ToxCast Profiling in a Human Stem Cell Assay For Developmental Toxicity

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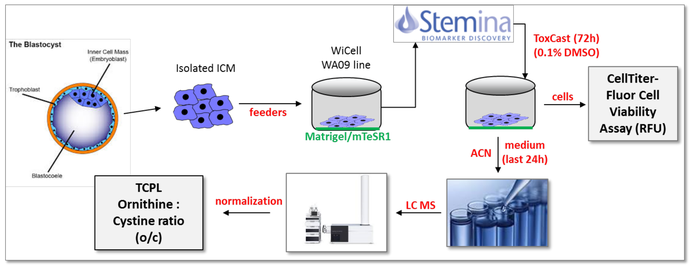
Introduction

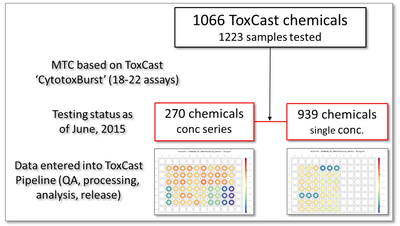
- ToxCast (release date December 2014) generated bioactivity profiles for 1060 diverse chemicals across 815 *in vitro* assay endpoints [1].
- To increase the diversity of ToxCast assays used to assess developmental toxicity, we tested the chemical library in a metabolic biomarker-based assay (Stemina devTOXqP). This platform utilizes the H9 human embryonic stem (hES) cell line to measure cellular release of ornithine (ORN) relative to cellular utilization of cystine (CYSS) as a predictive model for teratogenicity [2].
- To date, the raw and plate-normalized data for 286 samples in concentration series (269 chemicals plus replicates, n=3) and another 812 samples at a single concentration (n=4) have been collected for input to the ToxCast portfolio.
- GOAL: Here, we describe the ToxCast data pipeline (TCPL) for this dataset for QA, processing, analysis and eventual release to the public.

Methods



- METHODS: H9 cells were maintained undifferentiated in 96-well plates and exposed for 3-days to chemicals (blinded to the experimenter). Media from the last 24h of exposure was analyzed by HPLC-HRMS; C^{13} spike-in standards normalized ornithine (ORN) and cystine (CYSS) levels. Cell viability was determined by CellTiter-Fluor (Promega) assay.
- EXPOSURE: Each plate had controls for vehicle (0.1% DMSO), negative response (5 nM methotrexate (MTX), and positive response (1 µM MTX). ToxCast exposures were guided by cytotoxicity determinations [1]. Teratogen Index (TI) used the ORN/CYSS ratio (o/c ≤ 0.88) and related this to cell viability normalized to plate-level vehicle control [2].

ToxCast Pipeline (TCPL)



Testing Strategy: Maximum Test Concentration (MTC) for exposure was guided by AC50 concentration across multiple cytotoxicity-related assays in ToxCast [1].

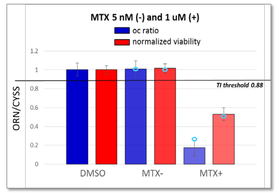
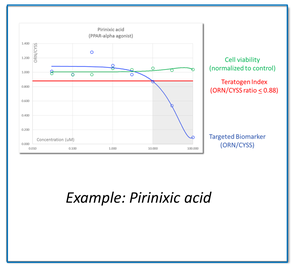
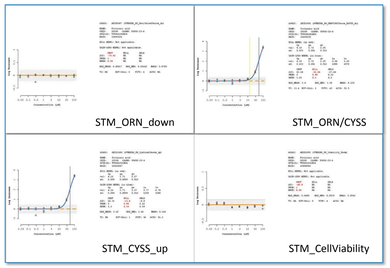


Plate References:
0.1% DMSO (n=846, 857)
MTX-negative (5 nM, n=425, 429)
MTX-positive (1 µM, n=424, 429)
MTX-ToxCast: 3 nM (-), 1 µM (+)

FEATURE ▶ LEVEL ▼	STM_ORN_down	STM_CYSS_up	STM_ORN/CYSS	STM_CellViability
0	Raw metabolite corrected to spike-in C^{13}-standard and normalized to median value of DMSO control		Direct Ratio computed from normalized raw data on the medium	Relative Fluorescent Units (RFU) on hES cell layer normalized to DMSO control
1	removed entries flagged for poor well-quality, empty ('0') cells, …			
2	Log2 transformation of raw data (individual measures, n)			
3	normalization (used contractor normalization); inverted relevant up/down features to look like ToxCast plots			
4	calculated parameters for automated curve fitting models (Constant, Hill, Gain-Loss)			
5	plot the winning model based on AIC and output µM conc. for Hit (0,1); TI (Teratogen Index); AC50; fold-change …			
6	manual flags for curve-fitting issues or data quality concerns (in progress)			



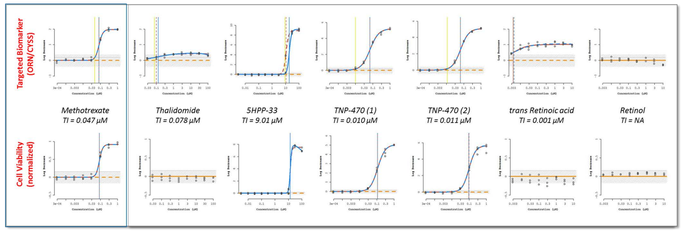
Conventional devTOXqP data representation [2].



TCPL representation [3].

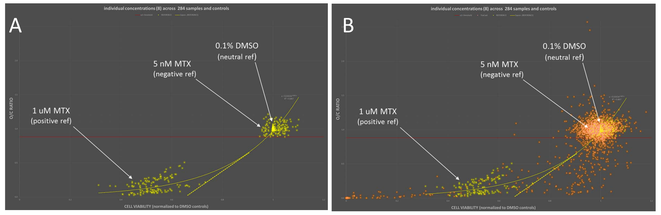
Results (TCPL Level 5)

Automated outputs for OCN/CYSS and hES CellViability shown for a few cases. Biomarker sometimes co-occurs with viability and other times not. TI conc. where ORN/CYSS falls to 0.88 [2] is imputed.



Individual data points graphed for ORN/CYSS as a function of CellViability.

A. Plate controls. MTX track projects to 65% viability.
B. 270 chemicals tested across 8-point conc. response [orange]; MTX projection defines sectors that do and don't co-occur with effects on viability.



Summary of Findings

- The devTOXqP dataset for ToxCast was shown to be of high-quality based on replicate samples and reference compounds.
- Overall, 136 chemicals (of 1066 tested) showed activity based TI ≤ 0.88. In 96.4% of these cases the concentration producing an effect on the biomarker (ORN/CYSS) fell below the AC50 for cell viability.
- Model performance (24 compound training set) showed a balanced accuracy of 83.3% (sensitivity 0.73, specificity 1.0); the increased specificity over sensitivity was consistent with the MTC testing strategy.
- For those 270 chemicals with concentration-response data, 75 actives tracked with decreasing cell viability and 48 chemicals tracked without an effect on cell viability.
- Some potent actives had low Emax (retinoic acid, thalidomide) relative to others where the targeted biomarker paralleled cytotoxicity (methotrexate, 5HPP-33).

References: [1] Judson et al. (2016) submitted
[2] Palmer et al. (2013) Birth Def Res B
[3] Knudsen et al. (2016) in prep.

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