Representation of Crizotinib and Pazopanib-mediated Drug-Induced Liver Injury (DILI) Using Quantitative Systems Toxicology (QST)

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INTRODUCTION

- Crizotinib and pazopanib are oral receptor tyrosine kinase inhibitors (TKIs).
- Crizotinib is used for treatment of locally advanced or metastatic non–small-cell lung cancer (NSCLC); pazopanib is prescribed for metastatic renal cell carcinoma and soft tissue sarcoma.
- In the clinic, serum ALT elevations were observed in 57% of patients (6% had >5X ULN) treated with standard doses of crizotinib {Source: *Livertox*}.
- In clinical trails of pazopanib, >5X ULN elevations in ALT and bilirubin were observed in only 1-2% of patients {Source: *Livertox*}.
- *In vitro* experiments to determine whether these TKIs inhibit bile acid transporters, induce production of reactive oxygen species, and/or inhibit mitochondrial electron transport chain activity were performed.
- DILIsym, a QST model of DILI that incorporates drug/metabolite disposition, multiple mechanistic pathways of DILI (e.g. oxidative stress, bile acid transporter inhibition, mitochondrial dysfunction), the hepatocyte life cycle, and liver injury biomarkers, was used to assess the potential contribution of each mechanism to the observed toxicity.

METHODS

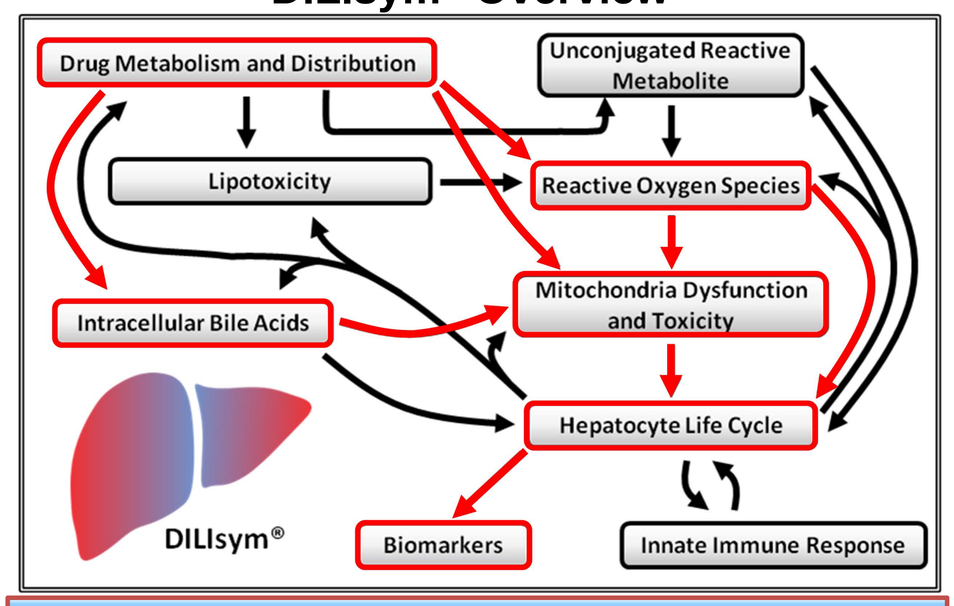
Determining TKI toxicity mechanism parameters from *in vitro* experiments MITOsym®, a mitochondrial modeling platform used to mimic *in vitro* experimental conditions[1], was used to determine parameter values for TKI-mediated mitochondrial uncoupling by fitting simulated results to Seahorse XF96 basal respiration data for HepG2 cells exposed to crizotinib and pazopanib[3]. Conversion factors translated the predicted results from HepG2 cells to human hepatocytes. The ROS production constant parameter was found by sweeping through potential values until the DILIsym "*in vitro*-like" simulation yielded ROS effects in line with the data. The BSEP/MRP3 IC_{50} for crizotinib and the BSEP IC_{50} for pazopanib were used to represent the appropriate inhibition constants (K_i) in DILIsym[2]. Further studies performed in transporter-overexpressing vesicles determined IC_{50} values for MRP3, MRP4, and NTCP which were used for both compounds. The mode of inhibition was assumed to be mixed with α = 5.

Physiologically-based pharmacokinetic (PBPK) model development Human PBPK models were developed in DILIsym using available *in vitro* and *in vivo* pharmacokinetic data. Distribution parameters were then optimized to get the best fit.

Simulation of crizotinib and pazopanib responses Previously constructed SimPops that include variability in species-specific parameters within the BA, ROS, and mitochondrial submodels in DILIsym were used to predict the hepatotoxic effects of crizotinib (250 mg BID, 60 days) and pazopanib (800 mg daily, 60 days) at the population level.

Mechanistic Analysis To identify the most important toxicity mechanism associated with TKI-mediated DILI in humans, each mechanism was individually inactivated and predicted ALT elevation frequencies for each simulation were compared.

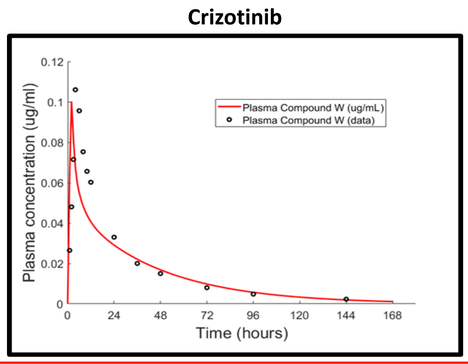
DILIsym® Overview

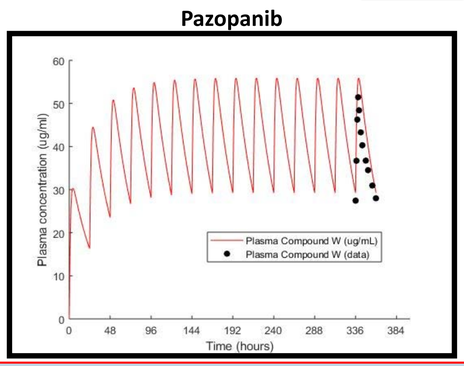


RESULTS

PBPK Simulations

HUMAN





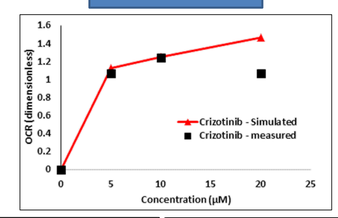
PBPK modeling reasonably predicts plasma concentrations at single dose for crizotinib (left) and multiple dose levels for humans administered pazopanib (right). Simulated plasma profiles (red) are within two standard deviations of the average measured profiles (black).

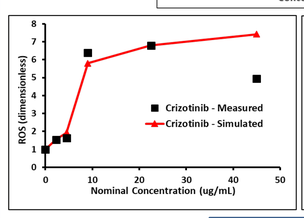
Liver/ Plasma Kp (Crizotinib) = 39
Liver/ Plasma Kp (Pazopanib) = 6.1

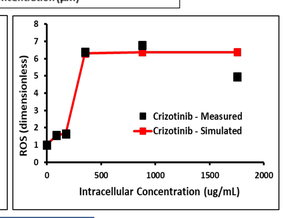
TKI Toxicity Parameters Used in DILIsym

Toxicity parameters				
Bile acid transporter inhibition constants (µM)				
	Crizotinib		Pazopanib	
	Nominal media assumption	PBPK derived Kp assumption	Nominal media assumption	PBPK derived Kp assumption
BSEP Ki	97.79	97.79	14.53	14.53
MRP3 Ki	-	-	-	-
MRP4 Ki	145.23	145.23	32.17	32.17
NTCP Ki	-	-	4.98	4.98
Mitochondrial toxicity parameters				
	Crizotinib		Pazopanib	
Uncoupler 1 effect Km (µmol/L)	492	492	-	-
ROS production parameters				
	Crizotinib		Pazopanib	
Liver RNS/ROS production rate Vmax 4 (1/hour)	0.21	0.29	0.06	0.06
Liver RNS/ROS production rate Km 4 (µmol/L)	14.38	600.00	4.30	26.27
Liver RNS/ROS production rate Hill 4	13.39	19	1.50	1.50
Liver RNS/ROS production rate Vmax 5 (1/hour)	0.26	0.05	-	-
Liver RNS/ROS production rate Km 5 (µmol/L)	33.76	150.00	-	-
Liver RNS/ROS production rate Hill 5	0.84	7.50	-	-

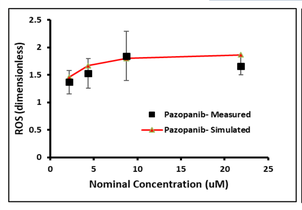
Crizotinib

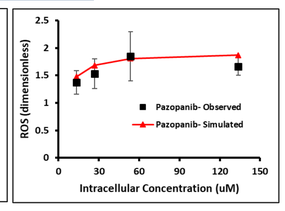






Pazopanib



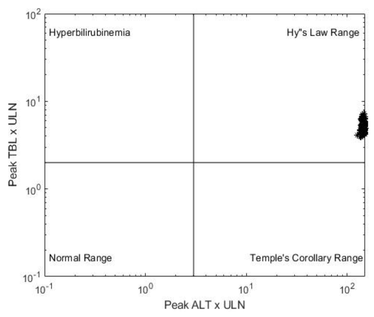


Observed and simulated percentage changes in OCR (top figure) and oxidative stress (bottom four figures) in response to crizotinib and pazopanib. Transporter inhibition constants pertaining to bile acid and bilirubin were used as K_i values in simulating TKI-mediated hepatotoxicity. The mitochondrial uncoupling and ROS production constant parameters were optimized to data using MITOsym and DILIsym, respectively.

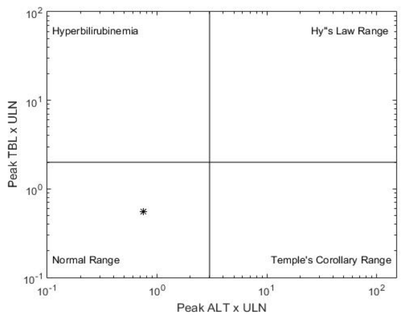
DILI predictions

Crizotinib

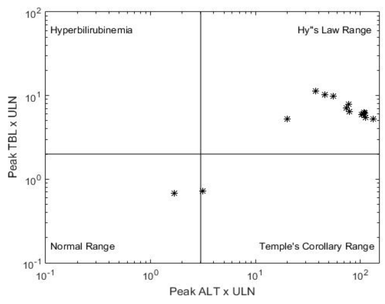
250 mg BID, 60 days, Nominal assumption



250 mg BID, 60 days, Kp assumption



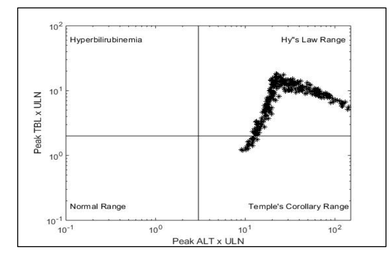
500 mg BID, 60 days, Kp assumption



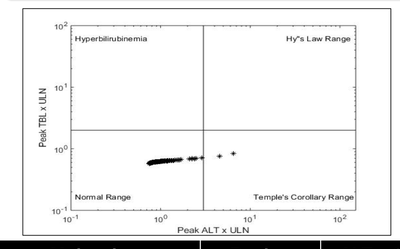
Compound	Dosing Scheme	ALT > 3x ULN
Crizotinib- Observed	250 mg BID, 60 days	66% (69/104)
Crizotinib- Simulated (Nominal assumption)	250 mg BID, 60 days	100% (285/285)
Crizotinib- Simulated (Kp assumption)	250 mg BID, 60 days	0% (0/285)
Crizotinib-Simulated (Kp assumption)	500 mg BID, 60 days (2X clinical dose)	87.5% (14/16)

Pazopanib

800 mg BID, 60 days, Nominal assumption



800 mg BID, 60 days, Kp assumption



Molecule	Dosing Scheme	ALT > 3x ULN
Pazopanib - Observed	800 mg, 60 days	7% (3/41)
Pazopanib – Simulated (Nominal assumption)	800 mg, 60 days	100% (285/285)
Pazopanib – Simulated (Kp assumption)	800 mg, 60 days	0.7% (2/285)

Each mechanism was simulated as the sole contributor to study its individual effect on toxicity in humans. ALT elevation frequency declined for pazopanib when ROS was inactivated; Mitochondrial Uncoupling was the main driver of ALT with crizotinib at 2X dose. The elimination of other mechanisms did not have an effect on the simulated ALT elevation frequency. Previously constructed SimCohorts that include variability in BA, ROS, and mitochondrial submodels within DILIsym (n=16) were used for this analysis.

Mechanistic Analysis

	Oxidative Stress	BA Inhibition	Mitochondrial Uncoupling	ALT >3X ULN
Pazopanib	Yes	Yes	-	1/16
Pazopanib	No	Yes	-	0/16
Pazopanib	Yes	No	-	1/16
Crizotinib (2X)	Yes	Yes	Yes	14/16
Crizotinib (2X)	No	Yes	Yes	14/16
Crizotinib (2X)	Yes	No	Yes	14/16
Crizotinib (2X)	Yes	Yes	No	0/16

CONCLUSION

- Mitochondrial Uncoupling and ROS production were the primary drivers of predicted increases in ALT with crizotinib and pazopanib administration respectively.
- In general, tox parameters derived from intracellular concentrations better predict clinically observed liver signals compared to nominal, but further investigations to understand discrepancies in crizotinib hepatotoxicity are ongoing.
- Quantitative systems toxicology (QST) models can be used to evaluate DILI mechanisms and investigate observed differences among drugs in the same class.

References: 1. Yang, Y. et al. "MITOsym®: A Mechanistic, Mathematical Model of Hepatocellular Respiration and Bioenergetics." *Pharmaceutical Research*(2015). 2. Howell, BA 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." *Journal of Pharmacokinetics & Pharmacodyn.* (2012). 3. Paech, Franziska, Jamal Bouitbir, and Stephan Krähenbühl. "Hepatocellular Toxicity Associated with Tyrosine Kinase Inhibitors: Mitochondrial Damage and Inhibition of Glycolysis." *Frontiers in Pharmacology* 8 (2017).

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