Integrating New Approach Methodologies into a Tiered Framework to Prioritize Chemicals for Hazard Assessment

J. D. Rogers[1,2], K. Paul-Friedman[1], L. J. Everett[1]

1. Center for Computational Toxicology and Exposure, Office of Research and Development, U.S. EPA, Research Triangle Park, NC
2. Oak Ridge Institute for Science and Education, Oak Ridge, TN

www.epa.gov

The views expressed in this presentation are those of the authors and do not necessarily represent the views or policies of the Agency.

Jesse Rogers | rogers.jesse@epa.gov | 0000-0001-6667-2342

Abstract

Background: Advancing a tiered strategy for chemical toxicity testing requires integrating multiple types of new approach methods (NAMs) into a framework for resource-efficient hazard identification. Pairing high-content assays encompassing broad biological activity (Tier 1) with complementary assays probing specific molecular targets (Tier 2) can improve confidence in NAM-based hazard identification. In this work, reference chemicals were used to identify putative mechanisms-of-action from Tier 1 transcriptomic screening data, and the results were subsequently compared to Tier 2 assays for confirmation.

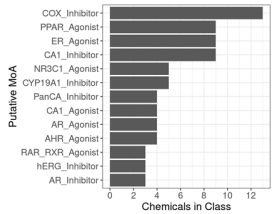
Results: Reference chemicals representing 13 molecular targets were identified from the RefChemDB database of chemical-target annotations, and transcriptomic profiles generated via the TempO-Seq platform in HepaRG and U2OS cell lines were used to develop new "reference signatures" associated with these reference chemicals. Of 1,218 chemicals screened in both cell lines, 271 chemicals demonstrated selective potencies for reference signatures representing 4 unique molecular targets. Comparison of Tier 1 transcriptomic responses and Tier 2 assays from the ToxCast program identified chemicals with selectivity for individual mechanisms in both tiers, including potential modulators of xenobiotic metabolism via aryl hydrocarbon receptor activation (41 test chemicals) and repressors of immune response via glucocorticoid receptor activation (4 chemicals). Over 60 chemicals were identified as potential modulators of aryl hydrocarbon receptor, retinoic acid receptor, and potassium ion channel activity, but were not previously screened in orthogonal ToxCast Tier 2 assays. Future screening of these chemicals in Tier 2 assays may confirm predicted molecular targets for these chemicals.

Conclusions: Our work demonstrates that Tier 1 transcriptomics can inform possible molecular targets and prioritize chemicals for specific hazards and confirmatory Tier 2 assays as part of a screening framework to support chemical risk assessment.

Reference Signature Development

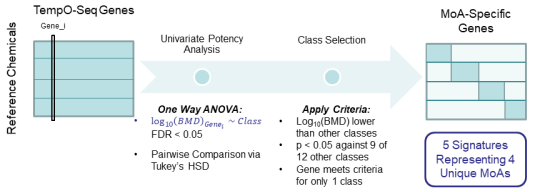
1) *Assign Reference Chemicals to Putative MoAs:*

- *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) *Generate Reference Signatures from High-Throughput Transcriptomics (HTTr) Potency Estimates*:

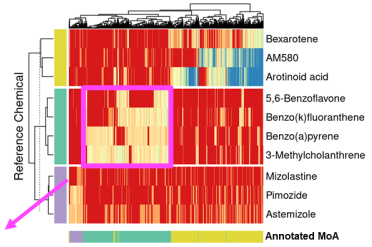
- 1218 chemicals screened in 8-point concentration response via TempO-Seq platform in HepaRG and U-2 OS cell lines (Yeakley *et. al. PLOS ONE* 2017, Harrill *et. al. Tox Sci* 2021)
- Benchmark Doses (BMDs) for 9418 concentration-responsive genes estimated via *tcplfit2* (Sheffield *et. al. Bioinformatics* 2022)
- Gene sets identified as uniquely potent for individual MoAs via univariate strategy



Reference Signatures Distinguish MoAs

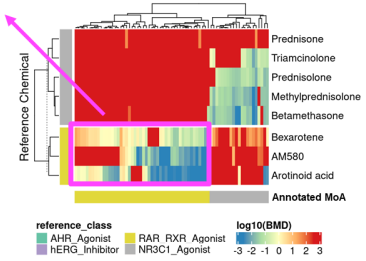
Gene-level comparison: Hierarchical clustering of gene BMDs reveals distinct potencies of reference chemicals towards selected genes

HepaRG Gene Clustering: 1173 genes identified



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

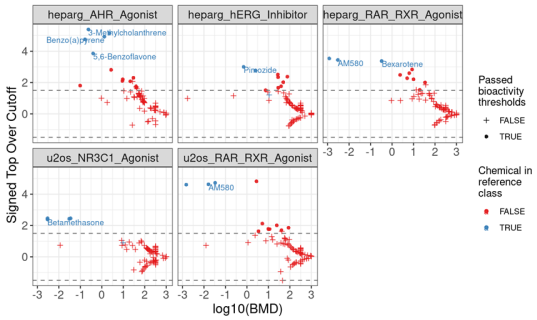
U-2 OS Gene Clustering: 69 genes identified



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

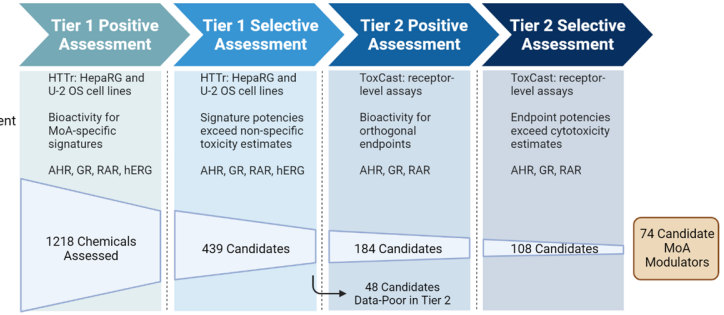


In-class chemicals: low BMD, high efficacy

Out-of-class chemicals: high BMD, low efficacy

Integration of Transcriptomics into Chemical Prioritization Framework

Primary Assessment Aim: Prioritize chemicals with selective effects on molecular targets across transcriptional and receptor-level readouts



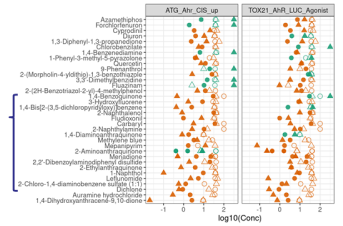
- "Cytotoxic burst" values estimated from BMDs for >10,000 publicly-sourced signatures (Judson *et. al. Tox Sci 2016*)
- Selectivity thresholds established for individual chemicals: $Mode(BMD_{public}) - \sigma(BMD_{public})$

Tier 2 Confirmation of Tier 1 Candidates

Molecular Target	Cell Line	Tier1+2-Selective Chemicals / Tier 1-Selective Chemicals
NR3C1	U-2 OS	8/8 (100%)
RAR/RXR	U-2 OS	12/35 (34.3%)
AHR	HepaRG	35/115 (30.4%)
RAR/RXR	HepaRG	24/52 (46.2%)

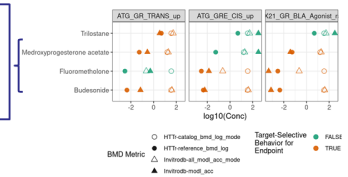
AHR ToxCast Assays: Transcription Factor Activity (ATG) and Receptor Activation (TOX21)

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



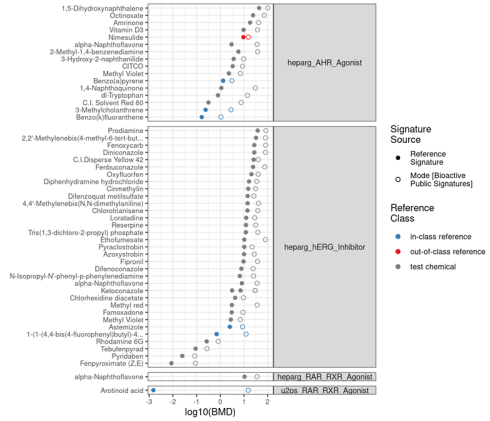
NR3C1 ToxCast Assays: Transcription Factor Activity (ATG) and Receptor Activation (TOX21)

NR3C1 Agonist Candidates: Prescribed synthetic glucocorticoids prioritized alongside minor agonists



Tier 1 Predictions of Data-Poor Chemicals

- Select candidates verified for AHR agonism, hERG inhibition in previous literature (Opitz *et. al. Nature* 2011, Sung *et. al. Biol Pharm Bull* 2012, Krishna *et. al. Biology* 2022)
- Subset of chemicals to be tested in Tier 2 assays for nuclear receptor binding, activation, and ion channel inhibition



Conclusions

- Univariate gene identification strategy paired with signature-level concentration response analysis allows for assessment of putative MoAs from high-throughput transcriptomic screening data
- Confirmation of transcriptional bioactivity via targeted 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

EPA United States Environmental Protection Agency

Disclaimer

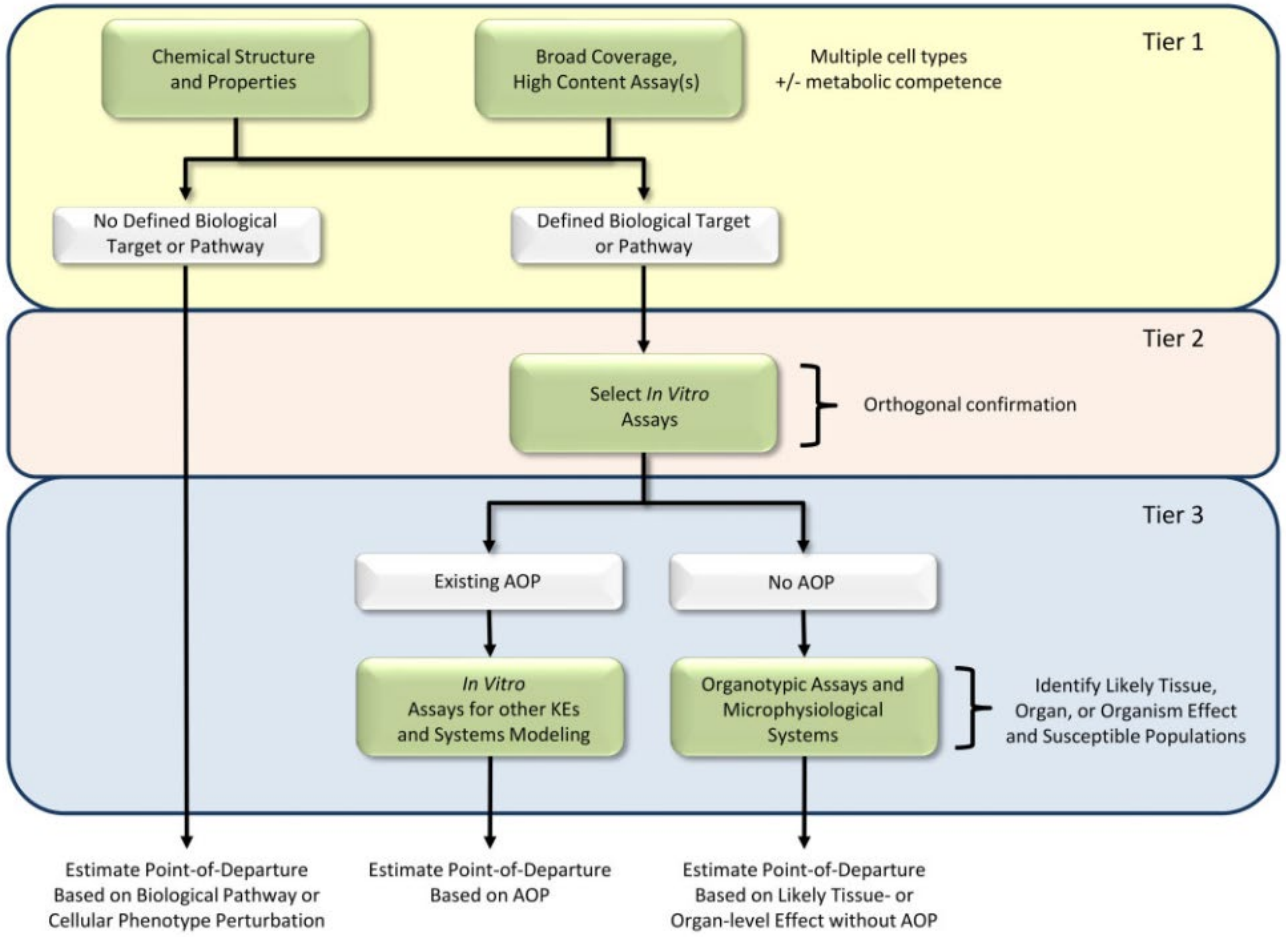
The views expressed in this presentation are those of the authors and do not necessarily represent the views or policies of the Agency.

Introduction

- Tiered testing of chemical toxicity requires a framework to integrate multiple types of New Approach Methods (Thomas et. al. *Tox Sci* 2019):
  - *Tier 1*: Broad biological coverage
  - *Tier 2*: Specific molecular targets

- Probing putative mechanisms-of-action (MoAs) in Tier 1 data forms basis for confirmation in Tier 2 testing

- Chemicals with selective signature potency can be linked to MoAs for Tier 2 confirmation



Thomas *Tox Sci* 2019

Methods: Reference Chemical Assignment

- Development of reference chemical classes based on RefChemDB associations with molecular targets (Judson et. al. *ALTEX* 2019):
  - Filter curated database for threshold level of support
  - Hierarchical clustering of molecular target annotations based on Jaccard distance
  - Assignment of chemicals to clusters based on support of constituent molecular targets

- **13 clusters represent unique mechanisms-of-action** after cross-referencing with current high-throughput transcriptomics screening data of 1218 chemicals

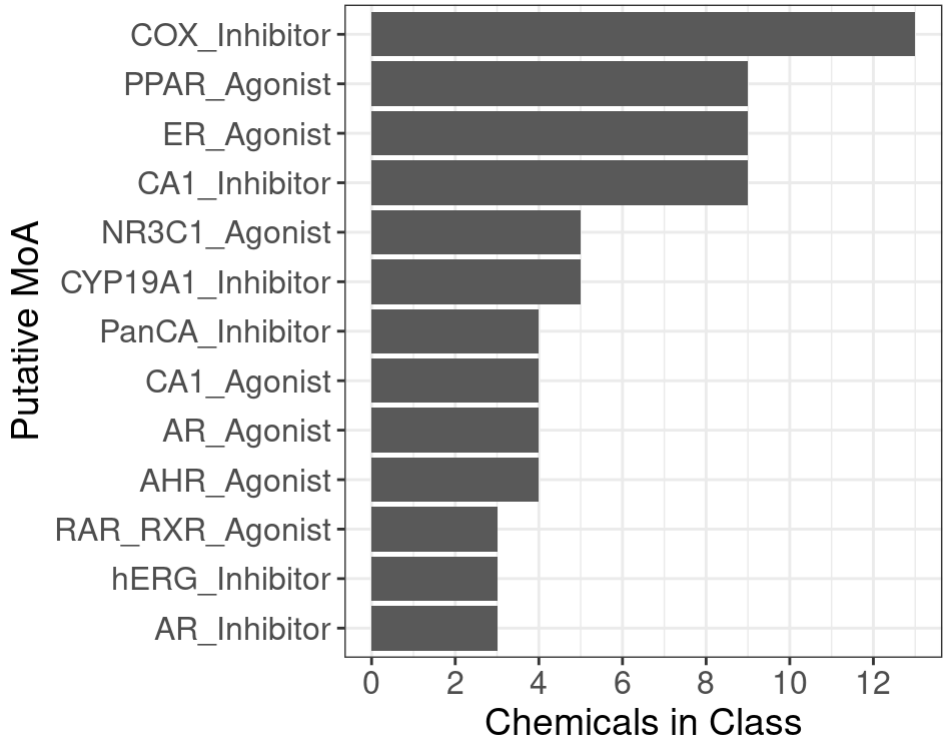
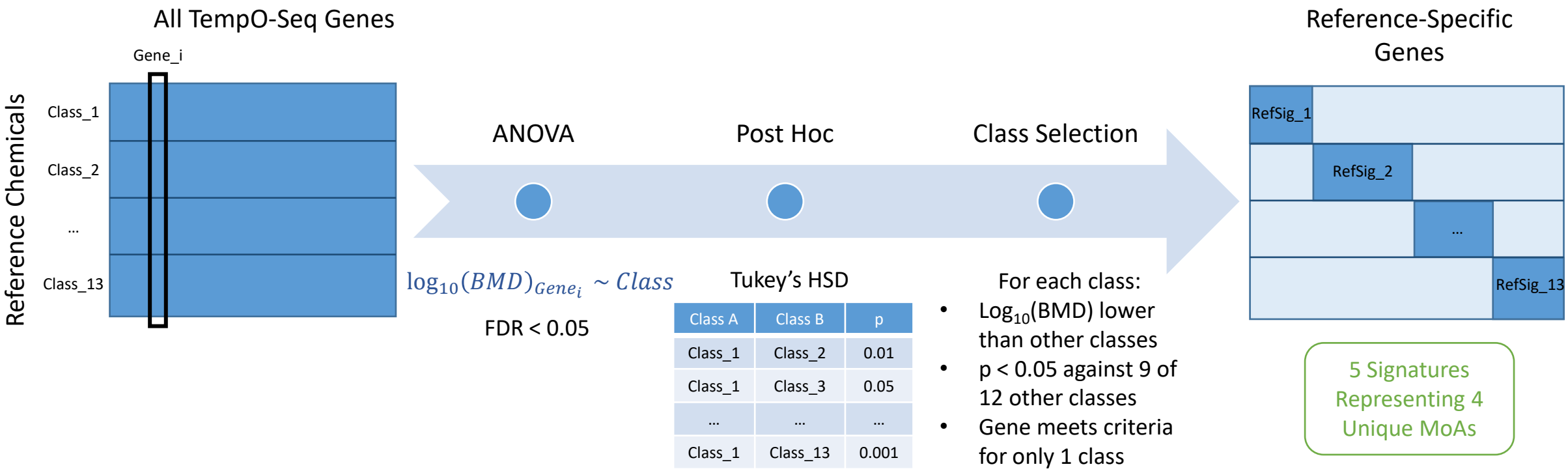


Figure indicates chemicals (selective and non-selective) associated with each signature (out of ~1200 screened chemicals)

Methods: Reference Signature Generation

- Previous transcriptomics screening data from HepaRG and U-2 OS cell lines used as basis for signature development (Yeakley *et. al. PLOS ONE* 2017, Harrill *et. al. Tox Sci* 2021)
- Gene sets **uniquely potent for individual MoAs** identified via univariate strategy:

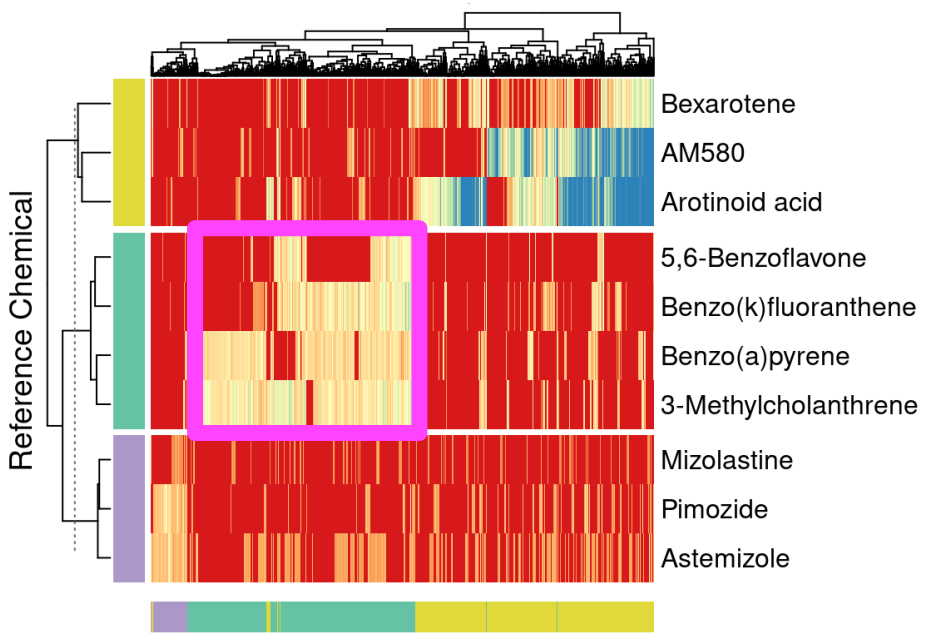


Results: Gene-level Comparison

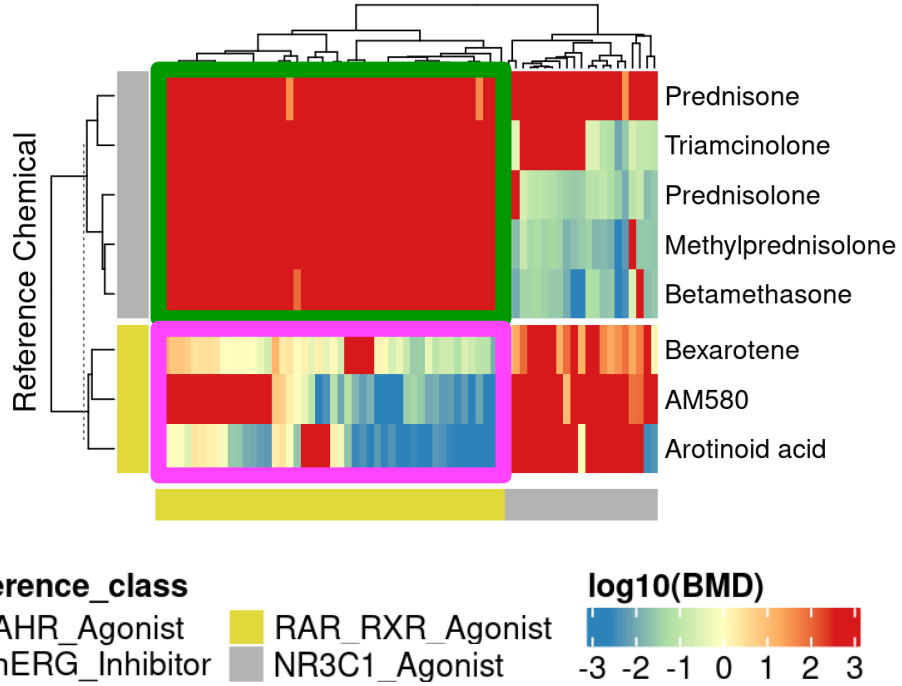
Reference Signature Comparison: gene-level BMDs show distinct similarities within annotated MoA via hierarchical clustering

- Chemicals annotated for same MoA as signature demonstrate activity at low concentrations
- Chemicals annotated for other MoA compared to signature show activity at high concentrations or no concentration-responsiveness

HepaRG Gene Clustering: 1173 genes identified



U-2 OS Gene Clustering: 69 genes identified



Results: Signature-level Comparison

- Concentration-response modeling of reference signatures via CompTox-httrpathway package
  - Enrichment scores estimated via ssGSEA (Barbie *et. al. Nature* 2009)
  - Benchmark dose/response estimated via tcplfit2 (Sheffield *et. al. Bioinformatics* 2022)

- Signature bioactivity determined via thresholding of confidence and efficacy metrics:
  - Curve-fit confidence: hitcall ≥ 0.9
  - Efficacy: top over cutoff ≥ 1.5

In-class chemicals: low BMD, high efficacy

Out-of-class chemicals: high BMD, low efficacy

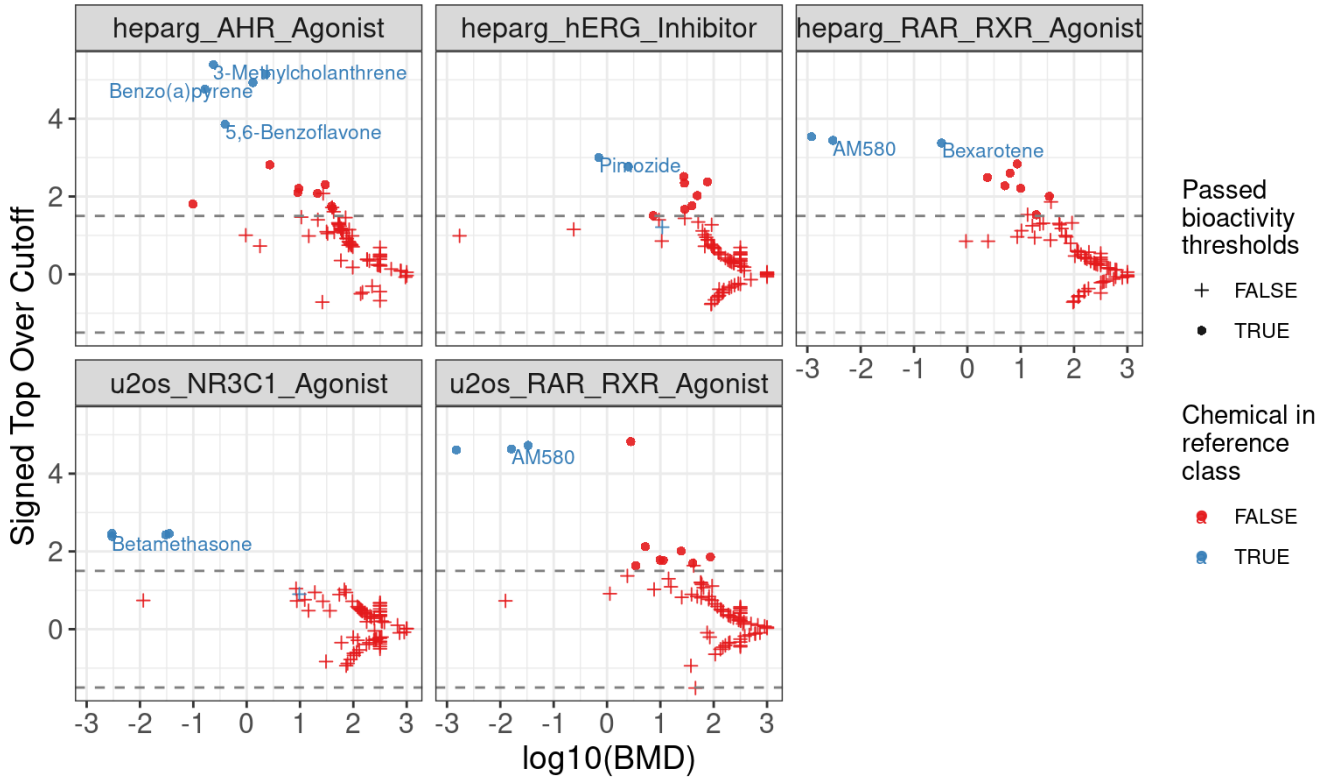
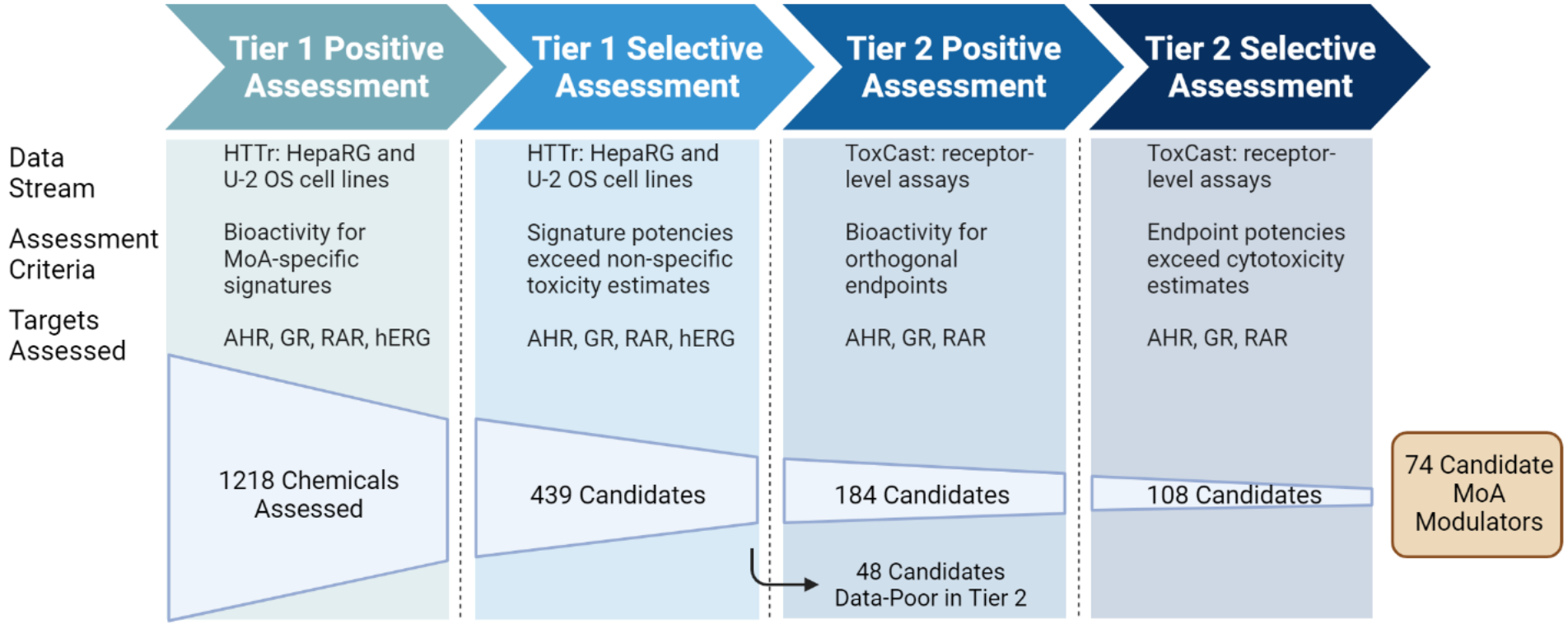


Figure indicates chemicals positive for each target passed threshold criteria for related signature, and few chemicals negative for each target passed criteria (except U2OS NR3C1, in which none passed)

Integration of Transcriptomics into Chemical Prioritization Framework

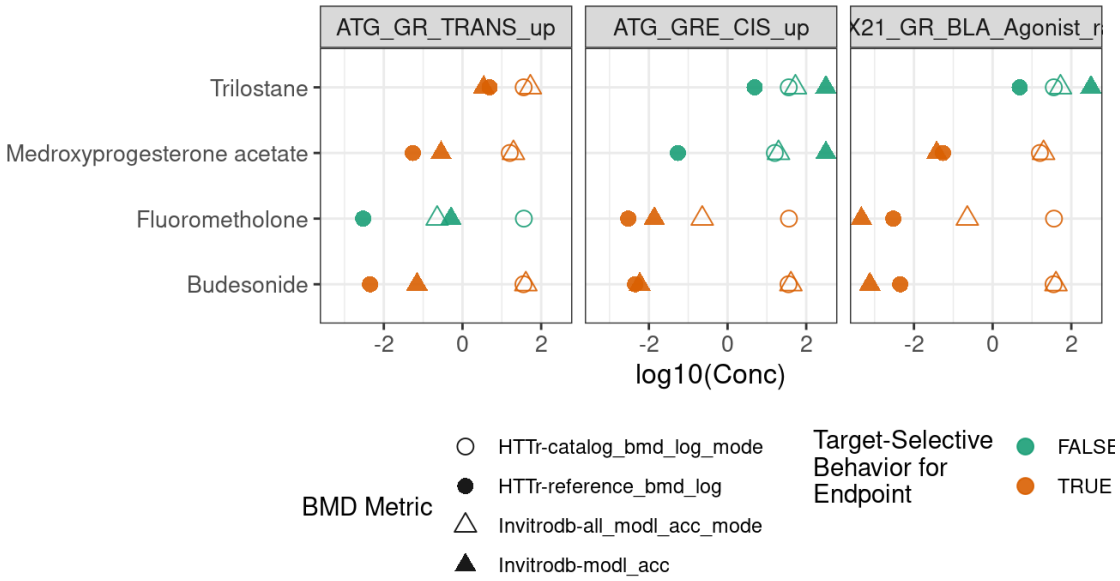
Primary Assessment Aim: identify chemicals with selective effects on molecular targets across transcriptional and receptor-level readouts

- Reference signature potencies compared to non-specific cytotoxic effects estimated from distribution of publicly-sourced signatures (Judson *et. al. Tox Sci 2016)*
- Selectivity thresholds established from non-specific estimates: $Threshold = Mode(BMD_{public}) - \sigma(BMD_{public})$

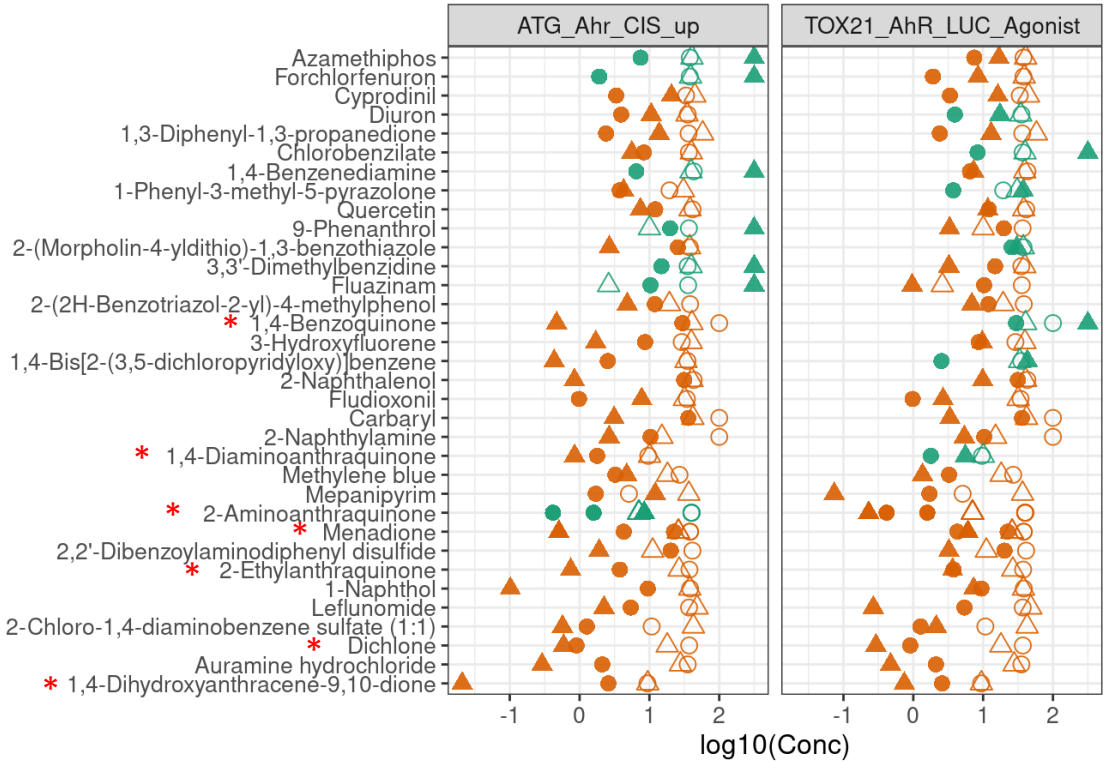


Results: Tier 1/2-Confirmed MoA Candidates Reflect Known Chemicals and Structural Features

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



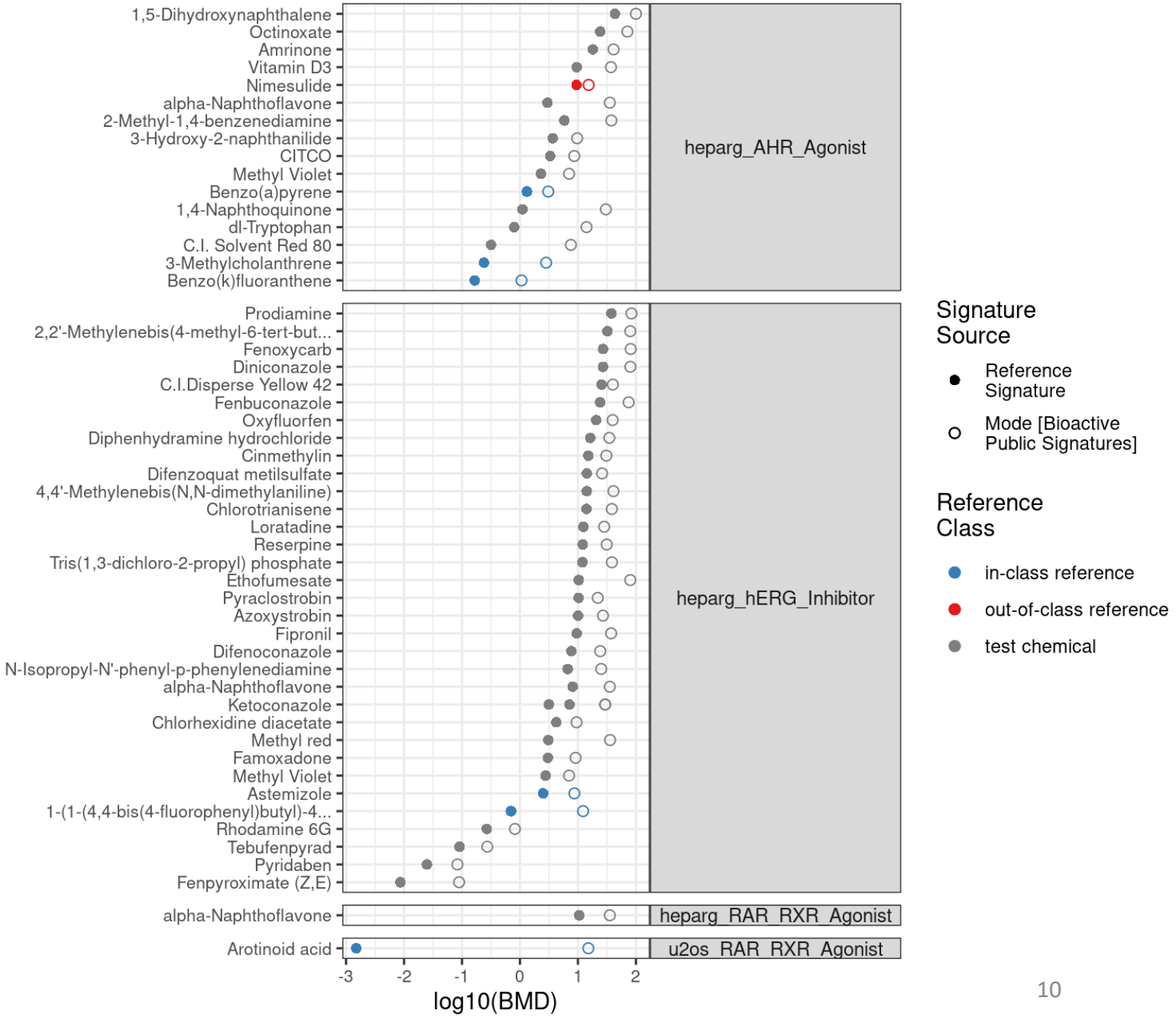
AHR ToxCast Assays: Transcription Factor Activity (ATG) and Receptor Activation (TOX21)



* Quinone or Anthraquinone Derivative

Results: Tier 1 Assessment Prioritizes Chemicals for Targeted Testing

- HTTr subset for chemicals with <2 orthogonal Tier 2 measurements for non-overlapping coverage
- Select candidates demonstrate for AHR agonism, hERG inhibition in previous literature:
  - *DL-Tryptophan*: metabolism to kynurenine causes AhR translocation and T cell proliferation in glioma cells and human glioblastoma tissue (Opitz *et. al. Nature* 2011)
  - *Ketoconazole*: concentration-dependent inhibition of hERG depolarization in rat ventricular cardiomyocytes via patch clamp assay (Sung *et. al. Biol Pharm Bull* 2012)
- Subset of candidates to be assessed in targeted Tier 2 assays for predicted molecular targets



Conclusions

- Univariate gene identification strategy paired with signature-level concentration response analysis allows for assessment of putative MoAs from high-throughput transcriptomic screening data

- Confirmation of transcriptional bioactivity via targeted 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