Accurate and interpretable in silico clinical risk assessment for drug-induced liver injury (DILI) from molecular structure

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Abstract

Drug-induced liver injury (DILI) is a leading cause of clinical trial failure and drug withdrawal, but current methods fail to provide accurate and interpretable clinical risk at human-relevant exposure.

- We profiled >100,000 unique molecules in primary human hepatocytes (PHHs) using a multiplexed cytotoxicity assay with high content imaging & two biochemical assays (LDH & Realtime Glo/MT-Glo).
- We train *in silico* models to predict dose-dependent responses for >10 distinct cell stress and death features from the images solely from 2D molecular structure (SMILES string).

Our *in silico* features & thousands of clinical data points are used to train an ***in silico* clinical risk assessment model that outperforms industry-leading in vitro assays in accuracy & interpretability.**

Methods

Protocol: 2D PHH multiplexed cytotoxicity screen with LDH, MT-Glo, and cell painting

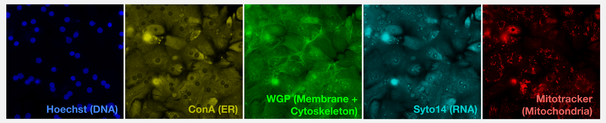
DIV0 Cell seeding → DIV0 (4 hours) Chemical perturbations → DIV2 (48 hours) Multiplexed readouts

- PHH, 6k/well
- n=5 donor pool (BioIVT)
- 384 well plates

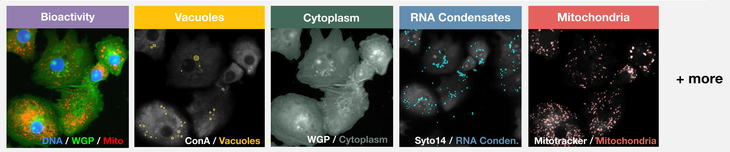
- Full 8 point dose-response
- Maximal concentration 100 µM
- 48 hr exposure

Biochemical
- MT-Glo (viability)
- LDH (necrosis)

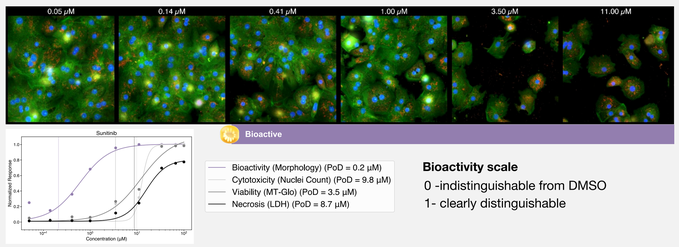
Image
- 4% PFA fixation
- Cell painting (40x)



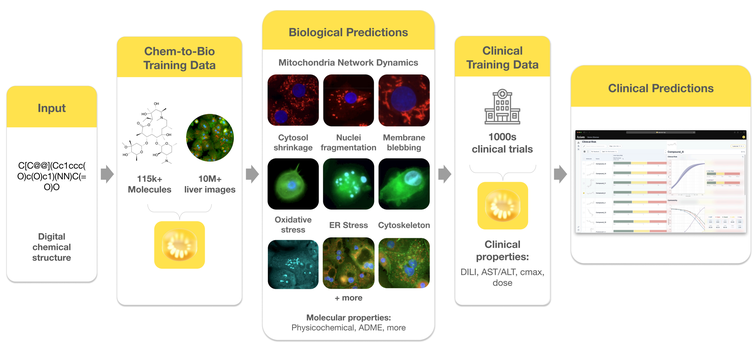
Phenotype quantification: We segment images with deep learning to quantify various morphologies



Bioactivity measure: We engineered a feature that uses deep-learning representations of all image channels to assess whether a perturbation induces any distinct morphological changes.

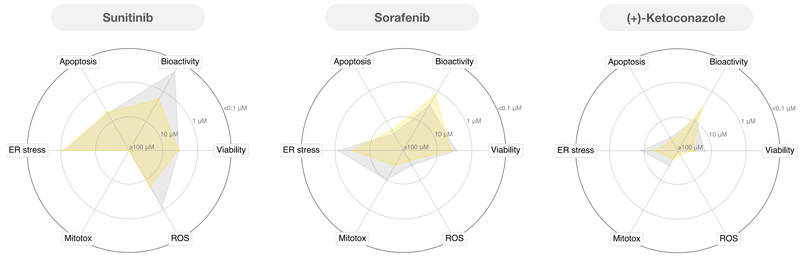


Bioactivity scale
0 -indistinguishable from DMSO
1- clearly distinguishable

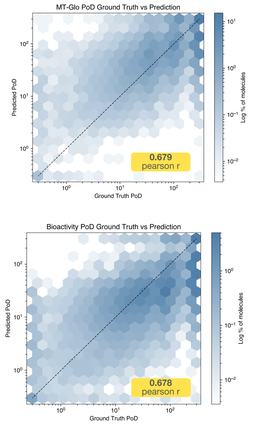


In Silico Prediction of Cell Morphology

Accurate, mechanistic in silico prediction: We train in silico models which use only the structural information of a compound (SMILES string) to predict diverse cytotoxicity features.



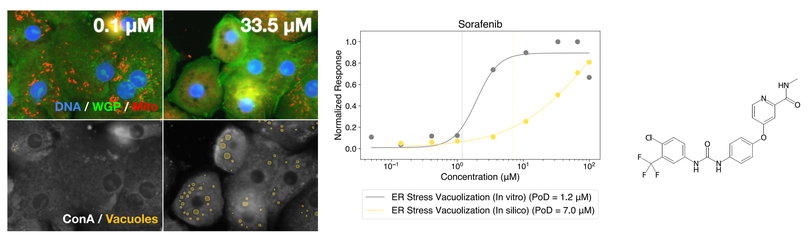
ER stress: cytoplasmic vacuolization
Mitotox: increase in fissioned mito
Apoptosis: cytosol area reduction
ROS: RNA condensate formation
Bioactivity: deep learning morphology assay
Viability: MT-Glo biochemical assay

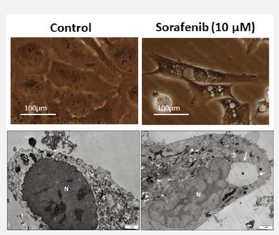


Response type	% of *in silico* assay PoDs within:		
	2x *in vitro* PoD	3x *in vitro* PoD	5x *in vitro* PoD
Viability (MT-Glo)	74	86	93
Cytotoxicity (Nuclei count)	82	90	95
Necrosis (LDH)	87	92	95
Apoptosis (Nuclei area)	86	92	96
Apoptosis (Cytoplasm area)	80	89	95
Mitotox (Fission)	81	90	95
ER Stress (Vacuolization)	67	81	90
ROS (RNA condensate)	81	89	94
Bioactivity	56	75	88

Case Study: Predicting ER stress for Sorafenib

Axiom's in-silico ER stress model accurately predicts the primary phenotypic driver of cytotoxicity for Sorafenib is endoplasmic reticulum (ER) vacuolization, indicating activation of the paratopic cell death pathway.



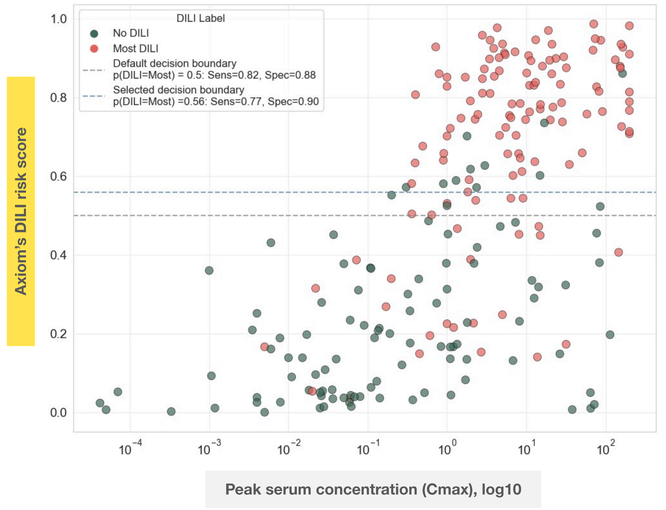


Dose dependent sorafenib induced cytoplasmic vacuolation in LX2 cells are due to ER stress. (a,b) Phase-contrast and Transmission electron micrograph (TEM) of untreated control and 10 µM sorafenib treated LX2 cells for 24 h.

Sharma et al, Cytoplasmic vacuolation with endoplasmic reticulum stress directs sorafenib induced non-apoptotic cell death in hepatic stellate cells. Sci Rep, 2021

Clinical Risk Assessment on FDA DILIrank

Combining our in silico cell stress and death features with predicted ADME properties and human exposure, we predict a DILI risk score that matches or outperforms in vitro models



0.92 AUC

77% sensitivity

90% specificity

Axiom's *in silico* model vs. *in vitro* assays

We compare our in silico DILI risk prediction model with published in vitro benchmarks, choosing a predictive threshold with 90% + specificity.

Benchmark Publication	Benchmark Assay	Assay Performance (auc, sens, spec)	Axiom Performance (auc, sens, spec)
F Shah, *Toxicol Sci* (2015)	2D HepG2	– / 34% / 91%	**0.89 / 75% /** 90%
S Schadt, *Toxicol In Vitro* (2015)	2D PHH imaging	– / 41% / 86%	**0.85 / 71% / 91%**
WR Proctor, *Arch Toxicol* (2017)	3D Spheroid	– / 48% / 93%	**0.83 / 71% / 93%**
L Fa, *Toxicol Sci* (2024)	3D Spheroid	0.80 / 72% / 90%	**0.90 / 77% / 91%**
Walker, *Archives of Toxic*ol (2020)	3D Spheroid	– / 71% / 100%	**0.91 / 74% /** 93%

AUC: area under the ROC curve. 0.5=random guessing, 1.0=perfect classification.
Sens: **sensitivity**, or true positive rate: the percentage of DILI compounds correctly classified as DILI
Spec: **specificity**, or true negative rate: the percentage of no-DILI compounds correctly classified as no-DILI

Conclusion

We developed in silico models of cell biology using a dataset of over 100,000 compounds tested in primary human liver cells with high-content imaging. We then combined these digital readouts with thousands of clinical datapoints to train a model that predicts DILI risk at human-relevant exposure levels.

Our AI system delivers purely digital clinical risk assessment with superior accuracy and interpretability to traditional in vitro assays. This tool will empower scientists to make better-informed decisions and develop safer drugs faster.