Exploring the Use of Cell Painting and Transcriptomics in a Battery of Human Cell Lines for Bioactivity-Based Chemical Prioritization

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Background and Design

- Advances in new approach methods (NAMs) have led to more efficient, information rich, and cost-effective strategies for testing chemicals to inform next generation risk assessment (NGRA):

In vitro NAM	Endpoint
High-Throughput Phenotypic Profiling (HTPP) with Cell Painting	Cell morphology Nyffeler *et al.,* 2020 (PMID: 31899216)
High-Throughput Transcriptomics (HTTr) with TempO-Seq™	Gene expression Harrill *et al.,* 2021 (PMID: 33538836)

- NAMs-based points of departure (PODs) can be derived from concentration-response modeling of chemical bioactivity data using these assays for use in NGRA applications such as bioactivity:exposure ratio (BER) analysis for chemical prioritization.

- 41 chemicals found in drugs and consumer products were screened at 8 concentrations based on an assigned dose band determined from available bioactivity data and were treated for 24 hours in both assays:

Dose Band	Test Concentration Range	# of Chemicals
HIGH	0.1 – 300 µM, semi-log	21
MEDIUM	0.01 – 30 µM, semi-log	8
LOW	0.01 – 3 µM, semi-log	4
VERY LOW	3 x 10^{-6} – 30 µM, full-log	8

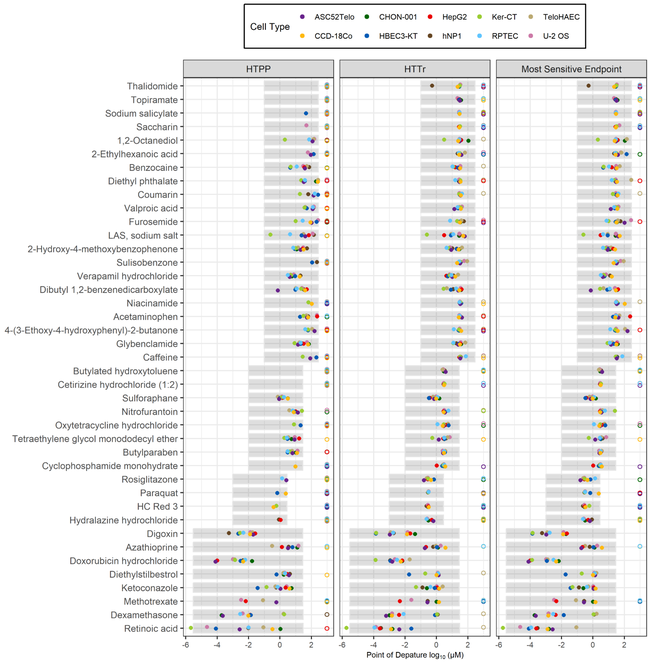
- These chemicals were screened across 10 biologically diverse human cell types to increase the coverage of molecular targets potentially perturbed by chemicals and to contribute to a greater likelihood that chemicals bioactive *in vivo* would be identified using these NAMs:

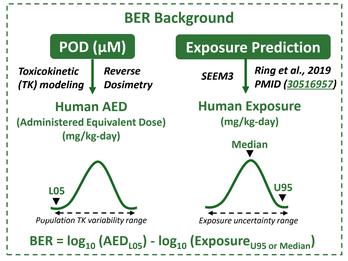
Cell Type	Background
ASC52Telo	hTERT-immortalized; adipose
CCD-18Co	Healthy donor; colon
CHON-001	hTERT-immortalized; long bone (cartilage)
HBEC3-KT	hTERT-immortalized; lung
HepG2	Cancer; liver
hNP1	Healthy donor; neural progenitor
Ker-CT	hTERT-immortalized; skin
RPTEC	hTERT-immortalized; kidney
TeloHAEC	hTERT-immortalized; heart
U-2 OS	Cancer; bone

- **Objective:** To assess how the individual or combined use of both high-throughput profiling assays and a cell line battery affected the ability to identify chemicals with overlapping bioactivity and human exposure potential

Using NAMs-Based Points of Departure (PODs) in Bioactivity:Exposure Ratio (BER) Analysis

Figure 1: The most sensitive endpoint POD provides the greatest coverage.





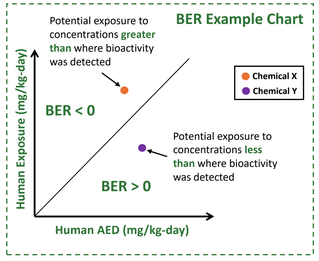
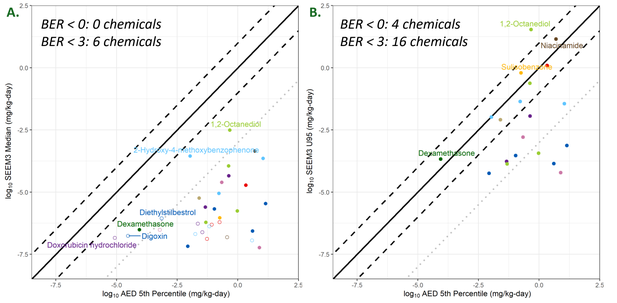


Figure 2: No individual cell line drives the most sensitive BER flags.



33/41 chemicals were incorporated into analysis due to lacking data for AED conversion and/or SEEM3 exposure predictions.

20/33 chemicals were incorporated into analysis due to 13 chemicals having unknown exposure pathway predictions for SEEM3.

Figure 3: Increasing biological coverage improves the ability to detect active and flagged chemicals.

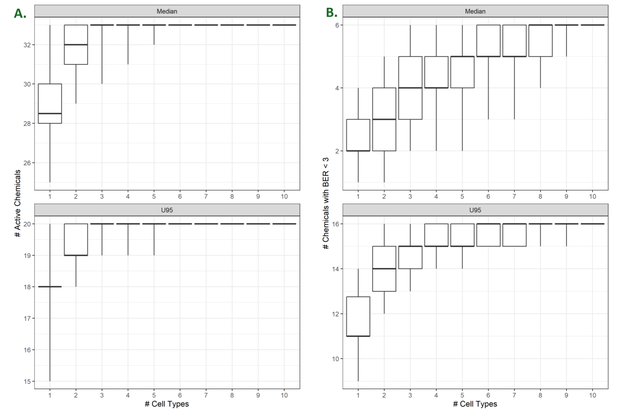


Figure 1: HTPP, HTTr and most sensitive HTP endpoint points of departure (POD). Each dot represents the $\log_{10}$ (POD) in µM for one cell type for all chemicals. Points are color coded by the associated cell type. Open circles represent that the POD for a given cell type was inactive, and values were arbitrarily assigned a $\log_{10}$ value of 3 for plotting purposes.

Figure 2: BER results using the 5th percentile AEDs derived from the most sensitive HTP endpoint. Results are plotted using exposure rates associated with SEEM3 median (A) or SEEM3 U95 (B). Each dot represents the results for one chemical that are associated with the color-coded cell type (see Figure 1) that had the smallest BER. Open circles represent chemicals that only had SEEM3 median exposure rates available. Chemicals with a BER < 3 (A) or a BER < 0 (B) are labelled. The solid line represents equivalent PODs between assays, and dashed lines represent one order of magnitude above or below equivalence.

Figure 3: Combinatorial analysis to assess how changing the cell line battery composition impacts BER findings. For each potential combination of cell types that could be used in analysis, the box plots represent how the maximum number of active chemicals (A) and maximum number of BER < 3 flags (B) varies by number of cell types included in the iteration from a single cell type to the full battery. Results are shown for data associated with the most sensitive HTP endpoint. There were a total of 1023 possible cell type combinations.

HTPP vs. HTTr Bioactivity

Figure 4: HTPP and HTTr provide complementary, but not redundant, information for screening-level assessment.

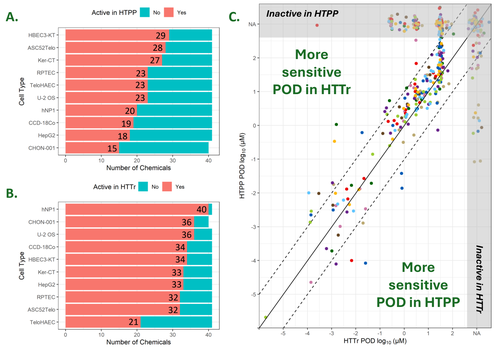


Figure 4: Comparing active chemicals and PODs between HTPP and HTTr. The proportion of test chemicals that were active (red) or inactive (blue) are shown for HTPP (A) and HTTr (B). The PODs derived from HTPP and HTTr for each cell type x chemical combination are plotted in C. The color of the dot represents the cell type (see Figure 1). The solid line represents equivalent PODs between assays, and dashed lines represent one order of magnitude above or below equivalence. Gray regions represent cases where a combination was only active in one assay.

Key Highlights

- VERY LOW dose band chemicals had the most diverse PODs across the cell line battery in both assays. These chemicals are potent drugs with known molecular target specificity.
- Complementary use of HTPP and HTTr (i.e., most sensitive endpoint) identified the greatest number of active chemicals for use in BER analysis.
- All cell lines had the most sensitive BER at least once across this chemical set despite differences in sensitivity observed across the full battery.
- Increasing the number of cell lines used in screening provided greater ability to detect active chemicals and chemicals with potential overlap between bioactivity and predicted human exposure levels.

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