devTOX *quick*Predict Accurately Predicts the Developmental Toxicity Potential of the ICH S5(R3) Guideline Reference Compounds

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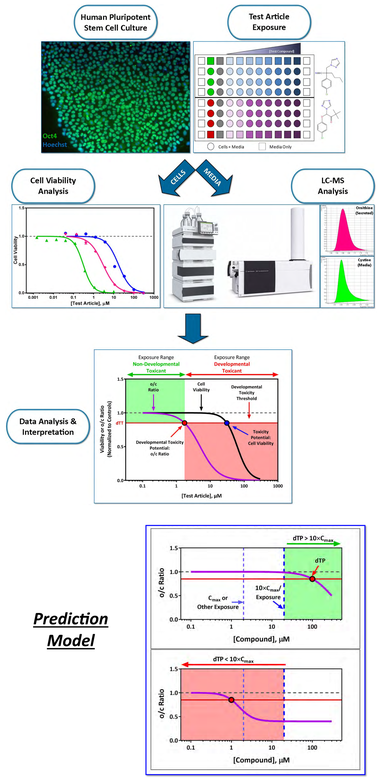
INTRODUCTION

- There have been increased efforts in the pharmaceutical and chemical industries to incorporate new approach methods (NAMs) (*in vitro, ex vivo,* or *in silico*) earlier in the product development pipeline prior to *in vivo* testing.
- New guidelines have been released by regulatory agencies that permit the use of NAMs in conjunction with or in place of the traditional *in vivo* embryo-fetal development (EFD) studies.
- In particular, the revised S5 (R3) guideline on Detection of Reproductive and Developmental Toxicity for Human Pharmaceuticals recently issued by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) defines specific scenarios where qualified NAMs can be used to defer or replace conventional *in vivo* testing or as part of a weight of evidence assessment.
- The devTOX *quickPredict* (devTOX^{qP}) assay is an *in vitro* human pluripotent stem (hPS) cell-based assay that predicts the developmental toxicity potential of chemicals based on changes in ornithine and cystine metabolism.
- In this study, the devTOX^{qP} assay was used to assess the developmental toxicity potential of the 29 ICH-positive reference compounds and 22 negative compounds. Assay accuracy was assessed by comparing the assay results to human, rodent, and rabbit plasma concentrations.

METHODS



- Human induced pluripotent stem (iPS) cells (HYR0103 or DYR0100; ATCC) were maintained in the undifferentiated state in mTeSR1 (StemCell Technologies) on Matrigel (Corning).
- Cells were plated in 96-well plates and exposed to 8 concentrations of each test article for 48 hours. Media ± test article were replaced approximately every 24 hours.
- Spent media from the last 24-hour treatment period was collected and cell viability was assessed using the CellTiter-Fluor Cell Viability Assay (Promega).
- Media samples were analyzed with UPLC-ESI-TOF-MS to determine ornithine (ORN) and cystine (CYSS) levels.
- ORN and CYSS were normalized to spiked-in internal standards (ISTD) and the median response of the reference treatment samples (0.1% DMSO). The o/c ratio was calculated by dividing the reference-normalized value of ORN by the reference-normalized value of CYSS
- Non-linear dose-response curves for the o/c ratio, ornithine and cystine response and cell viability were fit with GraphPad Prism 9 (GraphPad Software).
- The developmental toxicity potential (dTP, o/c ratio) and toxicity potential (TP, cell viability) concentrations were predicted from the respective dose-response curves using the developmental toxicity threshold (dTT).
- Accuracy was assessed by scoring the dTP concentration against the respective human rodent, or rabbit C_{max} using a 10-fold cutoff.
  - If the dTP concentration was > 10 × *in vivo* exposure, the drug was classified as a non-developmental toxicant (**green shaded box**).
  - If the dTP concentration was < 10 × *in vivo* exposure, the drug was classified as a developmental toxicant (**red shaded box**).

Prediction Model

RESULTS

devTOX^{qP} Accurately Predicts Developmental Toxicity Potential Across a Wide Range of Mechanisms

	Drug	CASRN	dTP (µM)	TP (µM)	Human Therapeutic Total C_{max} (µM) [2]	Human Dev Tox (1st Tri) [1]	devTOX^{qP} Prediction vs. Human C_{max} [A]	Rat NOEL C_{max} (µM) [2]	Rat LOEL C_{max} (µM) [2]	Rat *In Vivo* Dev Tox [1]	devTOX^{qP} Prediction vs. Rat C_{max} [B]	Rabbit NOEL C_{max} (µM) [2]	Rabbit LOEL C_{max} (µM) [2]	Rabbit *In Vivo* Dev Tox [1]	devTOX^{qP} Prediction vs. Rabbit C_{max} [C]	Rat & Rabbit Combined Classification	devTOX^{qP} Prediction vs. Rat/Rabbit Combined [D]
Developmental Toxicants (N=29)	Acitretin	55079-83-9	0.025	ND	2.4	DT	DT	4.6	9.2	DT	DT	n.d.	n.d.	DT	n.d.	DT	DT
	Aspirin	50-78-2	795	2,415	39.3	DT (HD only)	NON	139	222	DT	DT	n.d.	n.d.	NON	n.d.	DT	DT
	Bosentan	147536-97-8	128	165	2.0	Suspected	NON	8.2	16.3	DT	DT	2.6	NE	NON	NON	DT	DT
	Busulfan	55-98-1	2.5	6.3	0.5	DT	DT	NA	3.4	DT	DT	n.d.	n.d.	DT	n.d.	DT	DT
	Carbamazepine	298-46-4	0.6	ND	49.5	DT	DT	140	275	DT	DT	NA	123	DT	DT	DT	DT
	Cisplatin	15663-27-1	0.03	0.2	14.4	DT	DT	1.1	3.6	DT	DT	n.d.	n.d.	n.d.	n.d.	DT	DT
	Cyclophosphamide	50-18-0	ND	ND	406	DT	NON	NA	15.7	DT	NON	NA	578	DT	NON	DT	NON
	Cytarabine	147-94-4	0.04	0.2	11.5	DT	DT	23.8	47.7	DT	DT	n.d.	n.d.	n.d.	n.d.	DT	DT
	Dabrafenib	1195765-45-7	0.4	0.3	4.9	Suspected	DT	2.3	4.2	DT	DT	n.d.	n.d.	n.d.	n.d.	DT	DT
	Dasatinib	302962-49-8	0.003	0.05	0.3	DT	DT	NA	0.5	DT	DT	0.5	NE	NON	DT	DT	DT
	Fluconazole	86386-73-4	2,228	ND	29.6	DT (HD only)	NON	111	176	DT	NON	88.2	264	DT	DT	DT	NON
	Fluorouracil	51-21-8	1.3	1.6	223	DT	DT	20.0	29.8	DT	DT	NA	853	DT	DT	DT	DT
	Hydroxyurea	127-07-1	4.0	239	684	DT	DT	622	1,060	DT	DT	n.d.	n.d.	DT	n.d.	DT	DT
	Ibrutinib	936563-96-1	0.6	10.3	0.3	Suspected	DT	3.0	6.0	DT	DT	0.7	4.2	DT	DT	DT	DT
	Imatinib	152459-95-5	15.3	32.5	7.5	DT	DT	7.2	24.6	DT	DT	107	NE	NON	DT	DT	DT
	Isotretinoin	4759-48-2	0.003	ND	1.1	DT	DT	3.0	6.0	DT	DT	3.2	10.3	DT	DT	DT	DT
	Methotrexate	59-05-2	0.04	0.06	4.7	DT	DT	NA	0.5	DT	DT	NA	3.5	DT	DT	DT	DT
	Pazopanib	444731-52-6	0.2	2.1	133	Suspected	DT	7.9	23.8	DT	DT	0.3	2.4	DT	DT	DT	DT
	Phenytoin	57-41-0	119	173	57.5	DT	DT	53.1	106	DT	DT	107	135	DT	DT	DT	DT
	Pomalidomide	19171-19-8	0.01	N	0.3	Suspected	DT	NA	9.9	DT	DT	NA	0.3	DT	DT	DT	DT
	Ribavirin	36791-04-5	1.5	20.4	15.1	Suspected	DT	0.02	0.05	DT	NON	n.d.	n.d.	DT	n.d.	DT	NON
	Salicylic Acid	69-72-7	778	1,377	327	n.d.	DT	956	1,528	DT	DT	3,577	n.d.	NON	DT	DT	DT
	Tacrolimus	104987-11-3	27.0	12.4	0.04	n.d.	NON	0.004	0.02	DT	NON	0.001	0.004	DT	NON	DT	NON
	Thalidomide	50-35-1	0.09	ND	2.4	DT	DT	3.8	18.9	DT	DT	3.2	8.4	DT	DT	DT	DT
	Topiramate	97240-79-4	3261	4205	39.8	Inc./DT	NON	144	497	DT	DT	38.3	67.8	DT	NON	DT	NON
	Tretinoin	302-79-4	0.0001	ND	1.3	DT	DT	0.5	1.0	DT	DT	0.3	1.0	DT	DT	DT	DT
	Trimethadione	127-48-0	10,087	ND	299	DT	NON	411	1,642	DT	DT	n.d.	n.d.	n.d.	n.d.	DT	DT
	Valproic Acid	99-66-1	162	639	1422	DT	DT	512	1,574	DT	DT	2,843	5,636	DT	DT	DT	DT
	Vismodegib	879085-55-9	58.8	ND	30.9	Suspected	DT	NA	17.1	DT	DT	n.d.	n.d.	n.d.	n.d.	DT	DT
Non-Developmental Toxicants (N=22)	Acyclovir	59277-89-3	ND	ND	3.0	NON	NON	7.1	NE	NON	NON	1.7	NE	NON	NON	NON	NON
	Amoxicillin	26787-78-0	ND	ND	20.5	NON	NON	1,800	NE	NON	NON	n.d.	n.d.	n.d.	n.d.	NON	NON
	Cetirizine	83881-51-0	29.2	ND	0.9	NON	NON	116	2,597	DT-E	DT	352	NE	NON	DT	DT	DT
	Chlorthalidone	77-36-1	ND	ND	18.6	NON	NON	10.7	NE	NON	NON	n.d.	n.d.	NON	n.d.	NON	NON
	Clindamycin	18323-44-9	ND	ND	7.1	NON	NON	n.d.	n.d.	NON	n.d.	n.d.	n.d.	NON	n.d.	NON	n.d.
	Cyclobenzaprine	303-53-7	0.3	ND	0.09	NON	DT	n.d.	n.d.	NON	n.d.	n.d.	n.d.	NON	n.d.	NON	n.d.
	Desloratadine	100643-71-8	16.3	19.7	0.01	Inc./NON	NON	1.6	5.0	DT-E	DT	1.5	3.8	DT-E	DT	DT	DT
	Doxylamine	469-21-6	53.7	ND	0.4	NON	NON	n.d.	n.d.	NON	n.d.	n.d.	n.d.	NON	n.d.	NON	n.d.
	Erythromycin	114-07-8	165	ND	2.7	NON	NON	n.d.	n.d.	NON	n.d.	n.d.	n.d.	NON	n.d.	NON	n.d.
	Hydrochlorothiazide	58-93-5	ND	ND	1.7	NON	NON	n.d.	n.d.	NON	n.d.	n.d.	n.d.	NON	n.d.	NON	n.d.
	Maraviroc	376348-65-1	ND	ND	1.7	n.d.	NON	14.7	NE	NON	NON	11.4	NE	NON	NON	NON	NON
	Metoclopramide	364-62-5	ND	ND	0.2	NON	NON	n.d.	n.d.	NON	n.d.	n.d.	n.d.	NON	n.d.	NON	n.d.
	Nizatidine	76963-41-2	ND	ND	4.1	NON	NON	n.d.	n.d.	NON	n.d.	n.d.	n.d.	NON	n.d.	NON	n.d.
	Oseltamivir	196618-13-0	ND	ND	0.2	n.d.	NON	12.1	42.6	NON	NON	23.4	29.8	DT-E	NON	DT	NON
	Saxagliptin	361442-04-8	ND	ND	0.08	NON	NON	789	NE	NON	NON	136	NE	NON	NON	NON	NON
	Sitagliptin	486460-32-6	ND	ND	1.0	NON	NON	26.5	56.9	NON	NON	54.8	NE	NON	NON	NON	NON
	Sulfasalazine	599-79-1	124	ND	15.1	NON	DT	n.d.	n.d.	DT-E	n.d.	n.d.	n.d.	NON	n.d.	DT	n.d.
	Tapentadol	175591-23-8	23.5	342	1.0	n.d.	NON	3.5	5.3	DT-E	DT	0.7	2.6	DT	DT	DT	DT
	Tegaserod	145158-71-0	3.1	4.3	0.01	NON	NON	0.2	NE	NON	NON	0.7	NE	NON	DT	NON	DT
	Vildagliptin	274901-16-5	ND	ND	2.2	n.d.	NON	n.d.	n.d.	NON	n.d.	n.d.	n.d.	NON	n.d.	NON	n.d.
	Zaleplon	151319-34-5	92.6	307	0.3	n.d.	NON	11.6	60.6	NON	DT	8.6	NE	NON	NON	NON	DT
	Zidovudine	30516-87-1	169	ND	5.0	Inc.	NON	36.6	110	DT-E	DT	103	347	DT-E	DT	DT	DT

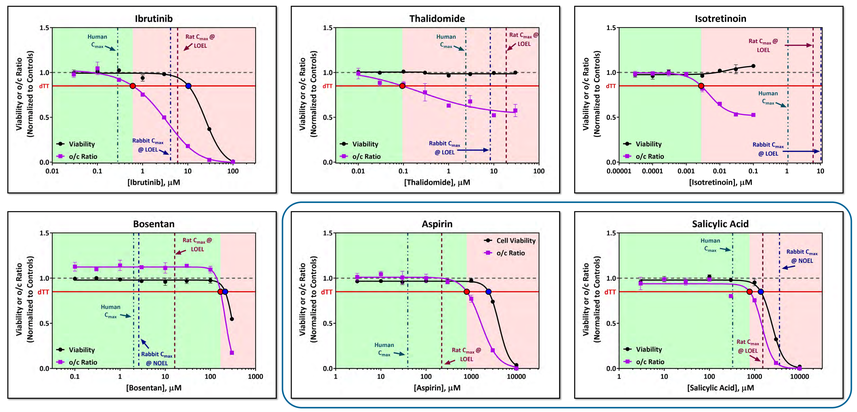
Notes: DT: Developmental Toxicant; **DT-E**: Developmental Toxicant main effect of chemical is embryo lethality; **Suspected**: Drug expected to cause developmental toxicity based on MOA; **NON**: Non-Developmental Toxicant; **Inc.**: Human studies have been inconclusive or inconsistent; **HD**: High doses; **ND**: no effect detected within the exposure range tested; **NE**: No developmental effects observed in studies; **NA**: NOEL dose not determined; **n.d.**: no data available or not tested/determined. **A**: Scored against human therapeutic C_{max}; **B**: Scored against rat NOEL or LOEL C_{max}; **C**: Scored against rabbit NOEL or LOEL C_{max}; **D**: Combined classification based on more sensitive species. Rat and rabbit C_{max} values used for prediction are highlighted in yellow.

devTOX^{qP} Performance Scored Against Human Therapeutic and In Vivo Exposures

	Human	Rat	Rabbit	Rat & Rabbit
# DT	29	33	19	34
# NON	22	9	11	8
Total #	51	42	30	42
Balanced Accuracy	83%	88%	67%	79%
Sensitivity	76%	88%	79%	82%
Specificity	91%	89%	55%	75%
PPV	92%	97%	75%	93%
NPV	74%	67%	60%	50%

- The accuracy of devTOX^{qP} was assessed in comparison to human, rat, and rabbit positive and negative exposures as described in the ICH S5(R3) guidelines.
- Seven developmental toxicants compounds were mis-classified when scored against the human therapeutic C_{max}.
  - Three of these compounds require metabolism to the suspected proximate developmental toxicant (aspirin, cyclophosphamide, and trimethadione). Salicylic acid, aspirin's active metabolite, was correctly classified in the assay, indicating the importance of testing the active metabolite *in vitro* in addition to the parent compound when possible.
  - Bosentan may be misclassified due to the timing of expected effects, which is during the 3rd trimester in humans.

Example devTOX^{qP} Results



CONCLUSIONS

- When scored against the human therapeutic C_{max}, the assay predicted the developmental toxicity potential of these compounds with 82% accuracy (76% sensitivity, 91% specificity).
- devTOX^{qP} was 88% and 67% concordant with rat and rabbit *in vivo* developmental toxicity results, respectively, when scored against the *in vivo* NOEL or LOEL C_{max} concentration.
- devTOX^{qP} identifies the developmental toxicity potential for a wide range of mechanisms, but may not be applicable in all cases (i.e., targets not expressed in human iPS cells).
- The results from this study provide further evidence of the accuracy of the devTOX^{qP} assay and support its use as an alternative method under the revised ICH S5 (R3) guideline.

References:
[1] Human, rodent and rabbit effects summarized from Drugs in Pregnancy and Lactation (Briggs et al., 2011), TERIS (https://deohs.washington.edu/teris/), and/or approved package inserts. [2] Exposure data were identified from the ICH S5(R3) guidelines, literature, drug-specific FDA pharmacology reviews, and/or approved package inserts. Full list of references available upon request.

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