EPA United States Environmental Protection Agency

# Interactions Between EU-ToxRisk and the Toxicology in the 21st Century (Tox21) Consortium

## Development of a Common Reference Chemical Dataset for Interpretation of High-Throughput Transcriptomics Screening Data.

# EPA Disclaimer

*The views expressed in this presentation are those of the author(s) and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency, nor does mention of trade names or products represent endorsement for use.*

# Tox21 Consortium


Focused on developing methods to rapidly and efficiently evaluate the safety of chemicals using New Approach Methods (NAMs).

Organizations with like needs / interests form partnerships and pursue joint projects.

## Active Tox21 Cross Partner Projects

**Developing a Common Reference Chemical Dataset for Interpretation of High-Throughput Transcriptomics (HTTr) Screening Data (CPP5).**

*Cell Line Selection for High-Throughput Transcriptomics*

*In Vitro Chemical Disposition*

*Toxicodynamic Variability in Developmental Neurotoxicity*

*Performance Based Validation of Alternative Test Systems and Models*

*Retrofitting Tox21 HTS Assay with Metabolic Capability*

*Expansion of Pathway Coverage by Tox21 HTS Assays for Better Prediction of Adverse Drug Effects*

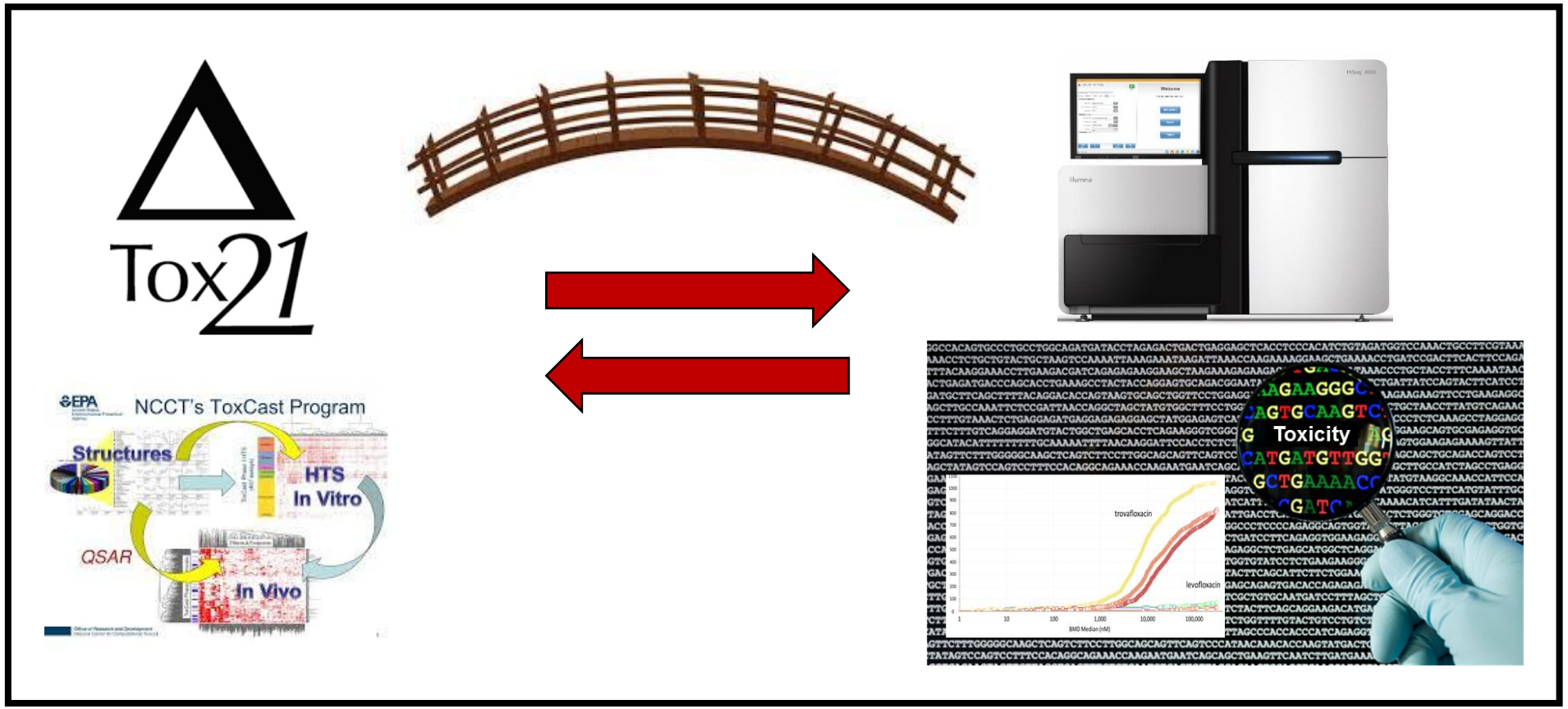
*Evaluating Thiol Reactivity of Tox21 Chemicals using the MSTI Assay*

*Predictive Toxicology of Retinoid Signaling Pathway*

*Investigation of Environmental Determinants of Pubertal Timing in Girls.*

# Project Overview

Development of a common set of transcriptional profiles from reference chemicals will allow more robust interpretation of high-throughput transcriptomic screens to link chemicals to biological-response pathways and molecular initiating events.



# Project Goals

- Identify and procure a diverse set of “reference” chemicals with known biological activities at discrete molecular targets or well characterized biological pathways.

- Build a robust dataset of transcriptome profiles for these reference chemicals across a range of concentrations and cell models, starting with test systems currently employed by Tox21 partners in high-throughput transcriptomic screens.

- Develop transcriptomic signatures that accurately identify specific molecular targets/biological pathways perturbed by the reference chemicals, and their respective ‘firing orders’ across the range of concentrations examined.

# Aim 1: Identify and Procure A Diverse Set of Reference Chemicals (1)

- **Aim 1:**
  - Develop a list of ~300 chemicals covering ~75-100 biological-response pathways (i.e., 3 or more for a given pathway) with well-annotated associations to specific molecular targets or biological-response pathways.

# Aim 1: Identify and Procure A Diverse Set of Reference Chemicals (2)

## RefChemDB

A database of ***chemical_target_mode_activity*** associations created by Judson et al. from information contained in the public domain.

- > 2900 biological targets
- > 37,000 unique chemicals

Intended to be used in a semi-automated workflow for development ***candidate reference chemical lists*** for a molecular target

Candidate lists are then refined using expert knowledge.

For a given chemical, more than one literature source may support a given chemical_target_mode association (i.e. ***level of support***).

Research Article ALTEX 36(2), 261-276. doi:10.14573/altex.1809281

**Workflow for Defining Reference Chemicals for Assessing Performance of *In Vitro* Assays**

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| Source | Chemicals | Targets | Chemical-target-mode-activity combinations | Mean multiplicity | PMIDs |
|---|---|---|---|---|---|
| ChEMBL | 28,832 | 2,238 | 310,984 | 1.16 | 11,520 |
| ChEMBL Drug | 1,187 | 738 | 4,099 | 1 | 0 |
| CTD | 2,317 | 7,904 | 25,606 | 1.22 | 5,280 |
| DrugBank | 1,630 | 1,169 | 3,623 | 3.41 | 6,274 |
| Eurofins Biochemical | 206 | 570 | 925 | 1 | 0 |
| Eurofins Functional | 211 | 239 | 706 | 1 | 0 |
| Iuphar BPS | 1,860 | 941 | 5,081 | 1 | 0 |
| KEGG Drug | 661 | 263 | 1,201 | 1 | 0 |
| KIDB | 535 | 450 | 6,532 | 1 | 0 |
| KInaseDB | 133 | 168 | 676 | 1 | 1 |
| LitDB | 2,654 | 88 | 8,348 | 4.94 | 27,909 |
| Open Targets | 1,031 | 820 | 3,973 | 1 | 0 |
| Prodrug | 41 | 33 | 41 | 1 | 1 |
| Repurposing Hub | 2,279 | 2,172 | 10,209 | 1 | 0 |
| ToxCast | 9,136 | 343 | 852,470 | 1.03 | 0 |
| TTD | 3,916 | 1,575 | 11,557 | 1.00 | 0 |
| Web Curation | 3,940 | 1,059 | 5,617 | 1.01 | 0 |
| **Total** | **37,301** | **11,055** | **123,4580** | **1.02** | **49,883** |

# Aim 1: Identify and Procure A Diverse Set of Reference Chemicals (3)

- Targets and target classes were confirmed / refined following procurement.
- 139 Targets
- 9 Target Classes
- Predominantly "negative" biological activities (e.g. inhibitors, antagonists).

| Target | Target Class | Activity | # Chemicals |
|---|---|---|---|
| CETP | Chaperone | Negative | 2 |
| HSP90 | Chaperone | Negative | 1 |
| ACE | Enzyme | Negative | 2 |
| AChE | Enzyme | Negative | 2 |
| AKR | Enzyme | Negative | 2 |
| ALOX5 | Enzyme | Negative | 3 |
| CA | Enzyme | Negative | 4 |
| COMT | Enzyme | Negative | 2 |
| COX2 | Enzyme | Negative | 4 |
| CTSK | Enzyme | Negative | 2 |
| CYP19A1 | Enzyme | Negative | 2 |
| CYP2C9 | Enzyme | Negative | 2 |
| CYP8B1 | Enzyme | Negative | 1 |
| DAO | Enzyme | Negative | 1 |
| DHFR | Enzyme | Negative | 1 |
| DHODH | Enzyme | Negative | 2 |
| DPP4 | Enzyme | Negative | 2 |
| EPHX | Enzyme | Negative | 2 |
| FAAH | Enzyme | Negative | 2 |
| FDPS | Enzyme | Negative | 2 |
| FKBP | Enzyme | Negative | 1 |
| FNT | Enzyme | Negative | 2 |
| GAA | Enzyme | Negative | 2 |
| GSK3B | Enzyme | Negative | 1 |
| HMGCR | Enzyme | Negative | 2 |
| IMPDH | Enzyme | Negative | 2 |
| LDM | Enzyme | Negative | 3 |
| MAO | Enzyme | Negative | 2 |
| MMP | Enzyme | Negative | 2 |
| NEU | Enzyme | Negative | 1 |
| NOS | Enzyme | Negative | 3 |
| PARP | Enzyme | Negative | 1 |
| PDE | Enzyme | Negative | 2 |
| PLA2 | Enzyme | Negative | 2 |
| POL | Enzyme | Negative | 1 |
| PTGS | Enzyme | Negative | 3 |
| ROCK | Enzyme | Negative | 2 |
| SIRT1 | Enzyme | Negative | 1 |
| SIRT2 | Enzyme | Negative | 2 |
| SQLE | Enzyme | Negative | 2 |
| TMP | Enzyme | Negative | 1 |
| TOP | Enzyme | Negative | 5 |
| TPO | Enzyme | Negative | 2 |
| TYR | Enzyme | Negative | 2 |
| VKORC | Enzyme | Negative | 2 |
| CACN | Ion Channel | Negative | 2 |
| KCN | Ion Channel | Negative | 1 |
| SCN | Ion Channel | Negative | 2 |
| TRPV1 | Ion Channel | Negative | 5 |
| TRPV1 | Ion Channel | Positive | 3 |

| Target | Target Class | Activity | # Chemicals |
|---|---|---|---|
| AKT | Kinase | Negative | 2 |
| ALK | Kinase | Negative | 3 |
| AURKA | Kinase | Negative | 2 |
| AURKB | Kinase | Negative | 2 |
| BRAF | Kinase | Negative | 2 |
| CDK | Kinase | Negative | 3 |
| CHEK1 | Kinase | Negative | 2 |
| CSNK2 | Kinase | Negative | 1 |
| FGFR | Kinase | Negative | 1 |
| FLT3 | Kinase | Negative | 3 |
| JAK2 | Kinase | Negative | 1 |
| MEK | Kinase | Negative | 3 |
| MET | Kinase | Negative | 2 |
| mTOR | Kinase | Negative | 3 |
| p38 | Kinase | Negative | 3 |
| p53 | Kinase | Negative | 2 |
| PI3K | Kinase | Negative | 3 |
| PKC | Kinase | Negative | 2 |
| SPHK | Kinase | Negative | 2 |
| 5-HTR | Neurotransmitter Receptor | Negative | 10 |
| 5-HTR | Neurotransmitter Receptor | Positive | 10 |
| ADRA | Neurotransmitter Receptor | Negative | 2 |
| ADRA | Neurotransmitter Receptor | Positive | 4 |
| ADRB | Neurotransmitter Receptor | Negative | 3 |
| ADRB | Neurotransmitter Receptor | Positive | 3 |
| CHRM | Neurotransmitter Receptor | Negative | 7 |
| CHRM | Neurotransmitter Receptor | Positive | 2 |
| CHRN | Neurotransmitter Receptor | Negative | 2 |
| CNR | Neurotransmitter Receptor | Negative | 2 |
| CNR | Neurotransmitter Receptor | Positive | 1 |
| DRD | Neurotransmitter Receptor | Negative | 3 |
| DRD | Neurotransmitter Receptor | Positive | 1 |
| GABAR | Neurotransmitter Receptor | Negative | 1 |
| GABAR | Neurotransmitter Receptor | Positive | 2 |
| HRH | Neurotransmitter Receptor | Negative | 8 |
| NMDAR | Neurotransmitter Receptor | Negative | 4 |
| AHR | Nuclear Receptor | Positive | 2 |
| AR | Nuclear Receptor | Negative | 2 |
| AR | Nuclear Receptor | Positive | 2 |
| CAR | Nuclear Receptor | Positive | 2 |
| ER | Nuclear Receptor | Negative | 2 |
| ER | Nuclear Receptor | Positive | 2 |
| FXR | Nuclear Receptor | Positive | 4 |
| GR | Nuclear Receptor | Positive | 5 |
| HNF4A | Nuclear Receptor | Negative | 2 |
| NR3C2 | Nuclear Receptor | Negative | 1 |
| NRF2 | Nuclear Receptor | Positive | 2 |
| PGR | Nuclear Receptor | Positive | 2 |

| Target | Target Class | Activity | # Chemicals |
|---|---|---|---|
| PPARA | Nuclear Receptor | Positive | 2 |
| PPARG | Nuclear Receptor | Positive | 2 |
| PXR | Nuclear Receptor | Positive | 2 |
| RAR | Nuclear Receptor | Positive | 2 |
| ADORA | Receptor | Negative | 1 |
| ADORA | Receptor | Positive | 1 |
| AGTR | Receptor | Negative | 2 |
| AGTR2 | Receptor | Negative | 2 |
| AVPR | Receptor | Negative | 2 |
| CASR | Receptor | Positive | 1 |
| CCKR | Receptor | Negative | 2 |
| CYSLTR | Receptor | Negative | 2 |
| EGFR | Receptor | Negative | 2 |
| ENDR | Receptor | Negative | 2 |
| FFAR | Receptor | Positive | 2 |
| FOLR | Receptor | Negative | 1 |
| GNRHR | Receptor | Negative | 2 |
| GPR35 | Receptor | Positive | 1 |
| ITGB3 | Receptor | Negative | 2 |
| P3R | Receptor | Positive | 1 |
| PDGFR | Receptor | Negative | 2 |
| S1PR | Receptor | Positive | 2 |
| SHH | Receptor | Negative | 2 |
| SUR | Receptor | Negative | 1 |
| TAAR | Receptor | Positive | 3 |
| TGFBR | Receptor | Negative | 2 |
| TLR7 | Receptor | Positive | 2 |
| VDR | Receptor | Positive | 2 |
| VEGFR | Receptor | Negative | 5 |
| CTNNB | Structural / Motor Protein | Negative | 1 |
| Kinesin | Structural / Motor Protein | Negative | 2 |
| TUBB | Structural / Motor Protein | Negative | 2 |
| 5-HTT | Transporter | Negative | 4 |
| BCRP | Transporter | Negative | 1 |
| BSEP | Transporter | Negative | 2 |
| DAT | Transporter | Negative | 2 |
| GABATR | Transporter | Negative | 2 |
| NET | Transporter | Negative | 6 |
| OATP2A1 | Transporter | Negative | 1 |
| PGP | Transporter | Negative | 3 |
| PMAT | Transporter | Negative | 1 |

Curation of target potencies:

- Harris Ioannidis (EMBL-EBI)
- IUPHAR web tool

- **Aim 2:**

  Build a robust dataset of transcriptome profiles for these reference chemicals across a range of concentrations and cell models, starting with test systems currently employed by Tox21 partners in high-throughput transcriptomic screens.

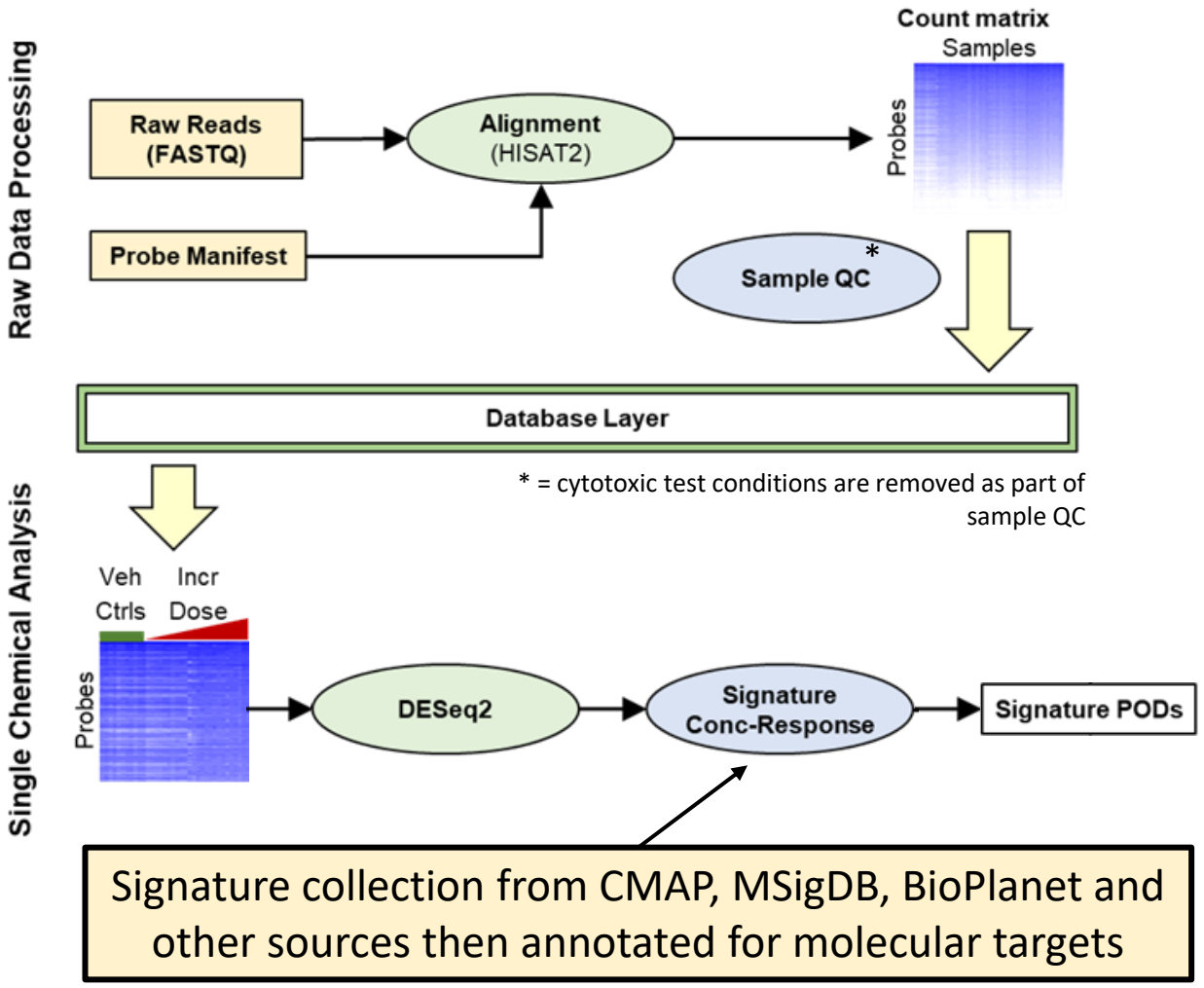
# EPA CPP5 Tier 1 Screening (To Date)

| Parameter | Multiplier | Notes | | | | |
|---|---|---|---|---|---|---|
| Chemicals | 336 | 313 unique chemicals + 23 duplicates | | | | |
| Cell Types | 4 | U-2 OS | | HepaRG-2D | | |
| Assay Formats | 2 | HTTr | HTPP | HTTr | LDH Release | Live Cell Imaging |
| Exposure Durations | Variable | 24 HR | | 24 HR | | |
| Concentrations: | 8 | 8 $\log_{10}$ units (0.01 nM – 100 µM) | | | | |
| Biological Replicates: | Variable | 3 | 4 | 3 | 3 | 3 |

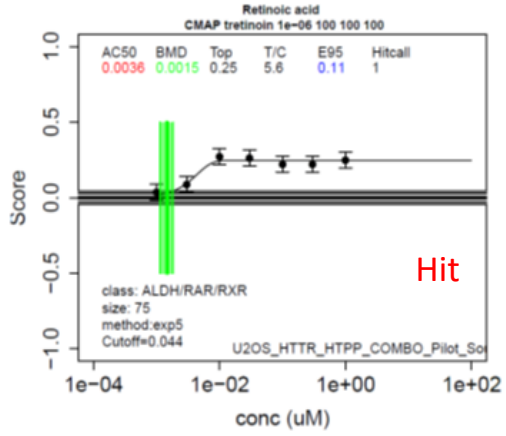
- High-throughput transcriptomics (HTTr) results have been pipelined for both cell types.
- Analysis of high-throughput phenotypic profiling (HTPP) data in progress.

# First Pass Analysis of CPP5 HTTr Data

## Analysis Overview

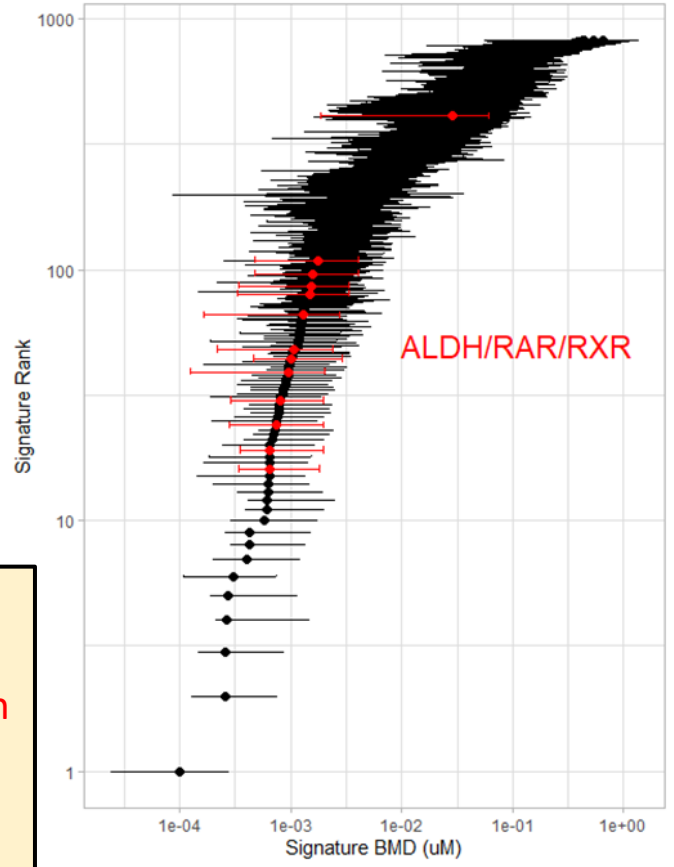



## Concentration-Response Modeling of Signature Scores




**Biological Pathway Altering Concentration (BPAC)**

Most sensitive signature
**OR**
Statistic based on distribution of active signatures (5th %ile)
**OR**
By target class

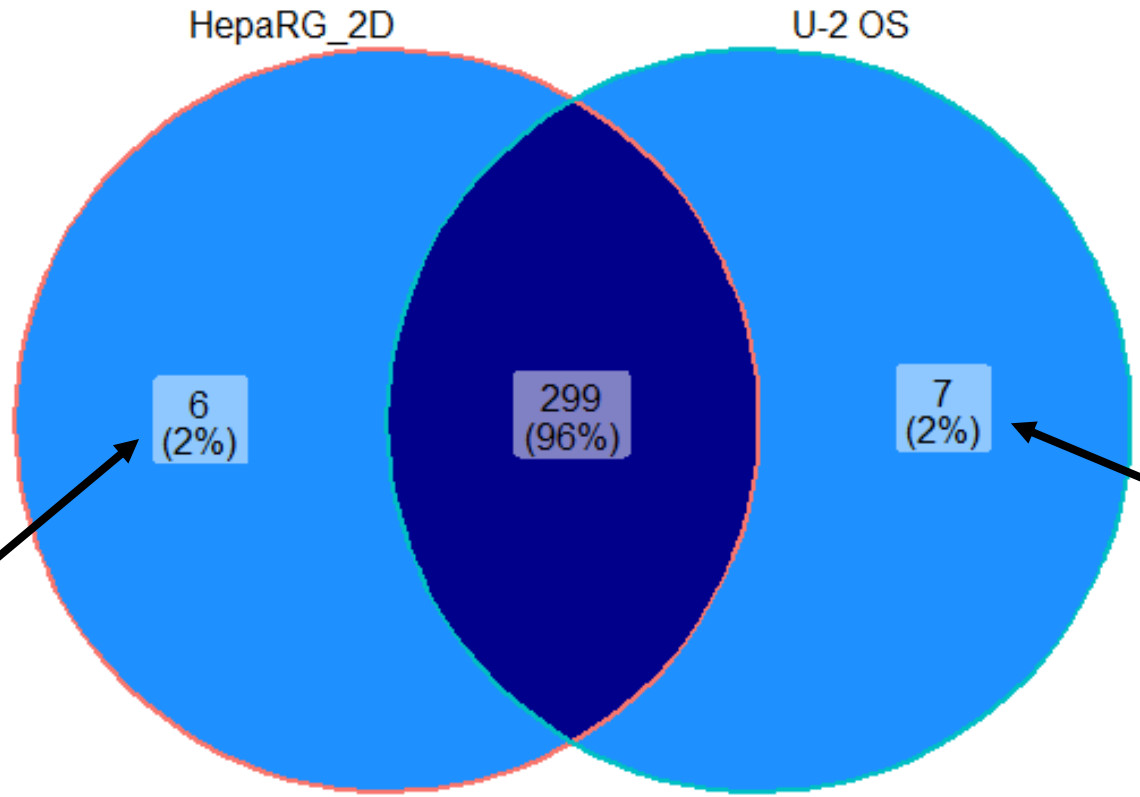



Adapted from Harrill et al. (2021) DOI: 10.1093/toxsci/kfab009

# EPA CPP5 HTTr Screening Results (1)

* Neomycin trisulfate salt hydrate was inactive in both cell types.

** Ispinesib was extremely cytotoxic to U-2 OS cells, not enough non-cytotoxic concentrations for modeling




Bambuterol hydrochloride (10)
Ispinesib (95)
Naproxen (7)
Rifamycin SV sodium salt (27)
Ticrynafen (28)
Tiotropium bromide (2)

*Active in HepaRG, but not U-2 OS*

Aminoguanidine (27)
Caffeine (42)
Captopril (1)
Methsuximide (21)
Pamidronic acid (20)
Tetrabutylammonium (7)
Tilarginine acetate (4)

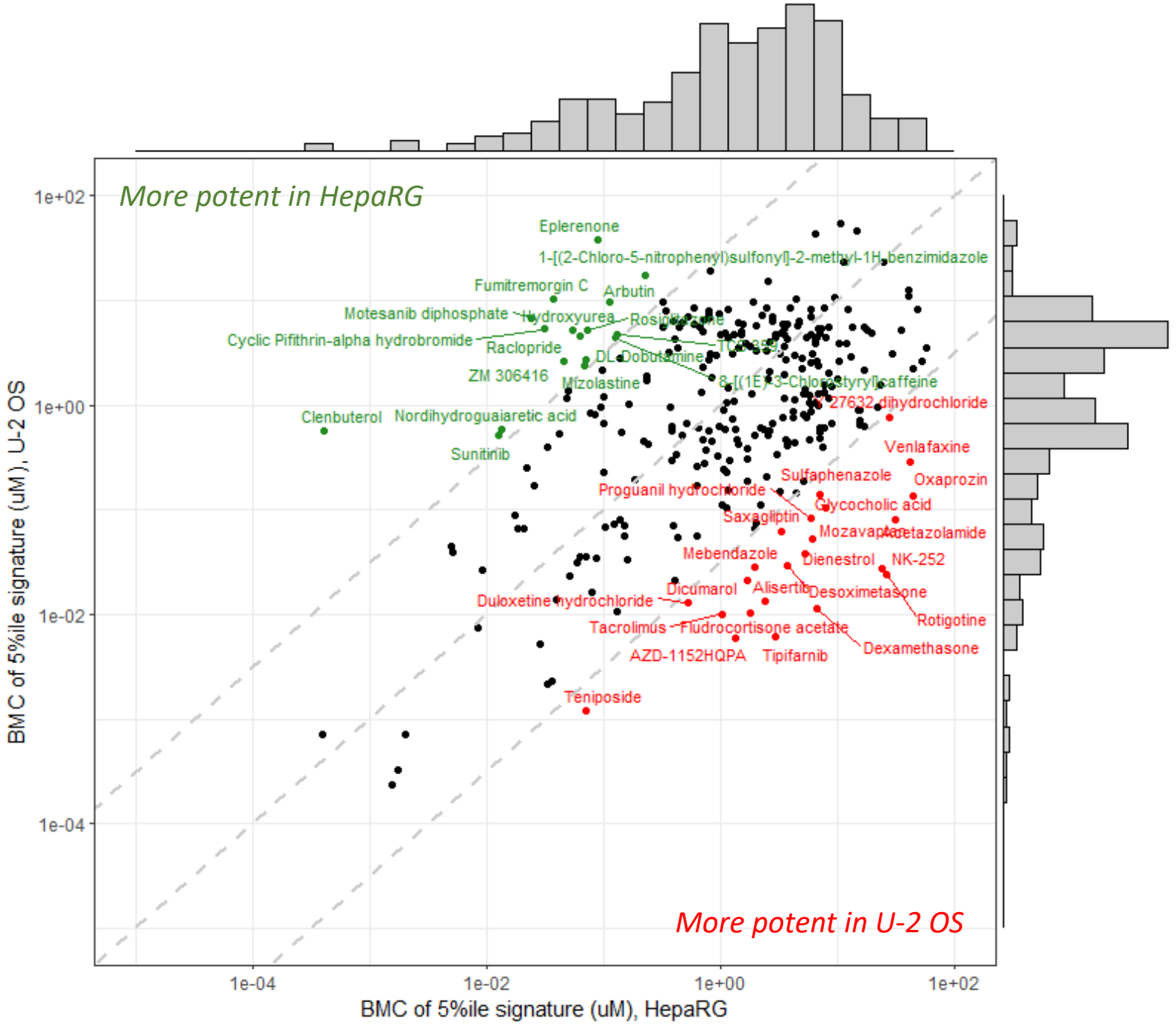
*Active in U-2 OS, but not HepaRG*

*Numbers in parenthesis are # of concentration-responsive signatures*

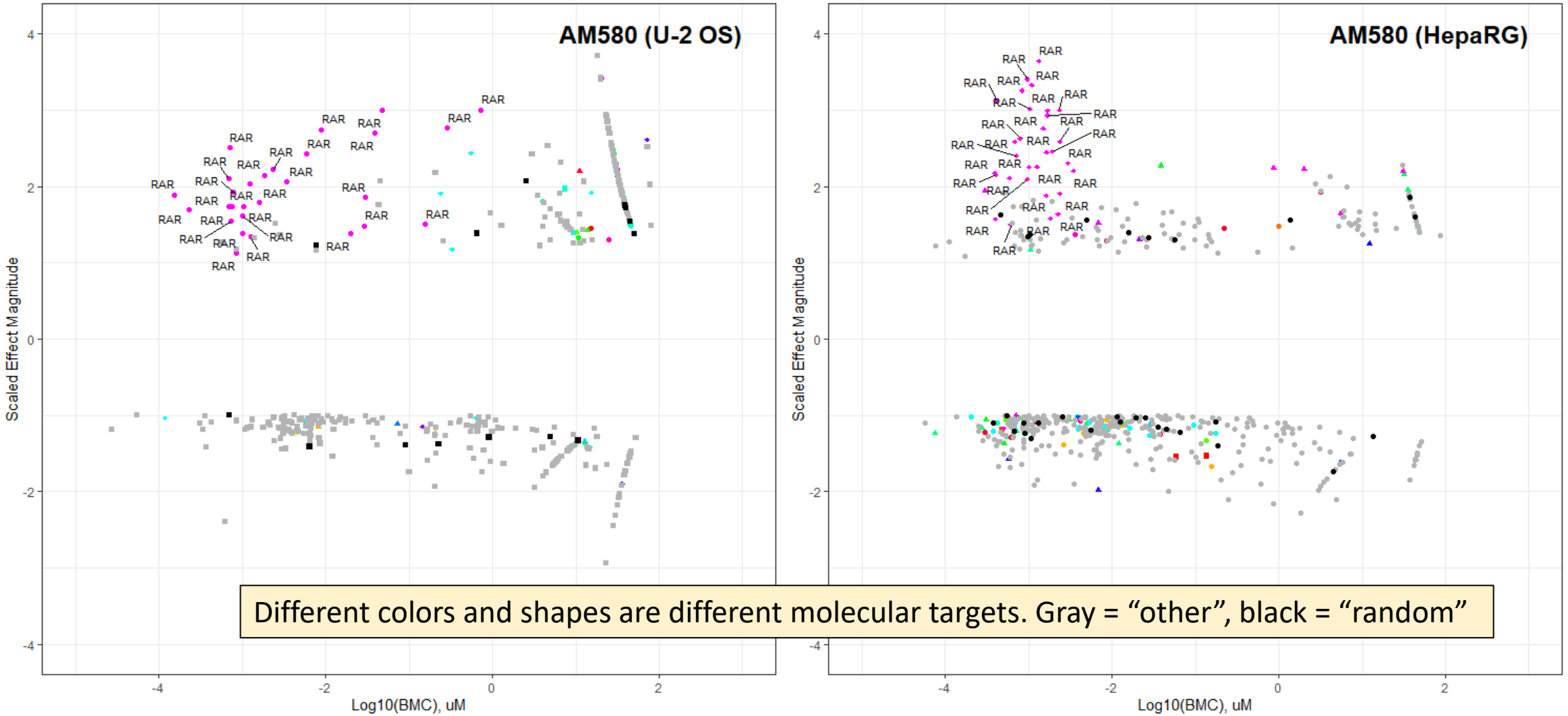
# EPA CPP5 HTTr Screening Results (2)

- The BPAC for many chemicals differs by more than two orders of magnitude ($log_{10}$) across cell types.

- Median BPAC across all chemicals:
  - HepaRG_2D → 1.7 µM
  - U-2 OS → 1.09 µM



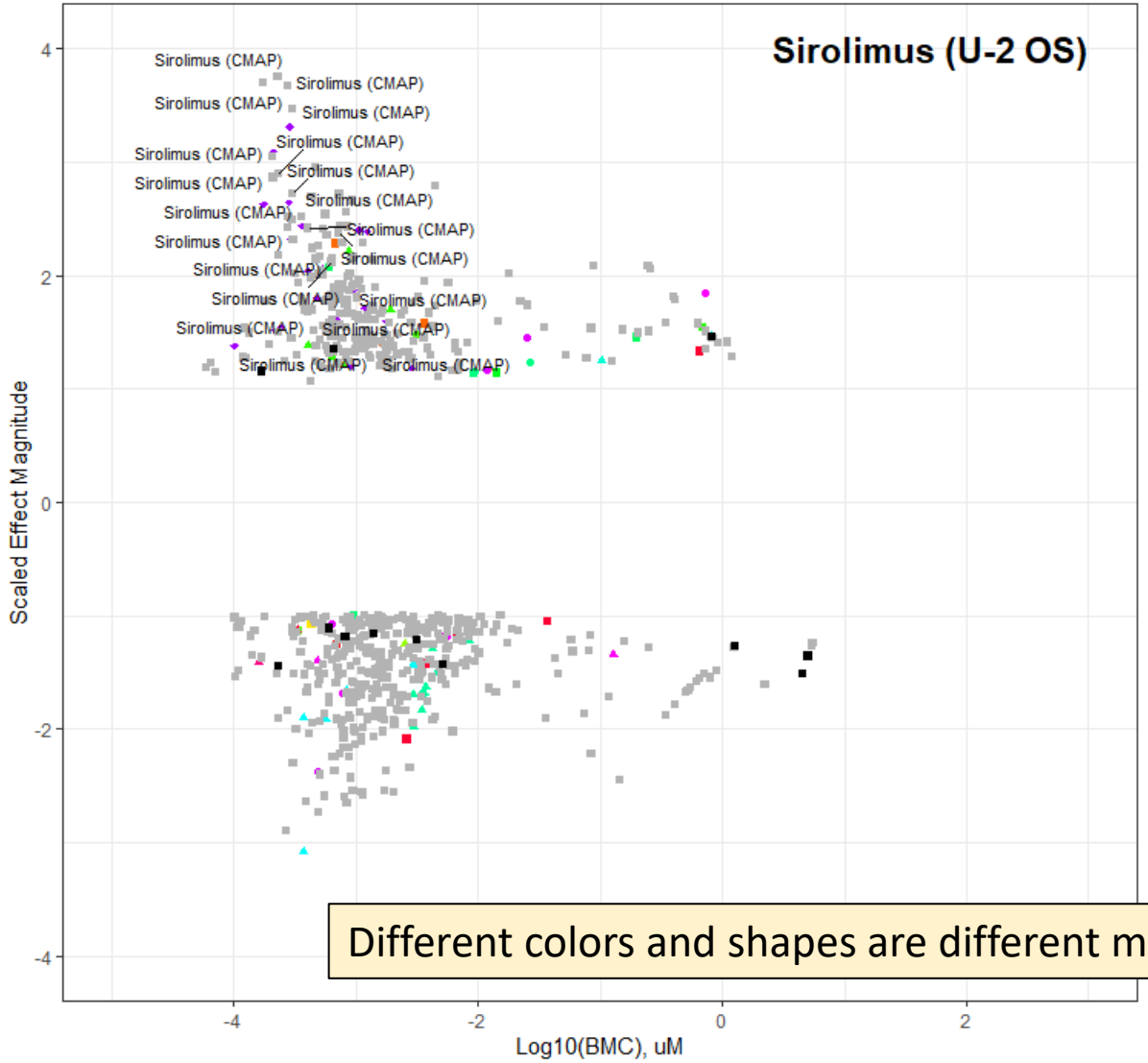
# Example Chemical, AM580 (RAR – Positive)

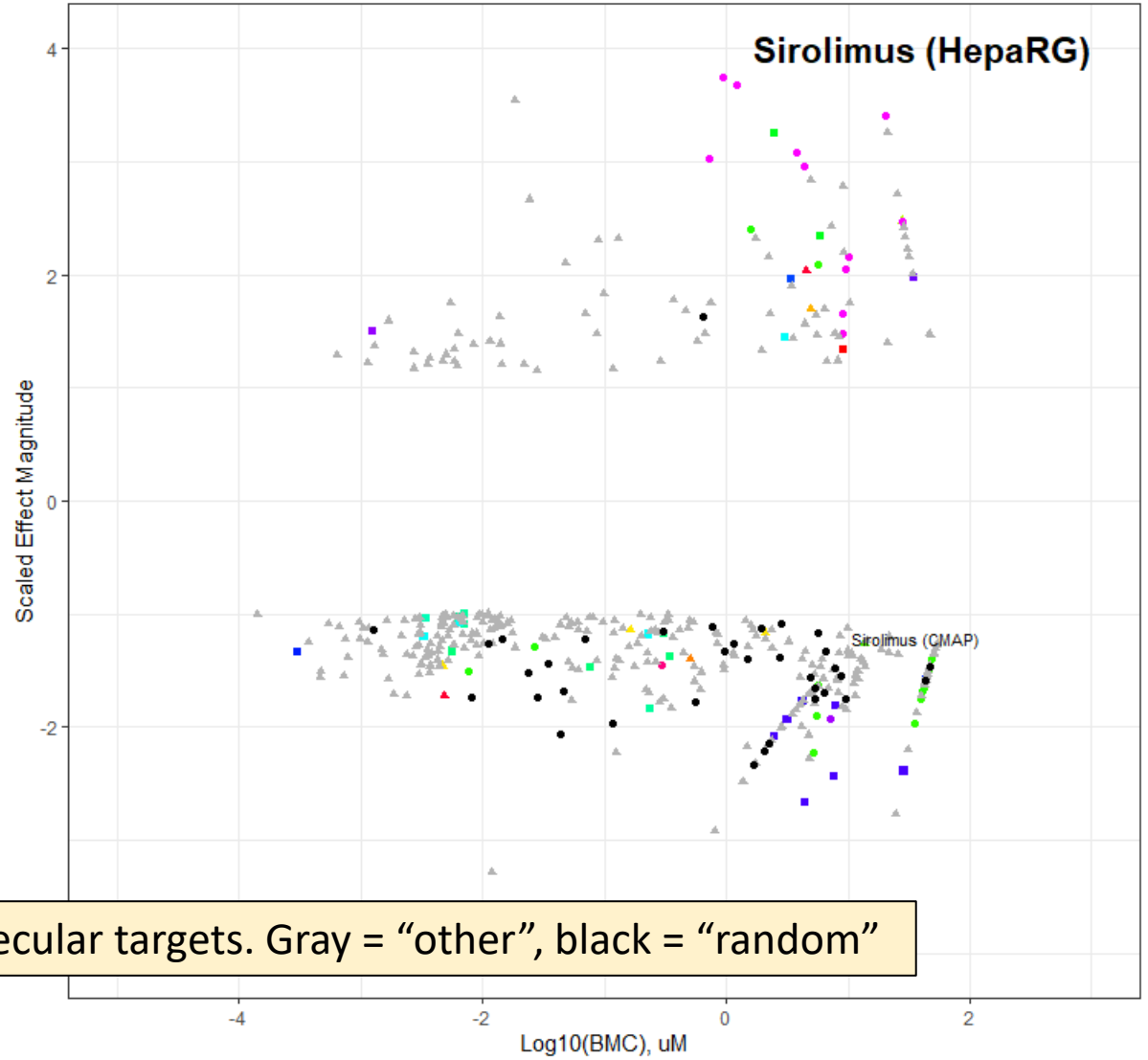



Different colors and shapes are different molecular targets. Gray = “other”, black = “random”

- AM580 is a retinoic acid receptor (RAR) agonist.
- Signature concentration-response modeling demonstrates that RAR signaling is affected at low concentrations of AM580 (10 nM) in both cell types.

# Example Chemical, Sirolimus (mTOR – Positive)




Different colors and shapes are different molecular targets. Gray = “other”, black = “random”

- Gene expression changes in U-2 OS cells following sirolimus treatment match to sirolimus signatures.
- Not so for HepaRG.  Results are dependent on cell type context.

# Next Steps at EPA for CPP5

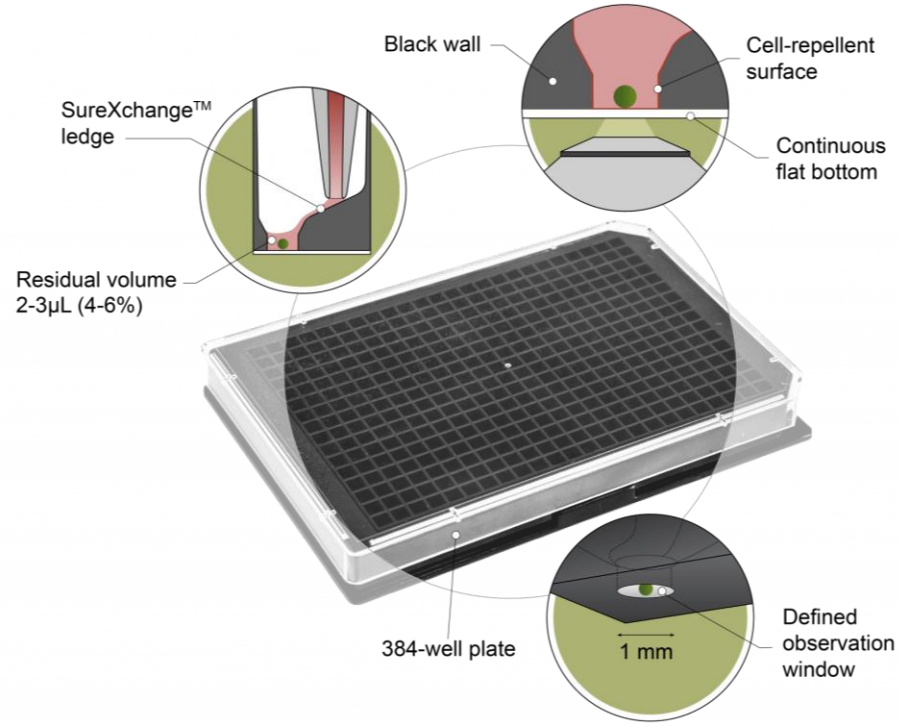
- **Continue analysis of U-2 OS and HepaRG_2D data**
  - Gene level HTTr analysis.
  - Build reference profiles.
  - Connectivity Mapping within the dataset.
  - Comparison to and interpretation of other TempO-Seq datasets.
  - Analyze HTPP data.

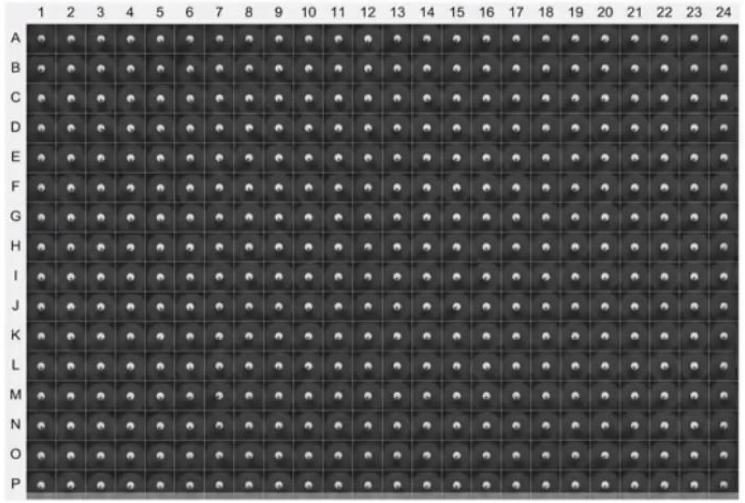
- **Continue curation of:**
  - Target_mode associations.
  - Potencies from HTS assays.
  - Signature catalog.

- **Continue testing of chemicals in additional cell types:**
  - Smaller number of concentrations, tailored to molecular target.

# DNTP Update for Tox21 CPP5

- Initial design to assess >300 reference chemicals with 3D HepaRG spheroids
- Revised strategy to use Akura™ 384
  - Engineered for spheroid screening/imaging
    - Imaging-centric design to localize spheroids within 1 mm reservoir, optically-friendly working distance
    - Pipetting ledge for confident media exchange
    - Reduced residual volume (2-3 µL)
      - Increased spheroid lysate concentrations
      - Enhanced transcriptomic read depths
  - Complementary to revised EPA focus on HepaRG cells (2D-Differentiated)



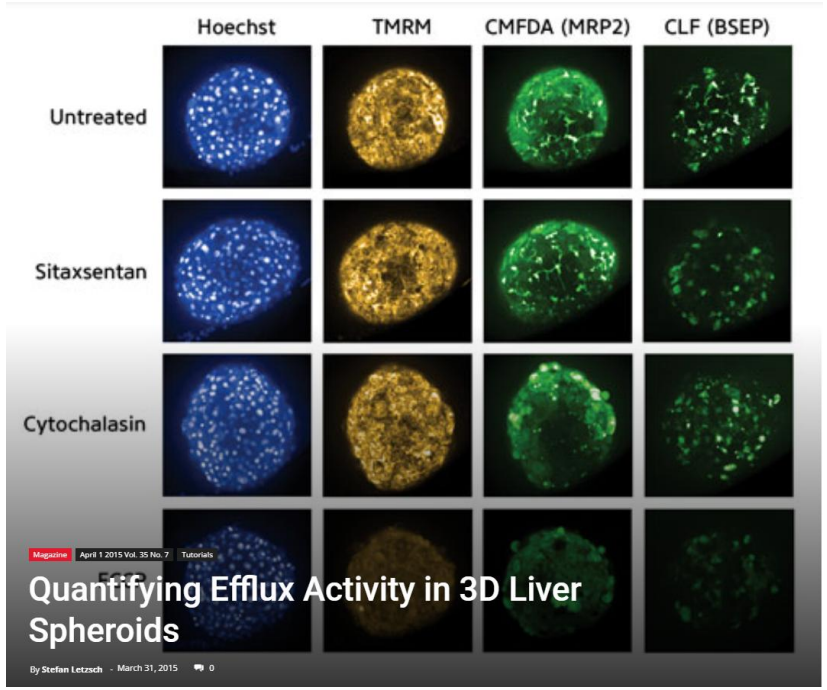
National Institute of Environmental Health Sciences
*Division of the National Toxicology Program*

# Tox21 Cross-partner Project #5

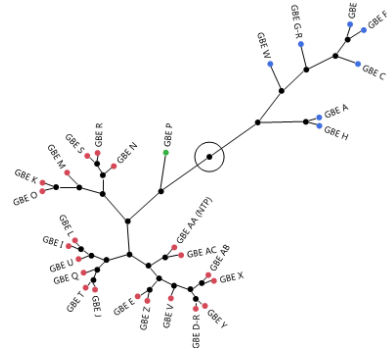
## Project Status

- Delays to initiate DNTP exposures
  - competing priorities
  - training/experience with Opera Phenix imaging system
  - availability of InSphero Akura plates
- Pilot Akura 384 plates:
  - Spheroid imaging (nuclei, cytoskeleton, biliary spaces)
  - Cell viability (ATP depletion)
  - Liver enzyme leakage (i.e., LDH-Glo)
  - Transcriptomics
- Reference chemical exposures to be initiated upon successful demonstration of pilot plate performance

Computational Collaborators Welcome!




- Create BRAVO computational tool to automate analysis:
  - **B**iological
    **R**esponse
    **A**nalysis
    **V**isualization
    **O**asis

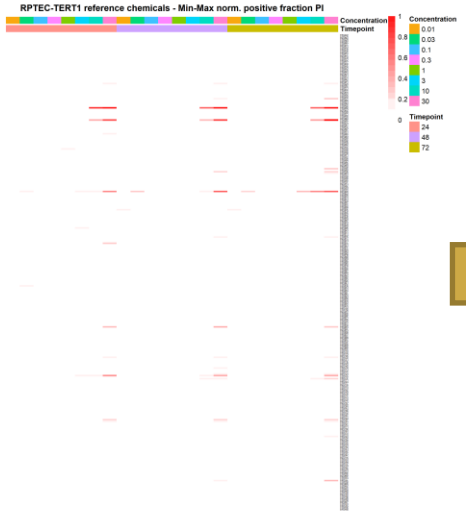


BRAVO Report

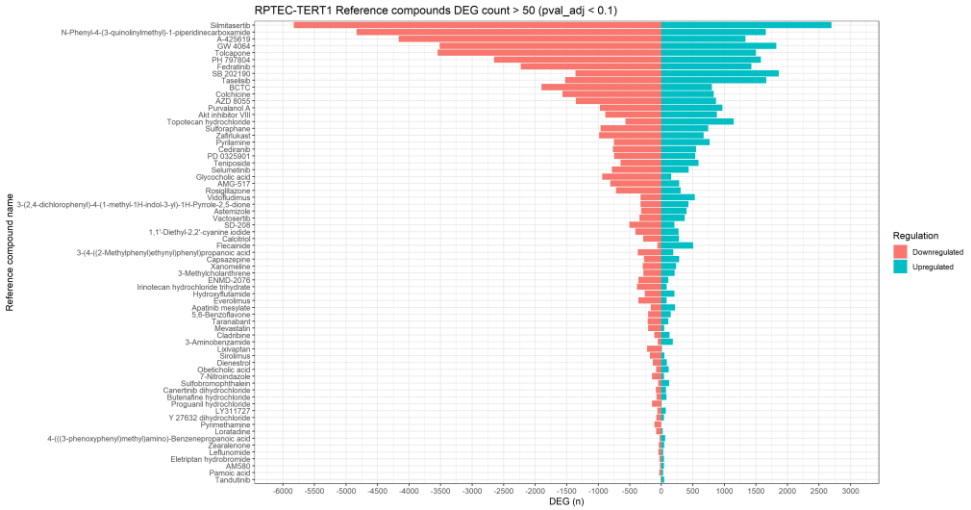
# Reference compound panel in RISK-HUNT3R: RPTEC

**Strategy reference compounds:**

- RPTEC-TERT1 cells
- Tox range finding
- Highest dose TempOseq
- Compound selection
- Concentration range TempOseq
- Target expression vs response
- TXG-MAPr projection
- Test system comparison



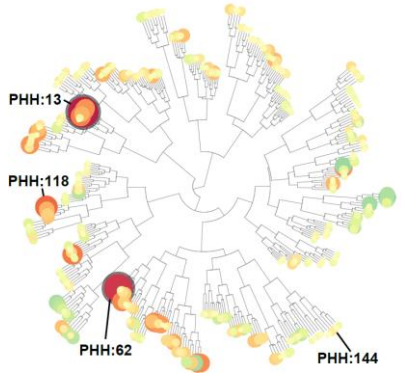

Toxicity profiling HCI




Highest conc WT TempOseq

Bob van de Water
Univ. Leiden

Other test systems
(e.g. LUHMES)




TXG-MAPr projection

Different concentrations for relevant compounds for WT TempOseq

# Acknowledgements


- **Ferguson (NTP)**, Harrill (EPA), Xia (NCATS) –provide overall leadership and oversight
- Paules (NTP), Simeonov (NCATS) and Thomas (EPA) – secure funding resources for HTT analysis
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- Waidyanatha (NTP), Collins (EPA), Richard (EPA), Coutros (EPA) and Huang (NCATS) – chemical library selection and securing
- Auerbach (NTP), Judson (EPA), Everett (EPA), Tong (FDA) and Huang (NCATS) – data analysis
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