Integrating High Throughput Transcriptomics into a Tiered Framework to Prioritize Chemicals for Toxicity Testing

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Background and Objectives

Study Background:

- 1000s of chemicals currently used in USA for commercial non-food/drug applications (https://www.epa.gov/tsca-inventory)
- Current toxicity testing requires multi-year animal studies, costing >$1 million per substance
- High-Throughput Transcriptomics (HTTr): broad-coverage assay for 1000s of chemicals in concentration-response format (Harrill *et. al. ToxSci* 2021)
- High-Throughput Screening (HTS): measurement of alterations in key molecular targets via US EPA ToxCast program (Richard *et. al. CRT* 2016)
- Computational integration of HTTr/HTS data streams for assessing key hazards is needed for further adoption in regulatory applications

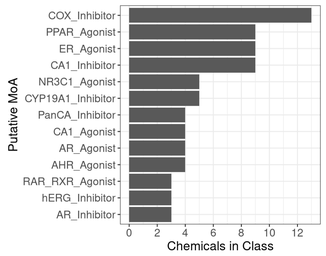
Study Objectives:

- Generate new signatures from HTTr screening data representing putative mechanisms-of-action (MoAs)
- Develop framework for validating HTTr signature results using HTS data streams
- Apply framework to current screening HTTr screening data to identify candidate modulators of key MoAs

Reference Signature Development

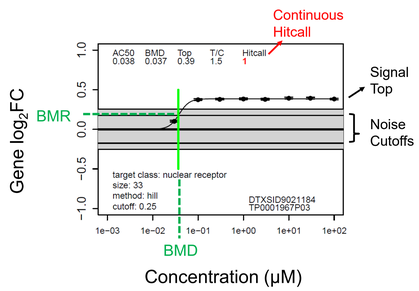
1) Assign Reference Chemicals to Putative MoAs:

- *RefChemDB*: curated literature associations of chemical-target pairs (Judson *et. al. ALTEX* 2019)
- Hierarchical clustering of similarly-represented targets (Bundy *et. al. BioData Mining* 2022)
- Chemical-cluster assignment: $Max(\sum Support_{Cluster})$



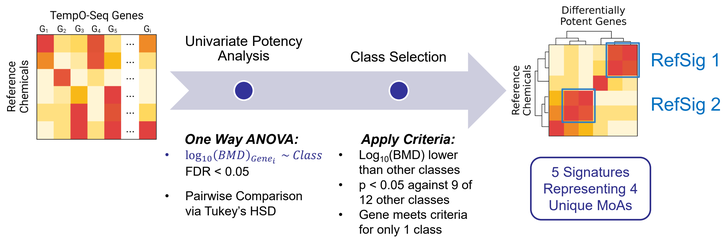
***2) Estimate Chemical Potency from HTTr Screening Data*:**

- 1218 chemicals screened in 8-point concentration response via TempO-Seq platform in HepaRG and U-2 OS cell lines (Yeakley *et. al. PLOS ONE* 2017, Harrill *et. al. Tox Sci* 2021)
- Benchmark Doses (BMDs) for DESeq2-moderated log2(FC) values estimated via *tcplfit2* (Sheffield *et. al. Bioinformatics* 2022)



3) Generate Reference Signatures from HTTr Potency Estimates:

- Apply univariate potency analysis to select genes uniquely potent for individual reference chemical sets:



One Way ANOVA:
- $\log_{10}(BMD)_{Gene_i} \sim Class$
- FDR < 0.05
- Pairwise Comparison via Tukey's HSD

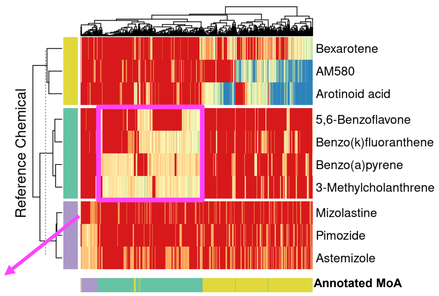
Apply Criteria:
- Log_{10}(BMD) lower than other classes
- p < 0.05 against 9 of 12 other classes
- Gene meets criteria for only 1 class

5 Signatures Representing 4 Unique MoAs

Reference Signatures Distinguish MoAs

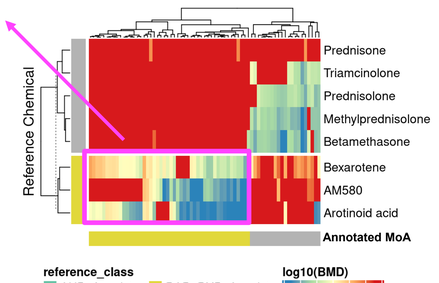
Gene-level comparison:
Hierarchical clustering of gene BMDs reveals distinct potencies of reference chemicals towards selected genes

HepaRG Gene Clustering: 1173 genes identified



Chemicals annotated for matching MoA demonstrate gene-level activity at low concentrations

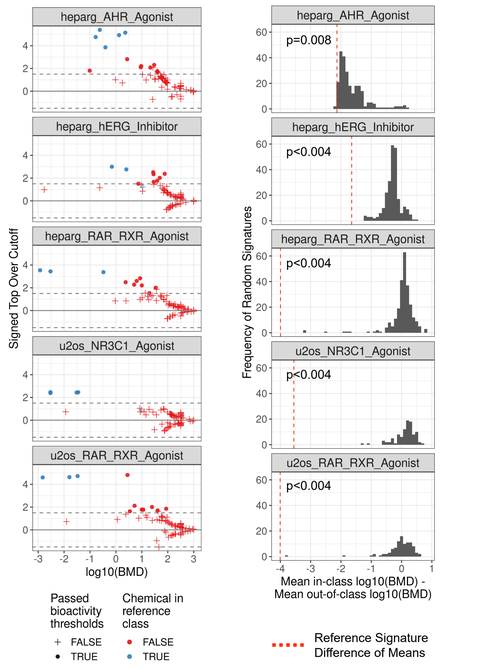
U-2 OS Gene Clustering: 69 genes identified



Distinct signatures across cell lines suggest need for profiling across multiple model systems

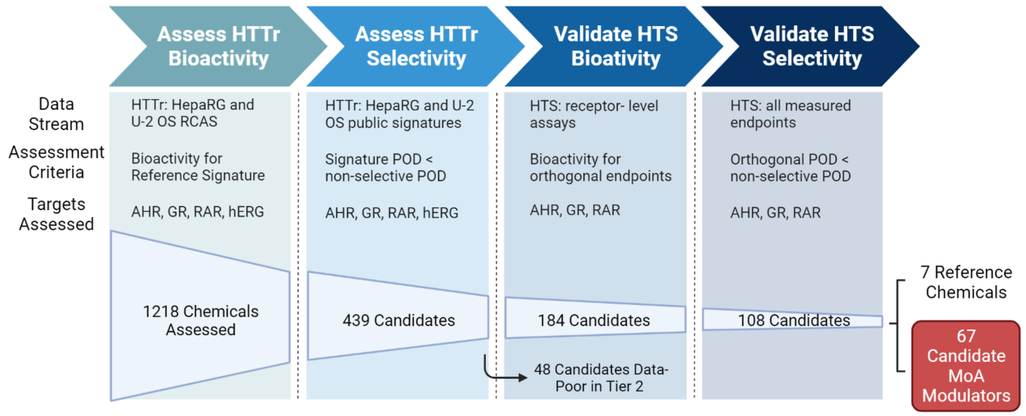
Signature-level comparison: Concentration-response modeling via *CompTox-httrpathway* R package (https://github.com/USEPA/CompTox-httrpathway)

- Enrichment score estimation via ssGSEA (Barbie *et. al. Nature* 2009)
- BMD estimation via *tcplfit2*, bioactivity determined by thresholding of hitcall and efficacy metrics (left)
- Randomization test against 250 randomly-generated signatures confirms ability of reference signatures to distinguish positive reference chemicals (right)



Integration of Transcriptomics into Chemical Prioritization Framework

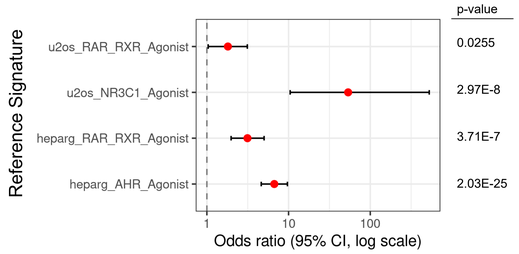
Primary Assessment Aim:
Prioritize chemicals with selective effects on molecular targets across transcriptional and receptor-level readouts by comparing targeted points of departure (PODs) against non-selective PODs



- Non-selective PODs estimated from BMDs for >10,000 publicly-sourced signatures (HTTr) or all measured ToxCast endpoints (HTS) (Judson *et. al. Tox Sci 2016)*
- Selectivity thresholds established for individual chemicals: $Mode(BMD_{nonselective}) - \sigma(BMD_{nonselective})$

HTS Confirmation of HTTr Predictions

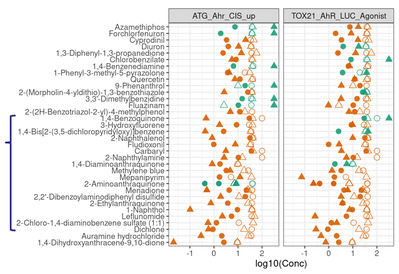
- Candidates designated as bioactive via HTTr show increased probability of bioactivity in orthogonal HTS assays via Fisher's exact tests:



- Individual candidates predicted as selective for individual MoAs in both HTTr/HTS represent known chemical classes or drugs for each target:

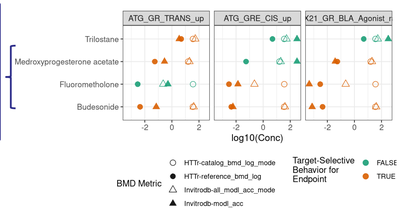
AHR HTS Assays: Transcription Factor Activity (ATG) and Receptor Activation (TOX21)

AHR Agonist Candidates: Anthraquinone derivatives indicate detection of similar aromatic features to known agonists, e.g. PAHs



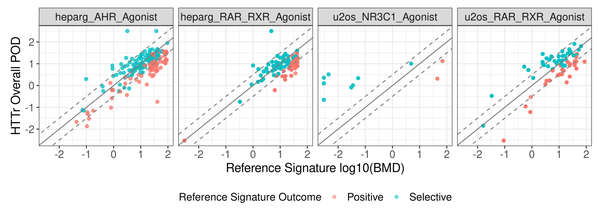
NR3C1 HTS Assays: Transcription Factor Activity (ATG) and Receptor Activation (TOX21)

NR3C1 Agonist Candidates: Prescribed synthetic glucocorticoids prioritized alongside minor agonists

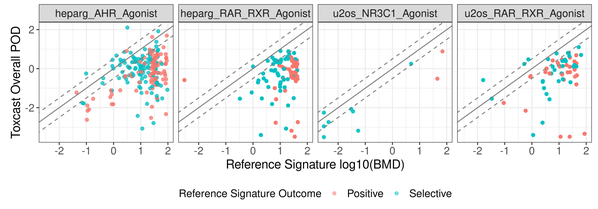


Comparison to Previous *in vitro* PODs

- ***Previous HTTr Overall POD***: 5th percentile BMD of bioactive signatures from >10,000 publicly-sourced signature catalog
- 80.0 ± 1.72% of bioactive chemicals demonstrate MoA-specific signature BMD within ± 0.5-log units of overall POD or below



- ***Previous HTS Overall POD***: 5th percentile BMD of bioactive endpoints from all measured ToxCast endpoints
- 20.1 ± 13.7% of bioactive chemicals demonstrate MoA-specific signature BMD within ± 0.5-log units of overall POD or below
- Narrow distribution of HTTr BMDs versus ToxCast consistent with previous transcriptional PODs



Conclusions

- Univariate gene identification strategy paired with signature concentration response analysis allows for assessment of putative MoAs from HTTr screening data
- Confirmation of transcriptional bioactivity via targeted HTS assays identifies selectively-acting environmental chemicals and pharmaceuticals
- Future testing of data-poor chemicals can be informed by broad-coverage assays for efficient chemical assessment

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