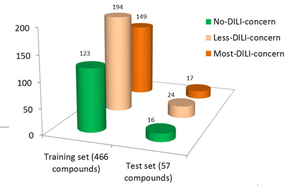
Development of DILI Prediction Tools for Humans and Mammals from the Vantage of Translational Safety Assessment

Wolfgang Muster[1], Doha Naga[1], James Rathman[2,3], Aleksandra Mostrag[2], Chihae Yang[2,3]

1 Hoffmann-La Roche Ltd, Pharmaceutical Sciences, Basel Switzerland 2 MN-AM - Molecular Networks GmbH, Nürnberg, Germany and Altamira LLC, Columbus OH USA 3 Chemical and Biomolecular Engineering Department, The Ohio State University

MOTIVATION

- Drug-induced liver injury (DILI) is challenging when translating pre-clinical stage knowledge to human clinical studies or in the market
- The developed liver toxicity prediction system was previously used to demonstrate that structural rules with metabolic reactivity can differentiate between mammalian and human DILI in cases of e.g., milnacipran and pyrantel[1]
- We present comparative analysis of drugs and effects spaces based on existing pre-clinical and clinical databases through a study within the eTRANSAFE (enhancing TRANslational SAFEty assessment through Integrative knowledge management) project[1]
- The liver toxicity prediction system was evaluated with in-house data focusing on translational relationships varying across the space defined by chemical structures, therapeutic categories, and metabolism



LIVER TOXICITY PREDICTION SYSTEM

Mammalian Training Set

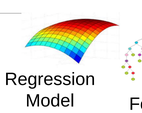
- 600 structures with treatment-related findings and 308 structures with no liver findings from over 1800 APIs from eTOX project[2]
- Ontology approach: over 400 findings descriptions linked to diseases were mapped into categories of steatosis, hepatitis, fibrosis/cirrhosis, etc and grouped to score the liver toxicity

Human Training Set

- From US FDA NCTR DILIRank dataset, containing 1036 drugs administered to humans classified in terms of their DILI risk based on FDA drug labels after verification for causality[3,4,5]
- DILI descriptions: serum enzyme elevations, no jaundice/jaundice, with/without hospitalizations, other severe symptoms, hepatic failure, liver transplant, etc..

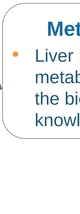
Human DILI QSAR Models

- Ordinal partial logistic regression
- Training set of 523 orally administered drugs originating from DILIRank dataset (ambiguous classifications were not used)
- Structural features: ToxPrint chemotypes[6]
- Quantum mechanical properties: HOMO/LUMO, heat of formation; physicochemical properties: logP, logS, TPSA, HBA, dipole moment, shape descriptors, etc.



Weight of Evidence [7]:

- Dempster-Shafer Theory
- Combine multiple evidence sources to derive the final outcome
- Estimate the uncertainty



Metabolism Prediction

- Liver BioPath – human liver metabolites generator based on the biotransformations knowledgebase

Structure Rules

- Represented as chemotypes[6]
- Based on human drug structure set (769) from FDA NCTR LTKB[4] and mammalian API dataset (908) from eTOX[2]
- Potency-aware rules by correlating with LO(A)EL

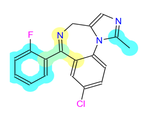
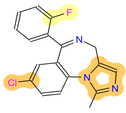
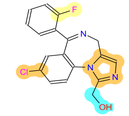
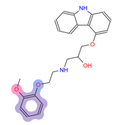
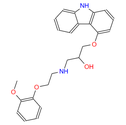
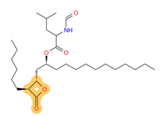
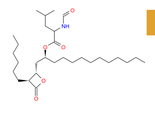
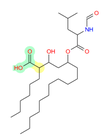
Chemotypes	Z-scores Human DILI (523)	Z-scores Mammal DILI (506)
Halide any	3.57 (152)	12.8 (264)
Halide aromatic	3.14 (122)	3.25 (223)
Halide alkyl	0.72 (33)	2.21 (66)
Carboxylic acid	3.10 (108)	0.57 (72)
Amine tertiary	-3.75 (156)	2.30 (191)
Amine aromatic primary	1.95 (28)	0.48 (26)
Amine alicyclic	-2.51 (108)	2.70 (159)
Furan	2.20 (13)	-1.00 (15)
Imidazole / Benzimidazole	2.18 (36)	-1.20 (34)
Nitro	1.96 (17)	-1.25 (10)
Piperazine	1.43 (27)	4.23 (59)
Pyrimidine	1.42 (27)	2.03 (54)
Benzyl alcohol	-4.29 (23)	1.78 (13)

Number in () represents frequency of each class.

$$z_i = \frac{\bar{y}_i - \bar{y}}{\sqrt{\left(\frac{n_0}{n_i}\right)\frac{S^2}{N}}}$$

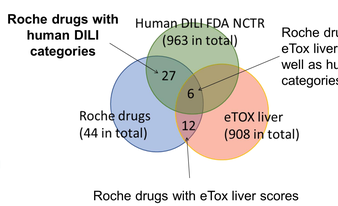
Dark orange: most potent (Z ≥ 2)
Light orange: above average (1 ≤ Z <2)
Grey: average (-1 < Z < 1)
Light blue: below average (-2 < Z ≤ -1)
Dark Navy blue: least potent (Z ≤ -2)

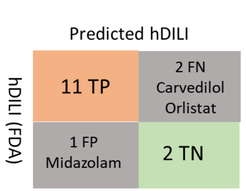
CASE STUDIES – RESULTS & ANALYSIS

	Mammalian DILI	Human DILI	Human metabolism	Human DILI alerts in parent	Human DILI alert in metabolite	Hypothesis / Conclusion
Midazolam (FP) **Pharmacology:** Short acting benzodiazepine, GABA A receptor modulator **Indication**: Anesthetic, hypnotic	Hepatotoxicity at high, clinically not relevant doses Species-specific effects (e.g. increase of Alkaline Phosphatase in dogs) **Preclinical effect not relevant for the clinical situation**	**No DILI concern**	 Oxidation at the 1- (alpha-) position mediated by cytochrome P450 3A4 in the liver and gut, followed by glucuronidation of the alpha-hydroxyl metabolite	 **DILI-positive alerts**: of "aromatic halide" and "imidazole" ring Benzodiazepine requires metabolic activation (not detected) - liver injury due to benzodiazepine is rare[8,9]	 **DILI-negative rule**: "bond:COH_alcohol_generic"	• Reliable mechanistic explanation for a non-relevant alert in preclinical studies and/or observed pre-clinical changes at doses which are higher then needed for efficacy • As the preclinical effect is not relevant for the clinical situation, the non-DILI alert for Midazolam is likely • FP result should be corrected: the DILI-negative rule for the main metabolite fits into the observed lack of DILI findings in the clinics, but the FP prediction is mechanism-related. The hydroxylation of methyl group occurs in humans as well as in animals
Carvedilol (FN) **Pharmacology**: Non-selective beta blocker - blocks beta-1 and beta-2 adrenergic receptors as well as the alpha-1 adrenergic receptors **Indication**: Treatment of mild to moderate congestive heart failure (CHF)	**Hepatotoxicity** in gavage studies in rat (from subchronic to chronic) Liver weight increase, enlargement, discoloration and congestion, hepatocytes vacuolation, focal liver cell and bile duct hyperplasia, enzymes elevation	**Low DILI concern** Reversible, mild hepatocellular injury with minimal clinical symptomatology	 Aromatic ring hydroxylation; highly lipophilic drug is rapidly and extensively metabolized to polar and mostly water soluble compounds	 **No alerts**	 **DILI-positive rule**: resorcinol	• FN for human-DILI should be corrected to human-DILI positive prediction after accounting for metabolism to AR-OH and hitting the "resorcinol" alert • Very low exposure to the metabolites – the DILI concern is very minor • This may not be a FN; but TN.
Orlistat (FN) **Pharmacology**: Inhibitor of pancreatic lipase, an enzyme that breaks down triglycerides in the intestine **Indication**: Anti-obesity agent	**No treatment related liver findings**	**Major DILI concern** Severe, but only rarely observed liver injury with hepatocellular necrosis, acute hepatic failure with some of the cases resulting in liver transplant or death, enzymes elevation, hepatitis that may be serious	 Rapid hydrolysis of beta lactone forming carboxylic acid metabolite	 **No alerts**	 **Strong DILI-positive** alert: Carboxylic acid	• Bioavailability of the API and its metabolites is very low - questionable importance of the metabolite for the liver effects • Bioavailability highly dependent on diet (low/high fat diet) –therefore the impact is unlikely, but cannot be fully excluded • Mechanism of liver injury is not known - immunological reactions hypothesized, but not confirmed

CASE STUDIES – DESCRIPTIONS

- 44 marketed drugs from Hoffman La Roche were identified within the mammalian (908) and human (963, all routes) DILI datasets.
- Human DILI Data for 27 drugs
  - 13 drugs - human DILI positive (oral); 3 – negative (oral)
  - 11 were either ambiguous or non-oral route (which needs further evaluation with metabolic rules)





- hDILI QSAR model results for 16 orally administered drugs
- We analyzed the FN/FP cases to present the development of structural knowledge (across species) to correct these false QSAR predictions

SUMMARY & PERSPECTIVES

- A liver knowledgebase prediction system containing multiple components (available from ChemTunes•ToxGPS and eTOXsys) was used for the development of knowledge supporting translating pre-clinical knowledge to human clinical studies
- We confirmed that the species variations highly depend on metabolic diversity and compound class

REFERENCES

(1) eTRANSAFE project, http://etransafe.eu/ **(2)** eTOXsys products at http://etoxsys.com/, eTOXsys sampler at https://etoxsys.eu/etoxsys.v3-demo-bk/dashboard/. **(3)** Willyard C. Nature Medicine, 22(5), 450-451, 2016. **(4)** Chen M et al. Toxicol Sci 136(1), 242-249, 2013. **(5)** Thakkar S et al. Expert Review of Gastroenterology & Hepatology, 12(1), 31-38, 2017. **(6)** Yang C et al. Journal of Chemical Information and Modeling, 55(3), 510-528, 2015. **(7)** Rathman J et al. Computational Toxicology, 6, 16-31, 2018. **(7)** Digestive Diseases and Sciences, 42(7), 1400–1404, 2000. **(8)** Drug Metabolism and Disposition 37(2), 345-351, 2009.

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Contact: chihae.yang@mn-am.com