as that observed with BAL30072) can display a highly nonlinear relationship between exposure and response. As a result, the extrapolation of toxicity data to alternative dosing schemes can be done with higher confidence when mechanistic information about the underlying biochemistry is incorporated into the simulation.

In this article, DILIsym, in combination with previously reported in vitro experimental data, was used to explain the etiology of observed liver injury signals caused by BAL30072 treatment. DILIsym was then used to suggest modifications to the proposed clinical dosing protocol that could make BAL30072 safer while still maintaining its efficacy. Thus, this research has demonstrated the utility of DILIsym, and QSP modeling in general, for improving the safety profile of drug candidates and helping potentially effective medicines reach the market while minimizing adverse events.

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1. Higgins, P.G., Stefanik, D., Page, M.G.P., Hacket, M. & Seifert, H. In vitro activity of the siderophore monosulfactam BAL30072 against meropenem-non-susceptible Acinetobacter baumannii. J. Antimicrob. Chemother. 67, 1167–1169 (2012).
2. Hofer, B., Dantier, C., Gehhardt, K., Desarbre, E., Schmitt-Hoffmann, A. & Page, M.G.P. Combined effects of the siderophore monosulfactam BAL30072 and carbapenems on multidrug-resistant Gram-negative bacilli. J. Antimicrob. Chemother. 68, 1120–1129 (2013).
3. Landman, D., Singh, M., El-Imad, B., Miller, E., Win, T. & Quale, J. In vitro activity of the siderophore monosulfactam BAL30072 against contemporary Gram-negative pathogens from New York City, including multidrug-resistant isolates. Int. J. Antimicrob. Agents 43, 527–32 (2014 Jan).
4. Paech, F. et al. Mechanisms of hepatotoxicity associated with the monocyclic β-lactam antibiotic BAL30072. Arch. Toxicol. e-pub ahead of print 23 May 2017.
5. Howell, B.A. et al. In vitro to in vivo extrapolation and species response comparisons for drug-induced liver injury (DILI) using DILIsym®: a mechanistic, mathematical model of DILI. J. Pharmacokinet. Pharmacodyn. 39, 527–541 (2012).
6. Longo, D.M., Yang, Y., Watkins, P.B., Howell, B.A. & Siler, S.O. Elucidating differences in the hepatotoxic potential of tolopacine and entacapone with DILIsym®, a mechanistic model of drug-induced liver injury. CPT Pharmacometrics Syst. Pharmacol. 5, 31–39 (2016).
7. Woodhead, J.L. et al. Exploring BSEP inhibition-mediated toxicity with a mechanistic model of drug-induced liver injury. Front. Pharmacol. 5, 240 (2014).
8. Yang, K., Woodhead, J.L., Watkins, P.B., Howell, B.A. & Brouwer, K.L. Systems pharmacology modeling predicts delayed presentation and species differences in bile acid-mediated troglitazone hepatotoxicity. Clin. Pharmacol. Ther. 96, 589–598 (2014).
9. Woodhead, J.L. et al. Mechanistic modeling reveals the critical knowledge gaps in bile acid-mediated DILI. CPT Pharmacometrics Syst. Pharmacol. 3, e123 (2014).
10. Yang Y. et al. MITOsym®: a mechanistic, mathematical model of hepatocellular respiration and bienergetics. Pharm. Res. 32, 1975–1992 (2015).
11. Shoda, L.K., Battista, C., Siler, S.O., Piesiky, D.S., Watkins, P.B. & Howell, B.A. Mechanistic modelling of drug-induced liver injury: investigating the role of innate immune responses. Gene Regul Syst Biol. 11, 1177625017696074 (2017).
12. Hietz, H. et al. Caspase-cleaved cytokeratin 18 and 20 S proteasome in liver degeneration. J. Clin. Lab. Anal. 21, 277–281 (2007).
13. Ulukaya, E., Yilmaztepe, A., Akgöz, S., Linder, S. & Karadag, M. The levels of caspase-cleaved cytokeratin 18 are elevated in serum from patients with lung cancer and helpful to predict the survival. Lung Cancer 56, 399–404 (2007).
14. Yilmaz Y. Systematic review: caspase-cleaved fragments of cytokeratin 18 – the promises and challenges of a biomarker for chronic liver disease. Aliment. Pharmacol. Ther. 30, 1103–1109 (2009).
15. Woodhead, J.L. et al. An analysis of N-acetylcysteine treatment for acetaminophen overdose using a systems model of drug-induced liver injury. J. Pharmacol. Exp. Ther. 342, 529–540 (2012).

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