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

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Addressing Gaps in Chemical Toxicity Testing

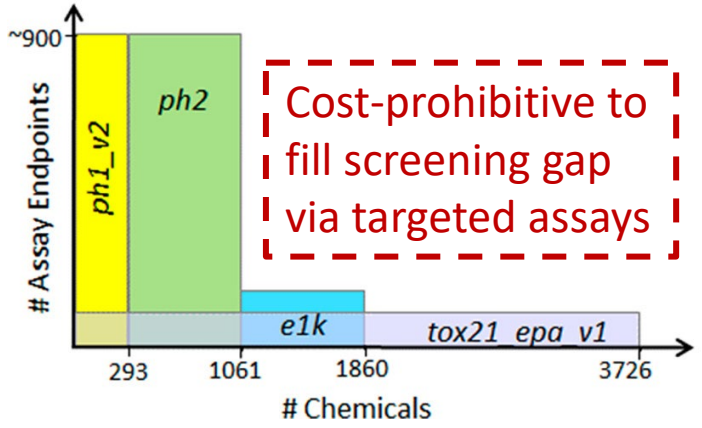
Toxicity Testing in the 21st Century (NRC 2007)

- Shift from traditional animal-based toxicity testing to New Approach Methodologies (NAMs) and predictive toxicology

US EPA ToxCast program (Dix et al. *Toxicol Sci* 2007)

- Broad bioactivity profiling of chemicals via high-throughput screening (HTS) assays
- Limited biological target coverage, reduced xenobiotic metabolism *in vitro* (Rice et al. *Environ Health Perspect* 2013)

Testing Phase	Chemical Set	Unique Chemicals	Assay Endpoints
ToxCast Phase I	*ph1_v1*	310	~700
ToxCast Phase II	*ph1_v2*	293	~200
	ph2	768	~900
	e1k	799	~50
Tox21	*tox21_epa_v1*	3726	~80



Cost-prohibitive to fill screening gap via targeted assays

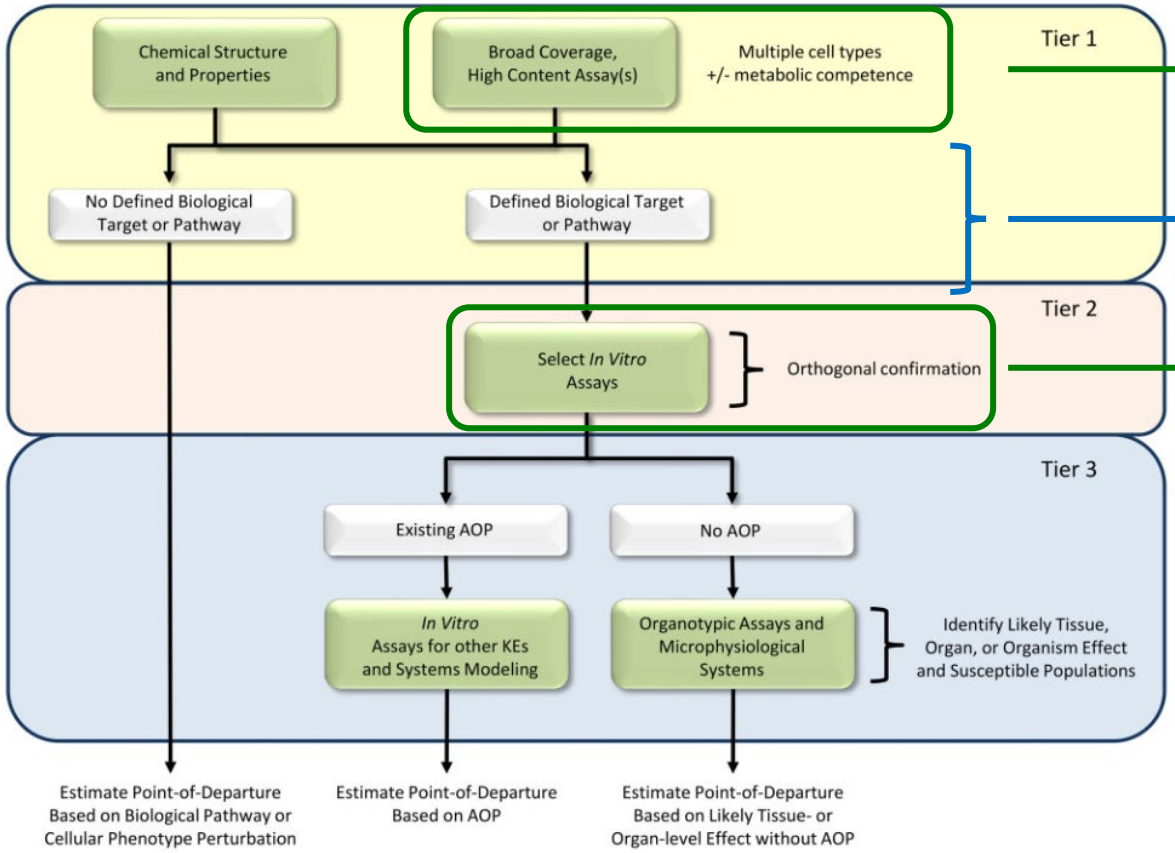
Next Generation Blueprint for Hazard Evaluation: Integrate multiple assay technologies into a single framework for efficient hazard screening (Thomas et al. *Toxicol Sci* 2019)

Slide adapted from Logan Everett

Richard *Chem Res Toxicol* 2016

Integrating Data Streams to Improve Scientific Confidence in NAMs

Tiered hazard evaluation framework: investigate potential mechanisms-of-action (MoAs) via high-throughput screening platforms and link verified chemicals to likely adverse outcomes (Thomas et al. *Toxicol Sci* 2019)



High-Throughput Transcriptomics (HTTr)
High-Throughput Phenotypic Profiling (HTPP)

?

Targeted High-Throughput Screening Assays (ToxCast)

Computational Needs:

- Derive high-confidence MoAs from transcriptomic NAMs
- Develop criteria to prioritize chemicals for key hazards based on Tier 1-2 NAMs

Thomas *Toxicol Sci* 2019

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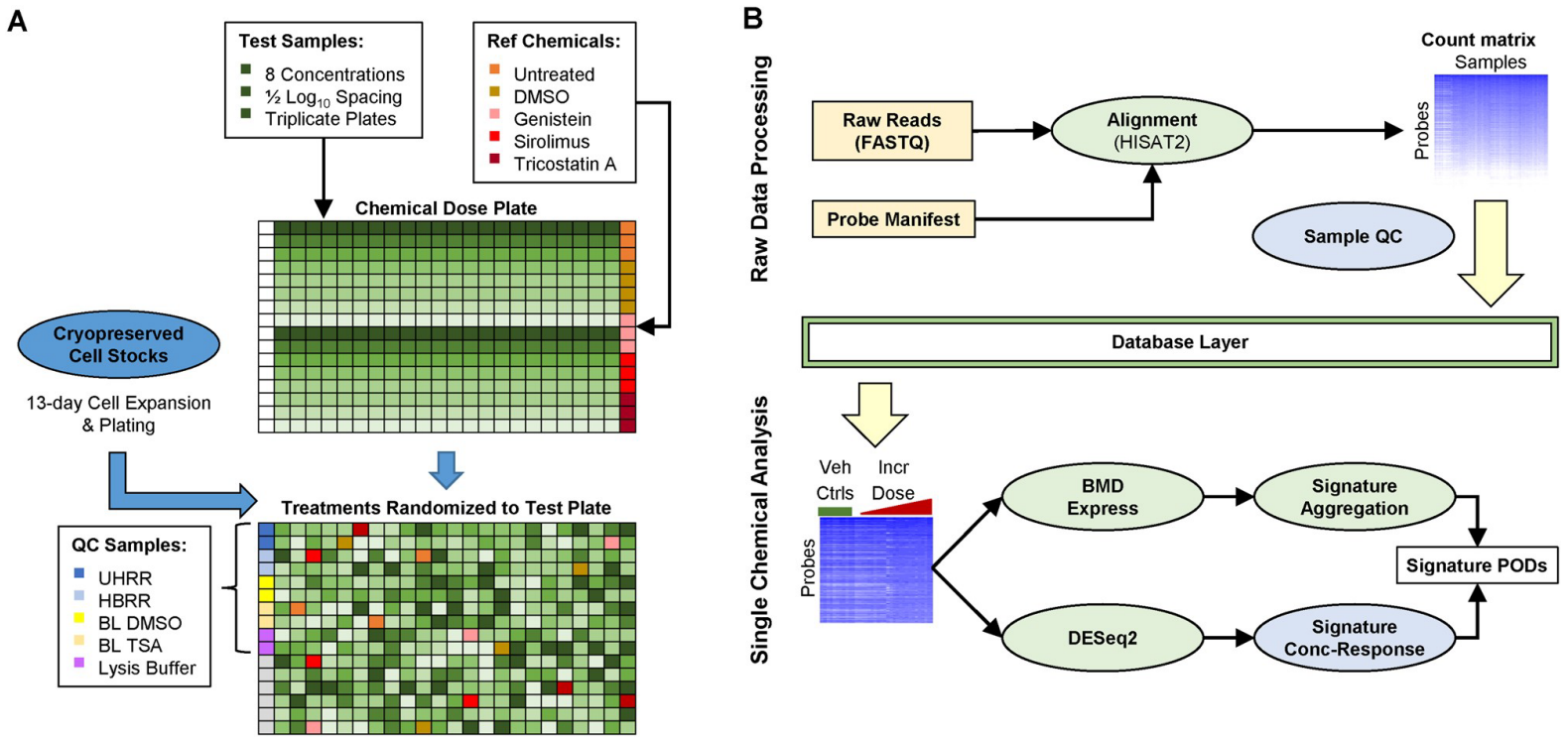
Project Outline

- Develop HTTr Signatures for MoA-Specific Activity
- Define Tiered Framework for Chemical Prioritization
- Apply Framework to Retrospective Tier 1-2 Screening Data
- Identify Candidates for Prospective Tier 2 Assessment
- Conclusions and Next Steps

High-Throughput Transcriptomics for Chemical Screening

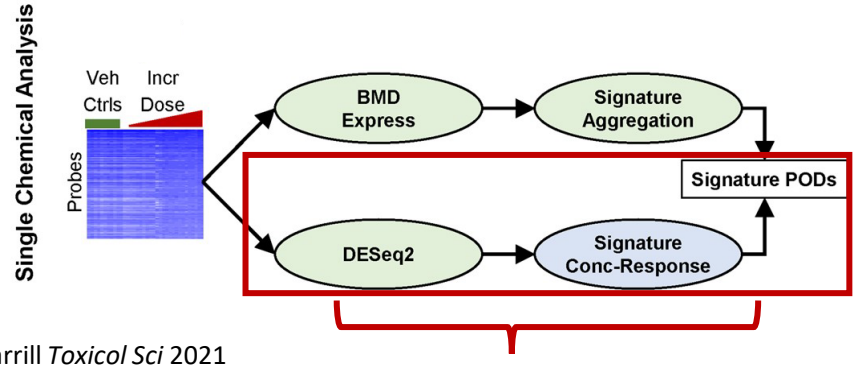
- TempO-Seq : Next-gen sequencing of >20,000 probes hybridized to expressed transcripts (Yeakley *et al. PLoS One* 2017)
- Up to 1,387 chemicals screened in multi-concentration format for multiple cell lines:
  - MCF7 Breast Carcinoma Cells (Harrill *et al. Toxicol Sci* 2021)
  - **U-2 OS Osteosarcoma Epithelial Cells** (Bundy *et al.* In Prep)
  - **HepaRG Hepatic Progenitor Cells** (Shah *et al.* In Prep)



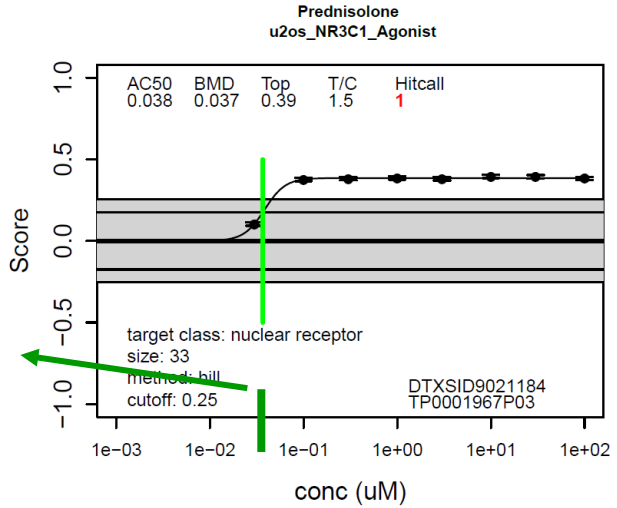
Harrill *Toxicol Sci* 2021

MoA Identification from Transcriptomic Data Streams

- Single-sample gene set enrichment analysis of compiled signatures (Barbie *et al. Nature* 2009)
- Concentration-response profiling of enrichment scores via *tcplfit2* (Sheffield *et. al. Bioinformatics* 2022)



Harrill *Toxicol Sci* 2021



Benchmark Dose (BMD)

Catalog of >11,000 public gene set signatures with toxicological relevance, annotated for known molecular targets:

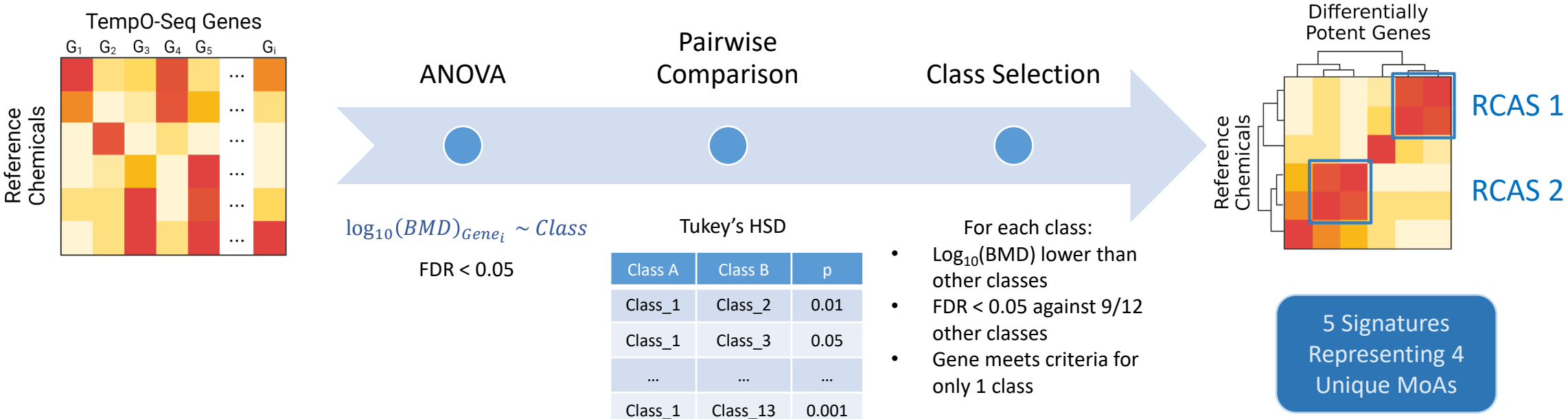
- **Bioplanet** (Huang, *et al. Front Pharmacol* 2019)
- **CMap** (Subramanian, *et al. Cell* 2017)
- **DisGeNET** (Pinero, *et al. Database* 2015)
- **MSigDB** (Liberzon, *et al. Cell Syst* 2015)

- Some public signatures may not be well-suited for probing MoAs in current assay
  - Cell lines used for derivation
  - Methods used for development, e.g. KEGG/Reactome
- ***Data-driven signatures may improve assay translation*** by profiling gene expression related to molecular initiating events

Data-Driven Signature Development Identifies Uniquely-Potent Features

Reference Class Associated Signatures (RCAS): gene sets uniquely potent for individual MoAs identified via univariate strategy

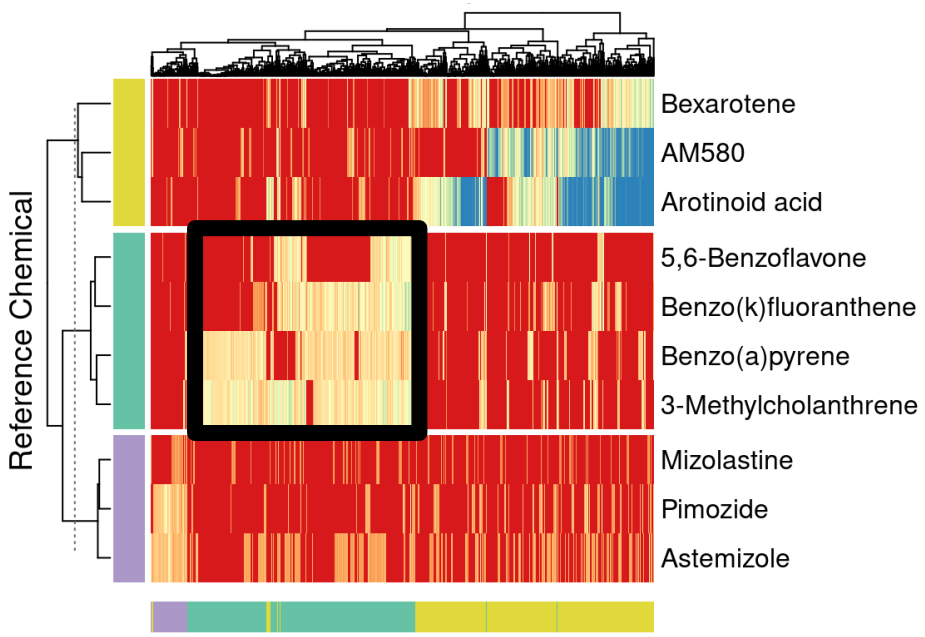
- Reference chemicals identified via *RefChemDB*: automated mining of literature databases for chemical-molecular target interactions (Judson et. al. *ALTEX* 2019)



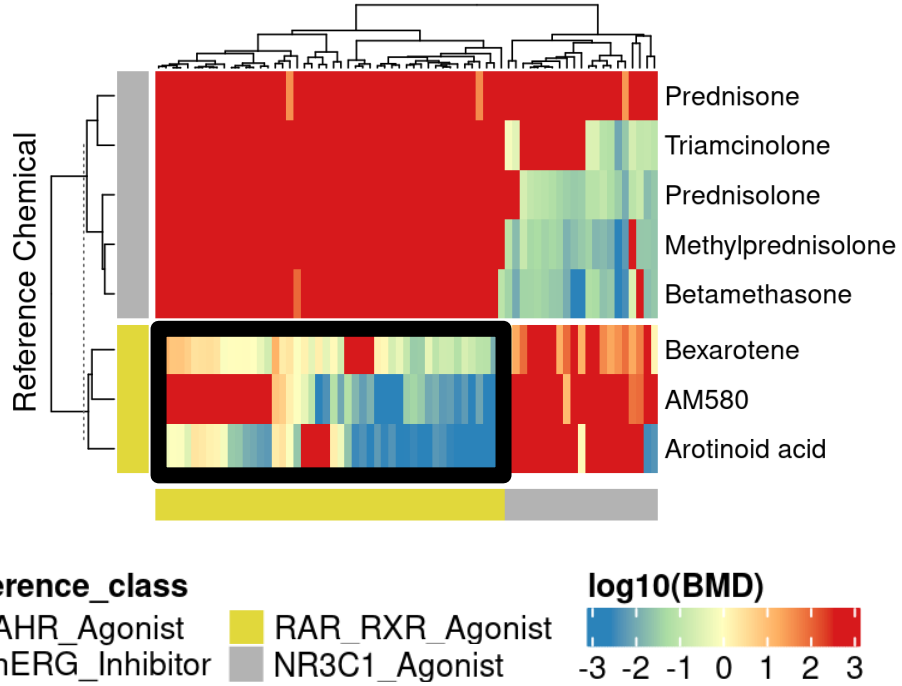
RCAS Gene Potencies Reveal Distinct Patterns by MoA

- Reference chemicals **annotated for same MoA** as signature demonstrate activity at low concentrations
- Reference chemicals annotated for other MoAs 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



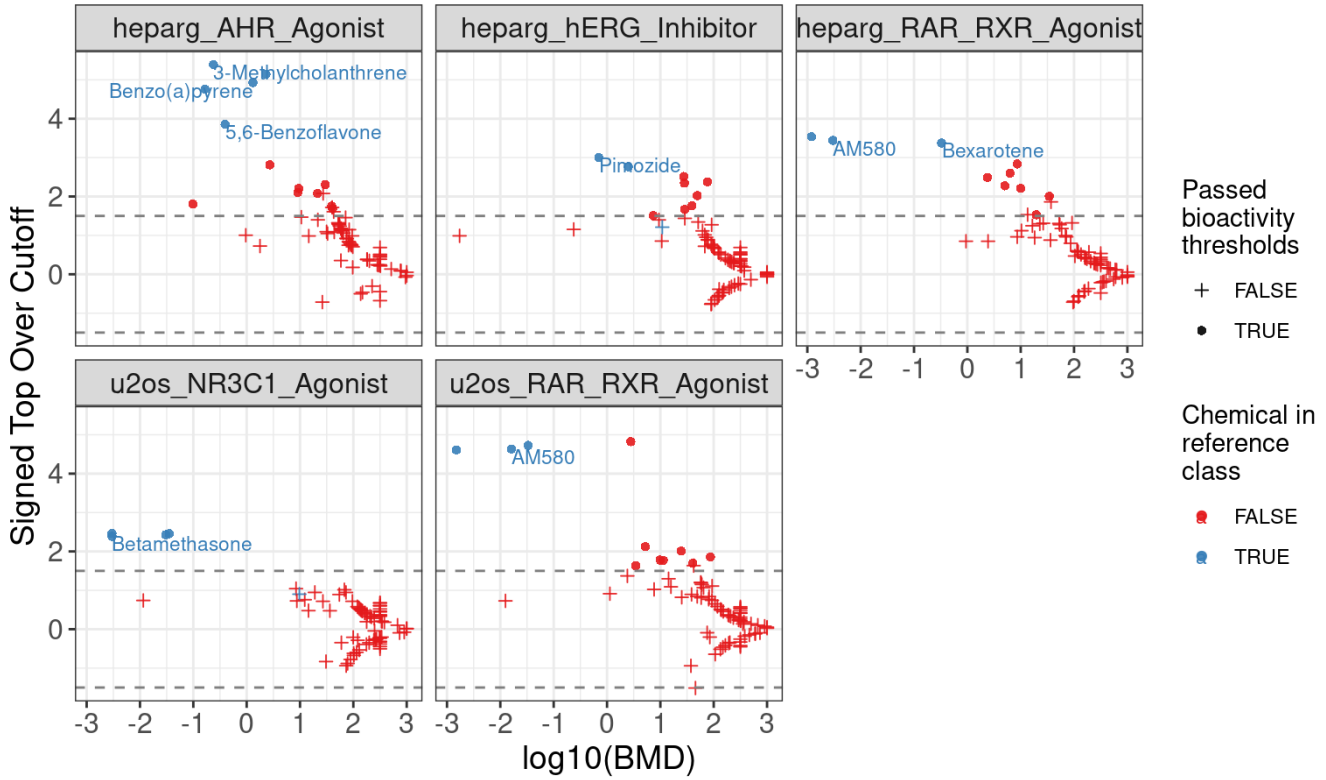
Efficacy and Potency for RCAS are Greatest for Matching Reference Chemicals

- Concentration-response modeling of reference signatures via CompTox-httrpathway package (https://github.com/USEPA/CompTox-httrpathway)
  - Enrichment scores estimated via ssGSEA (Barbie *et. al. Nature* 2009)
  - BMDs estimated from normalized enrichment scores 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

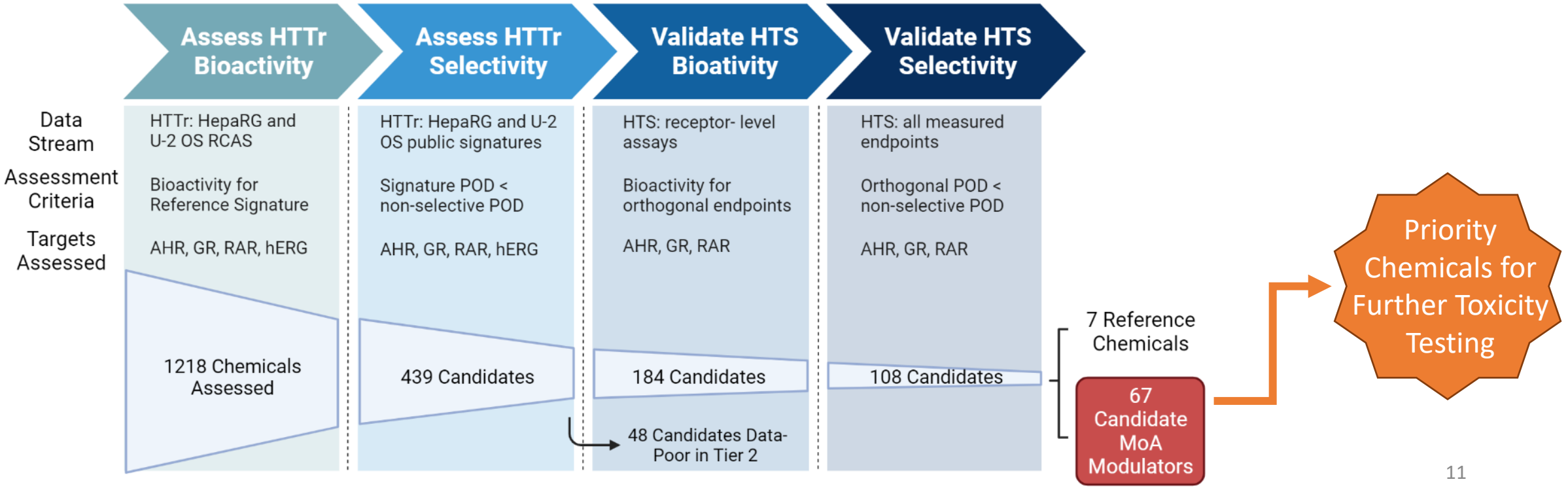


Chemicals annotated for each target passed threshold criteria for related signature, and few chemicals negative for each target passed criteria (except U2OS-NR3C1, in which none passed)

Integration of Transcriptomics into Chemical Prioritization Framework

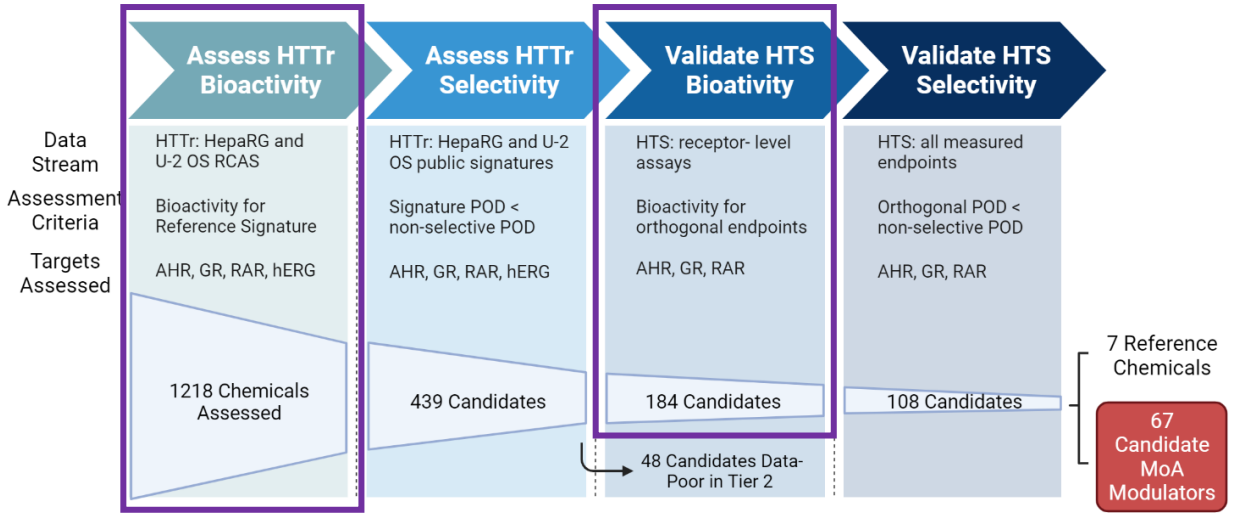
Primary Assessment Aim: identify chemicals with selective effects on molecular targets using transcriptional and receptor-level Points of Departure (PODs)

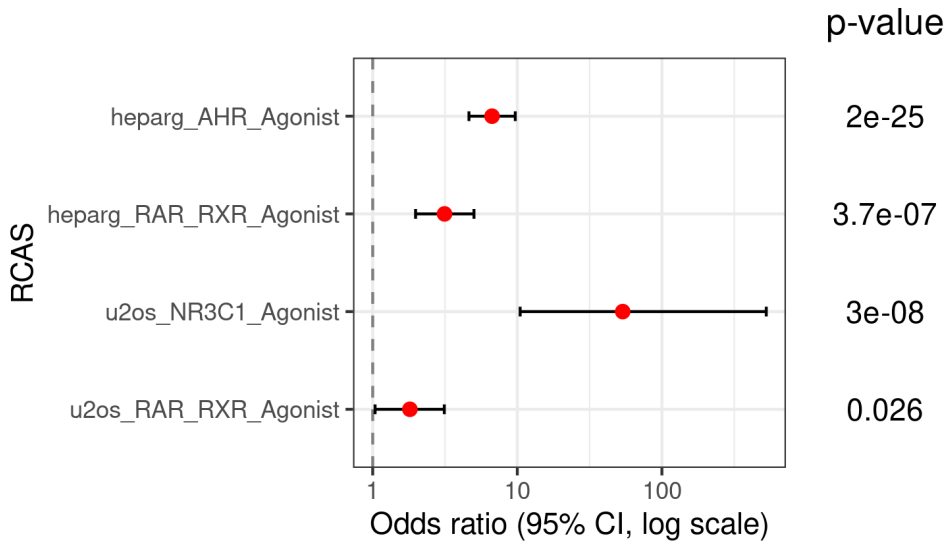
- Reference signature potencies compared to non-selective PODs estimated from distribution of >10,000 publicly-sourced signatures (Judson *et. al. Tox Sci 2016)*



Tier 1 Assessment Pre-Filters for Tier 2-Positive Chemicals

Association between Individual Tier Outcomes: Determine likelihood that Tier 1-bioactive chemicals are bioactive in at least one orthogonal Tier 2 assay



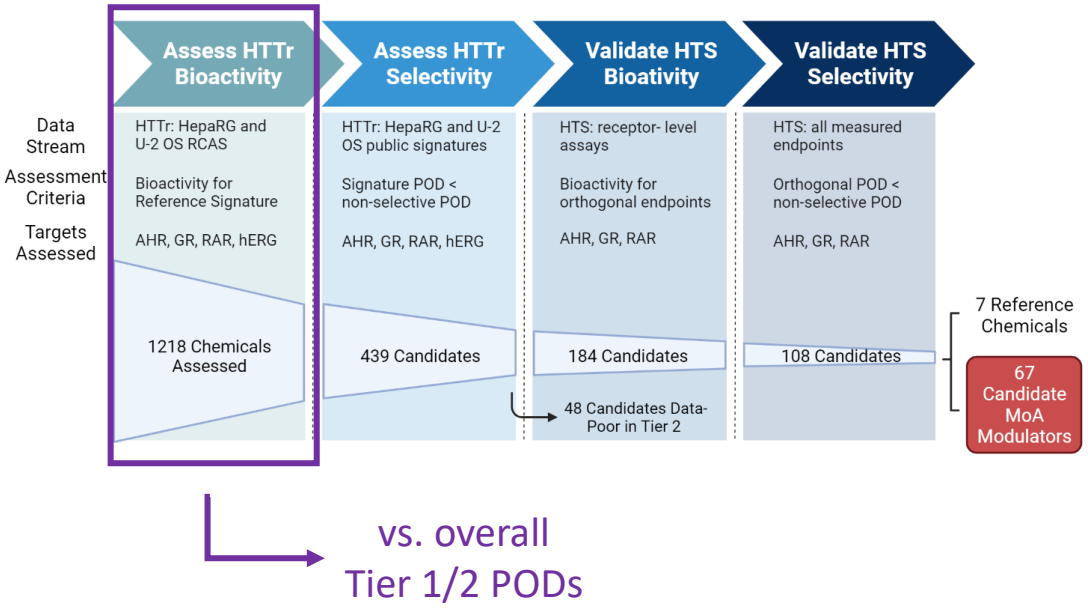


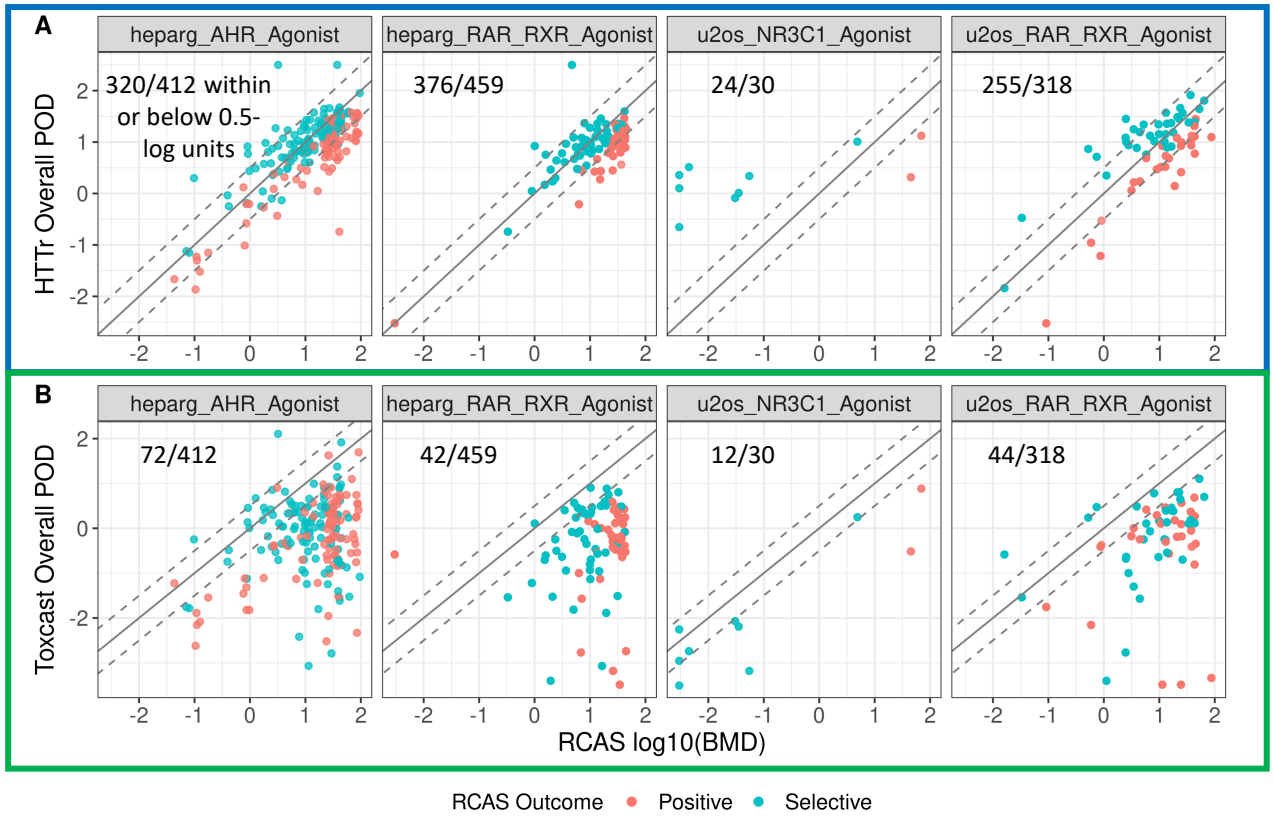
Chemicals positive for HTTr signatures were significantly more likely to show bioactivity in an orthogonal Tier 2 endpoint via Fisher's exact tests

Target-Specific Potencies Reflect Overall Transcriptomic PODs

Comparison to Previous PODs: Determine difference between Tier 1 potency estimates and overall PODs from Tier 1-2 Assays

- Tier 1: 5th percentile BMD from >10,000 publicly-sourced signatures
- Tier 2: 5th percentile ACC from all measured ToxCast endpoints

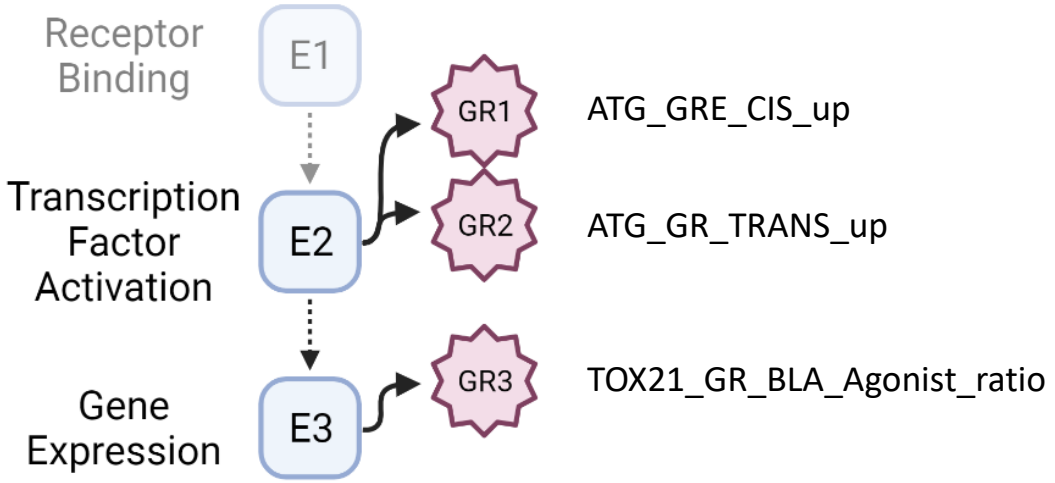


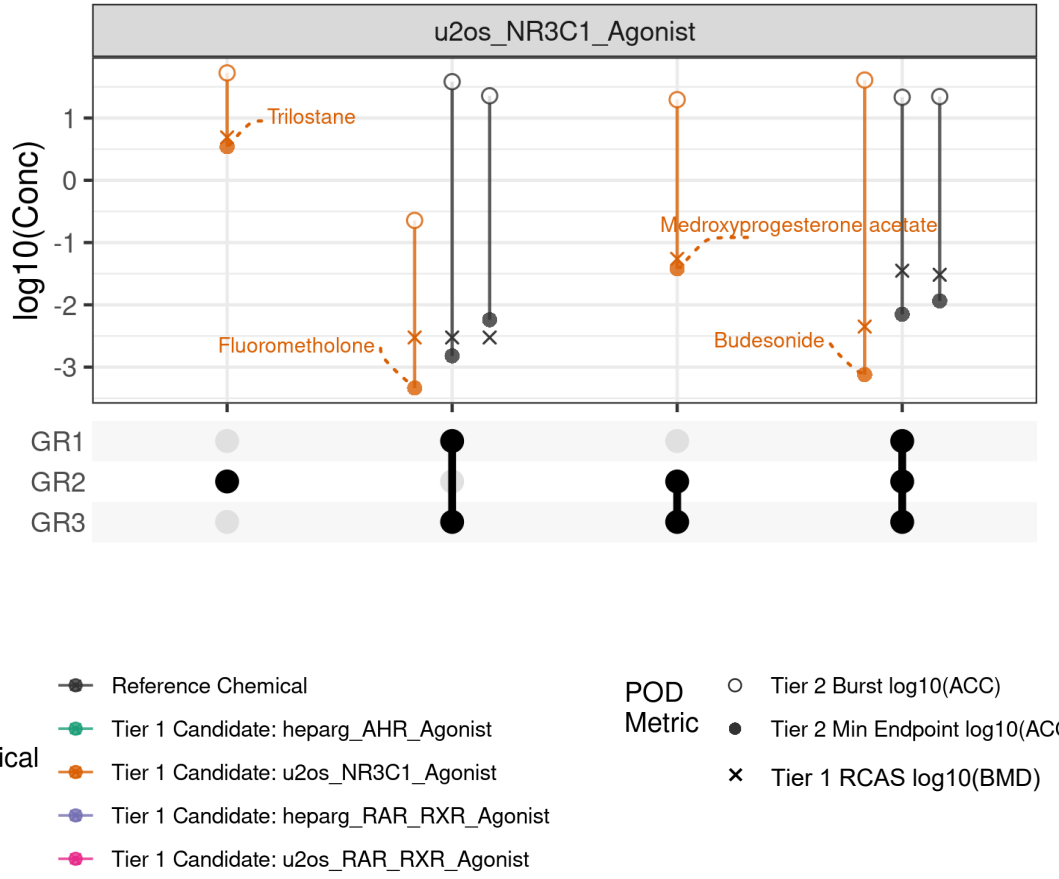


- **80±2%** of Tier 1-bioactive chemicals demonstrate MoA-specific BMD within 0.5-log units of overall HTTr POD or below
- **20±14%** of chemicals within 0.5-log units of overall ToxCast POD or below

Candidate NR3C1 Agonists Reflect Synthetic and Minor Glucocorticoids

Tier 2-Selective candidates demonstrate selective bioactivity in one or more orthogonal ToxCast endpoints:

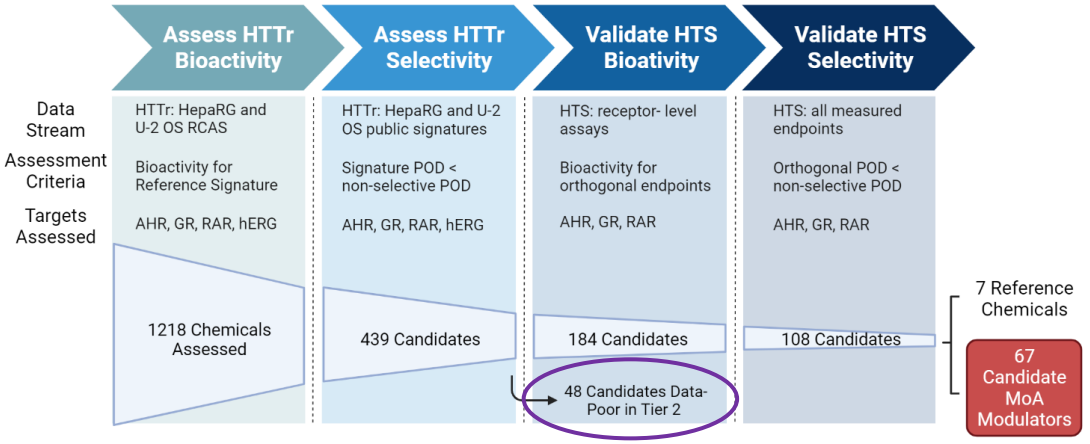




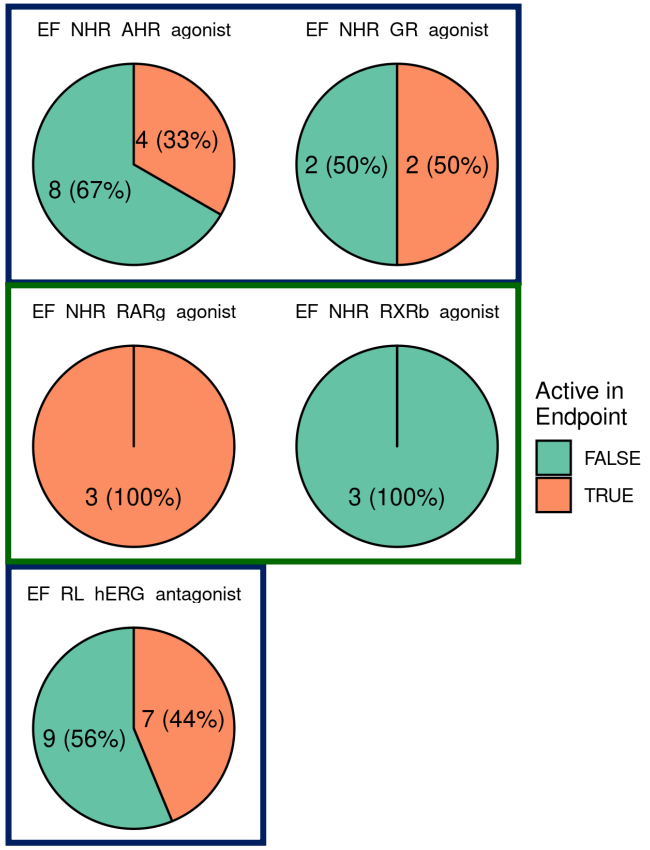
- *Fluorometholone*: active ingredient for treatment of eye inflammation
- *Medroxyprogesterone Acetate*: repression of interleukin secretion in normal human lymphocytes and amnion mesenchymal cells via minor GRE induction (Bamberger *et al. J Clin Endocrinol Metab* 1999, Marinello *et al. Front Physiol* 2020)

External Assessment of Data-Poor Chemicals Demonstrates Necessity of Multiple NAMs

Candidates with limited existing Tier 2 data profiled in orthogonal receptor-level assays:



Target	Vendor	Assay Type	No. Test Chemicals	Doses (uM)
AHR	Eurofins DiscoverX	Protein-Protein Interaction	12	0.3–30
GR	Eurofins DiscoverX	Protein-Protein Interaction	4	0.3–30
hERG	Eurofins Panlabs	Radioligand Binding	16	0.3–30
RARg	Eurofins Panlabs	Functional Coactivator	4	0.3–30
RXRb	Eurofins Panlabs	Functional Coactivator	4	0.3–30

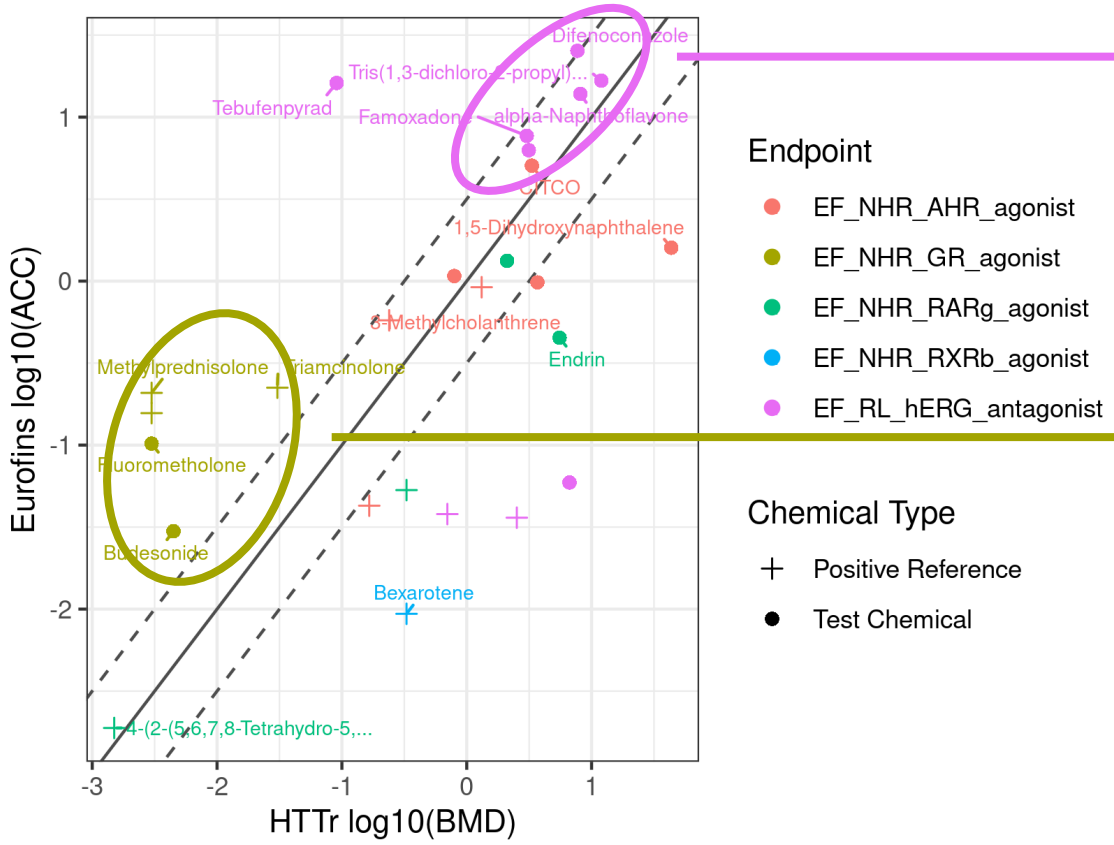


AHR/GR/hERG Candidates: Tier 2 endpoints can further support priority chemicals and de-prioritize others

RAR/RXR Candidates: Tier 2 endpoints distinguish between targets with similar transcriptomic profiles

External Assessment of Data-Poor Chemicals Demonstrates Necessity of Multiple NAMs

Estimated potency values from orthogonal endpoints compared to target-specific Tier 1 PODs:



hERG Inhibitors: Tier 1 PODs within 0.5-log units of orthogonal endpoint POD or below for 6/9 reference and test chemicals

GR Agonists: Tier 1 PODs at least 0.5-log units below orthogonal endpoint POD for 5/5 reference and test chemicals

- Sensitivity of HTTr towards individual mechanisms may be dependent on biological context
- Inclusion of multiple NAMs in pathway-specific PODs may be necessary to ensure confidence

Conclusions

- Univariate gene identification strategy paired with signature-level concentration response analysis allows for assessment of putative MoAs for transcriptomic-based toxicity testing

- Confirmation of transcriptional bioactivity via targeted Tier 2 assays identifies selectively-acting environmental chemicals and pharmaceuticals

- *Next Steps*: Inclusion of additional data streams to further support tiered testing (e.g. high throughput phenotypic profiling)

Acknowledgements

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- Bryant Chambers
- Laura Taylor
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Literature Mining Links Chemicals to Putative Targets

- *RefChemDB*: automated mining of multiple literature databases for chemical-molecular target interactions (Judson et. al. *ALTEX* 2019)

- Chemical assignment to molecular targets based on *support*, i.e. number of sources containing evidence of interaction
  - 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 (MoAs)** after cross-referencing with current high-throughput transcriptomics screening data

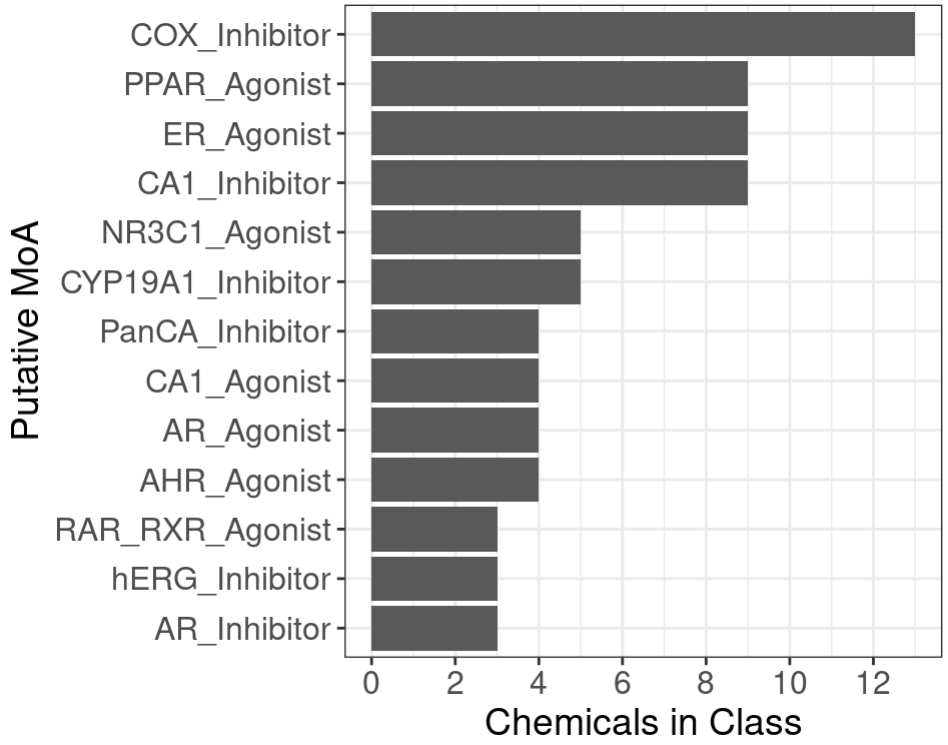
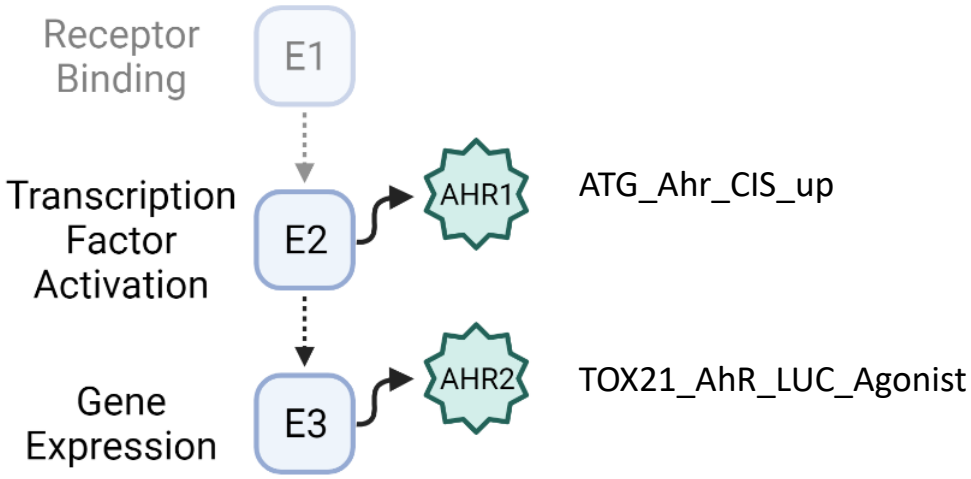
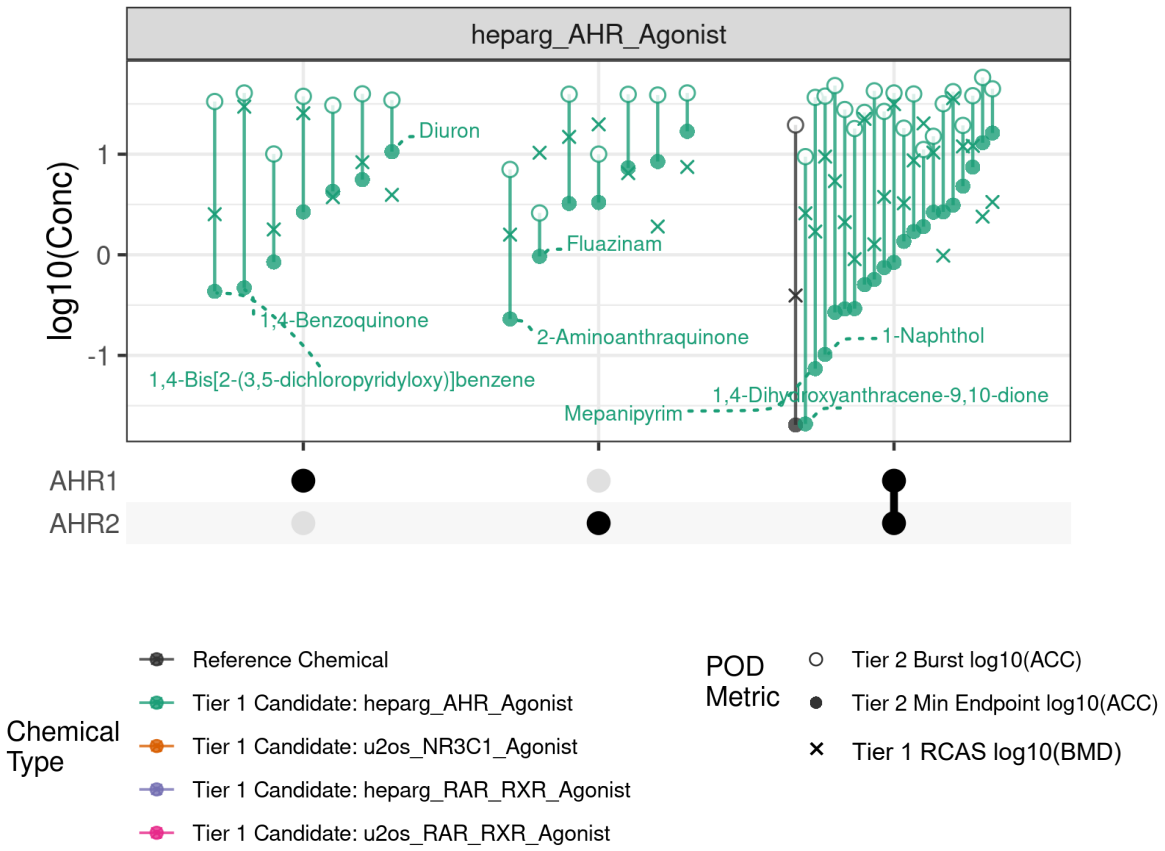


Figure indicates chemicals (selective and non-selective) associated with each signature (out of 1218 screened chemicals)

Candidate AHR Agonists Relate to Known Carcinogens

Tier 2-Selective candidates demonstrate selective bioactivity in one or more orthogonal ToxCast endpoints:





- *Anthraquinone Derivatives*: chronic oral exposure in Fisher rats increased rates of carcinogenesis, primarily in liver (Doi *et al J Environ Health B* 2006)

Candidate Retinoid Agonists Relate to …

Target	Cell Type	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%)**