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.

Office of Research and Development
Full Name of Lab, Center, Office, Division or Staff goes here.

EU-ToxRisk Final Symposium
2021-11-03

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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

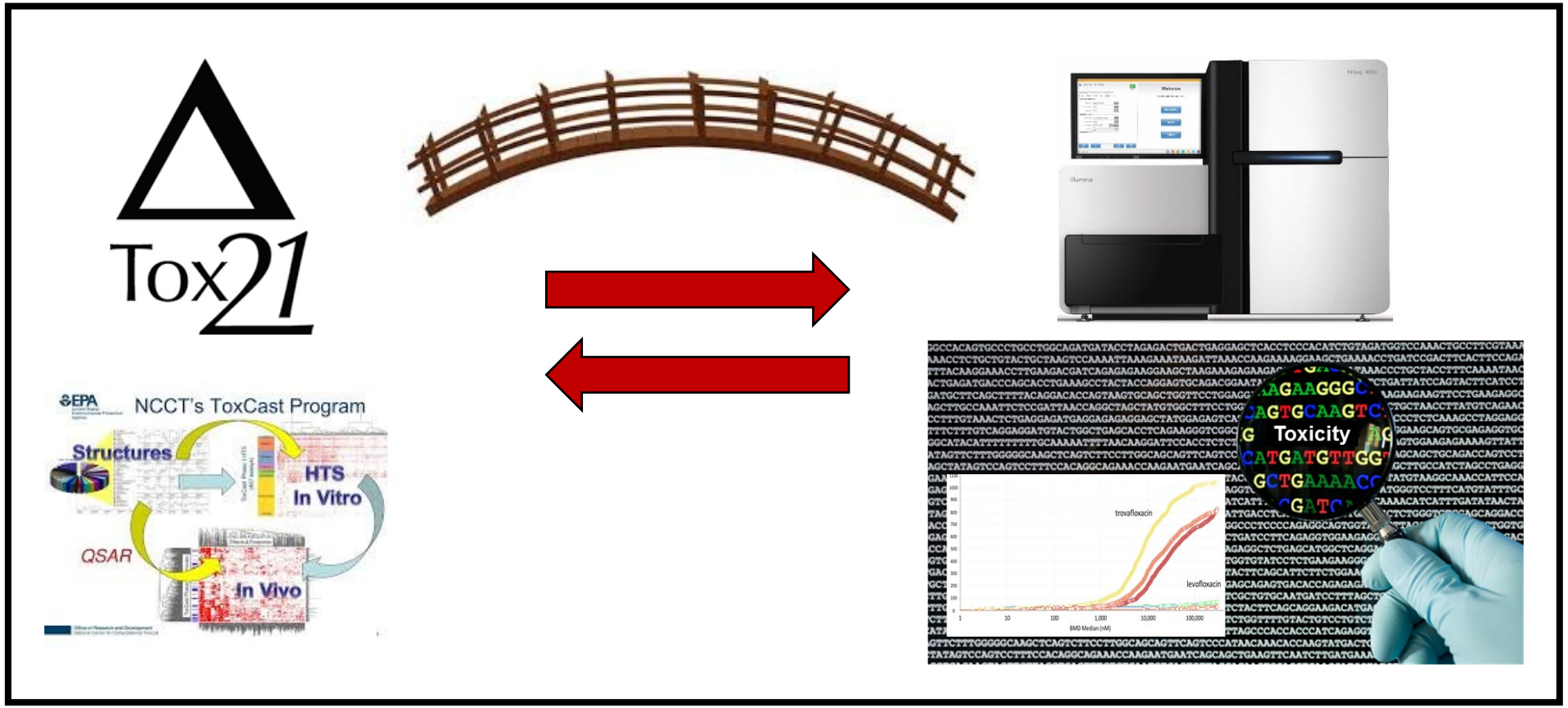
Evaluating Thiol Reactivity of Tox21 Chemicals using the MSTI Assay

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

Richard S. Judson[1], Russell S. Thomas[1], Nancy Baker[2], Anita Simha[3], Xia Meng Howey[3], Carmen Marable[3], Nicole C. Kleinstreuer[4] and Keith A. Houck[1]

[1]US EPA, National Center for Computational Toxicology, Research Triangle Park, NC, USA; [2]Leidos, Inc., Research Triangle Park, NC, USA; [3]ORAU, contractor to U.S. Environmental Protection Agency through the National Student Services Contract, Research Triangle Park, NC, USA; [4]National Toxicology Program, Interagency Center for the Evaluation of Alternative Toxicological Methods, Research Triangle Park, NC, USA

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.

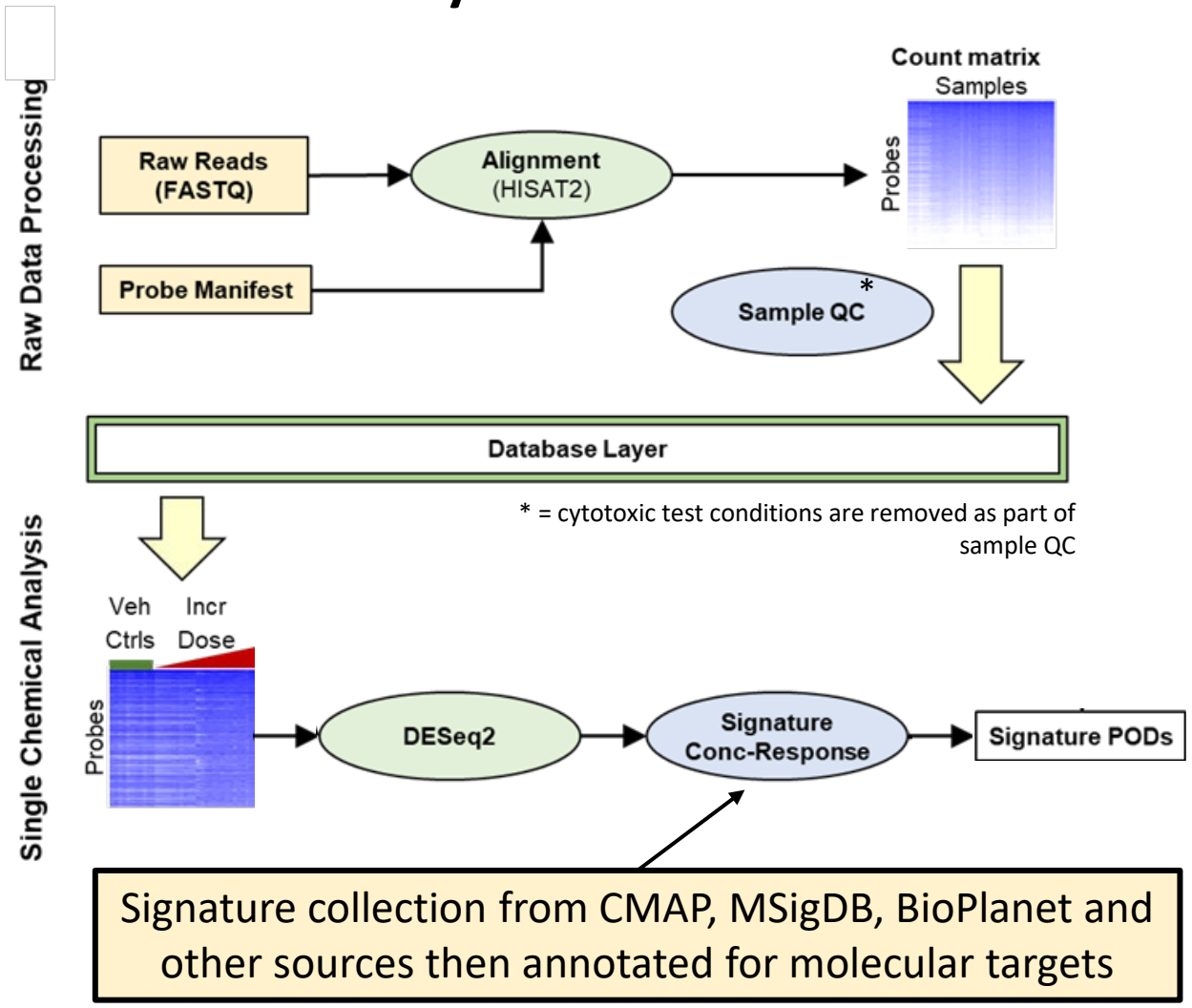
EPA CPP5 Tier 1 Screening (To Date)

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

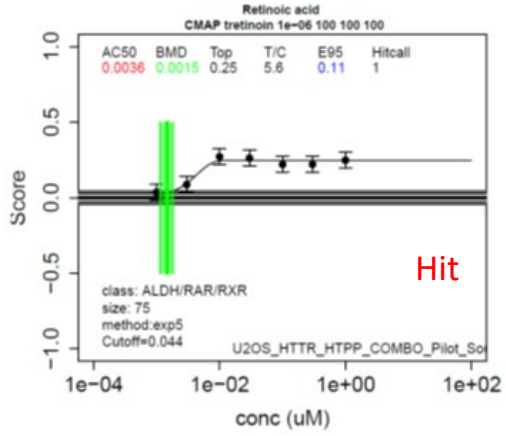
- High-throughput transcriptomics (HTTr) results have been pipelined for both cell types.
- Analysis of high-throughput phenotypic profiling (HTPP) data in progress.

First Pass Analysis of CPP5 HTTr Data

Analysis Overview

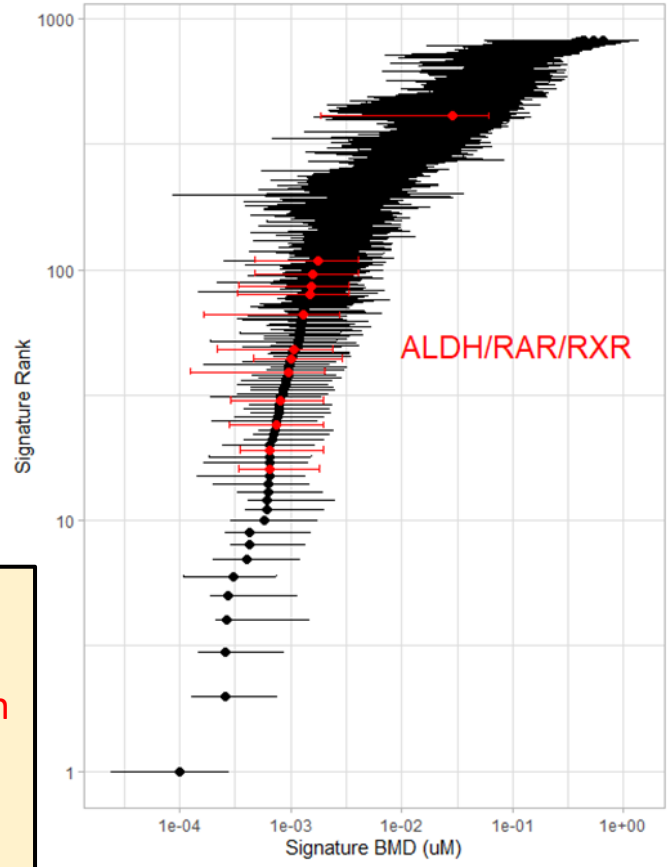


Concentration-Response Modeling of Signature Scores



Biological Pathway Altering Concentration (BPAC)

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

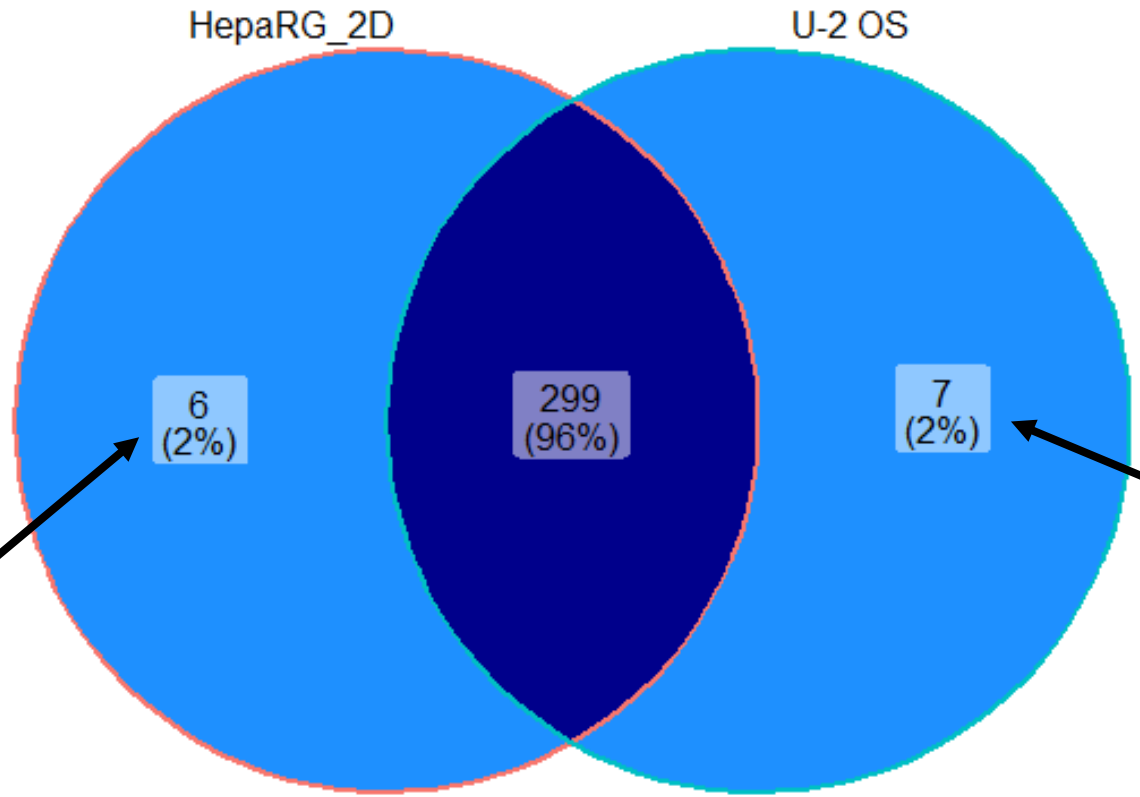


Adapted from Harrill et al. (2021) DOI: 10.1093/toxsci/kfab009

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)

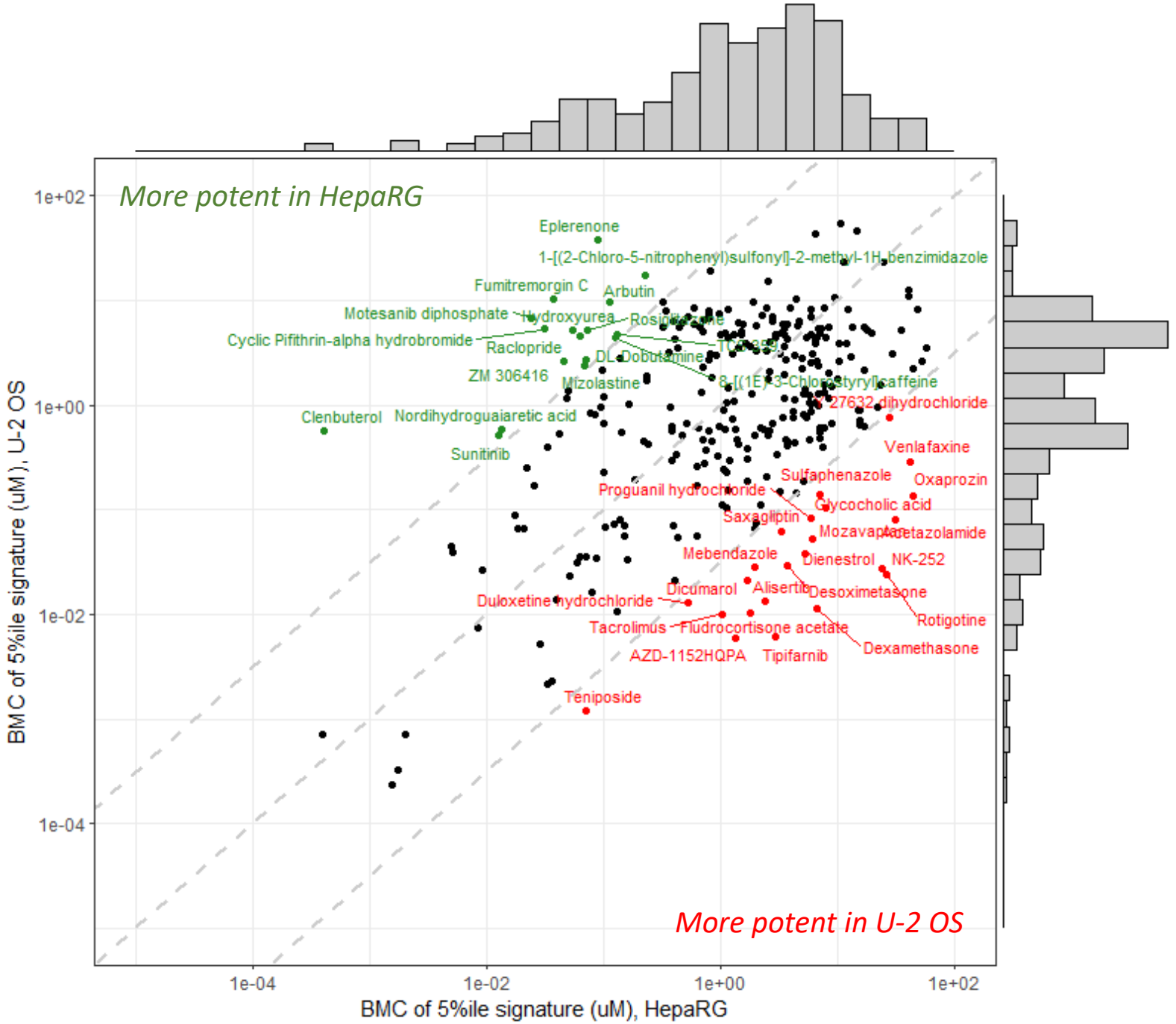
Active in U-2 OS, but not HepaRG

Numbers in parenthesis are # of concentration-responsive signatures

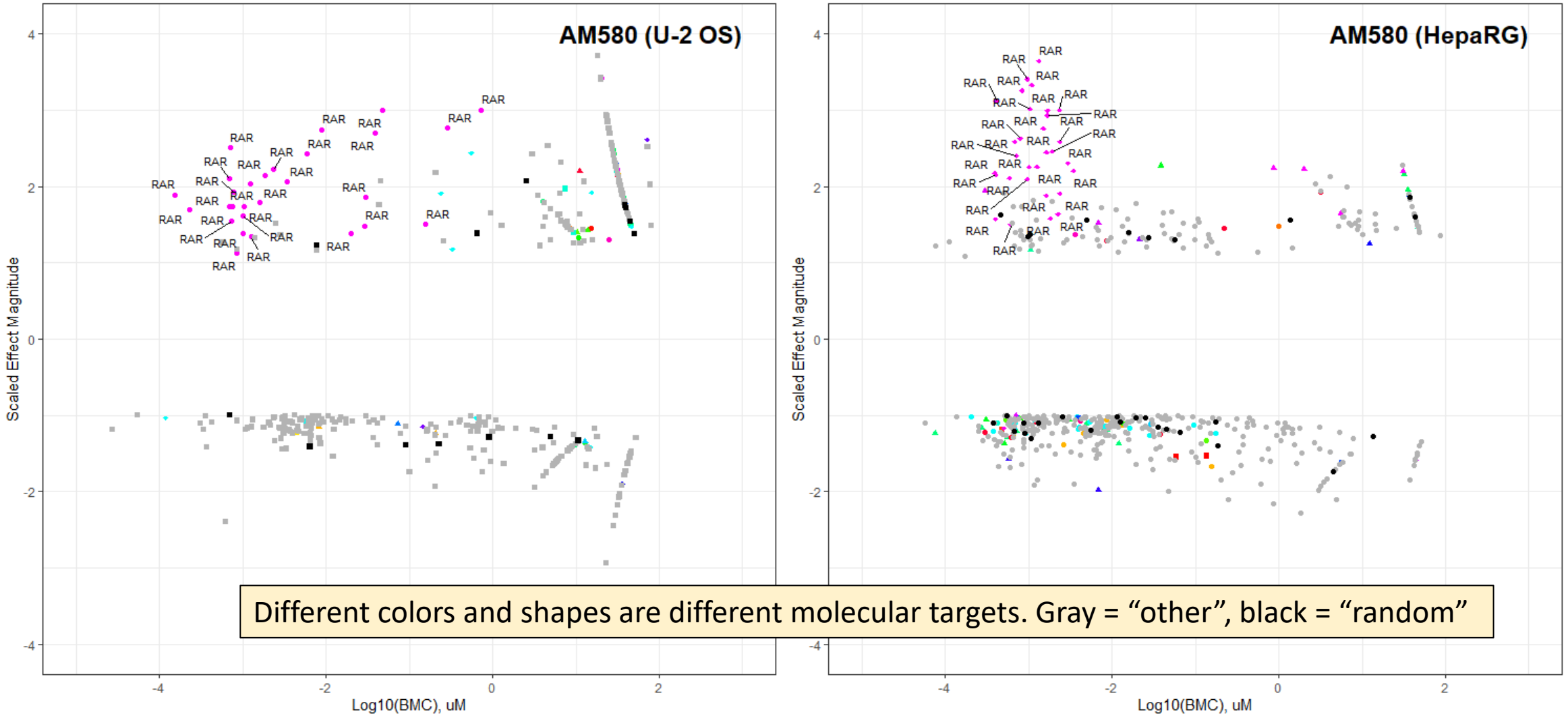
EPA CPP5 HTTr Screening Results (2)

- 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



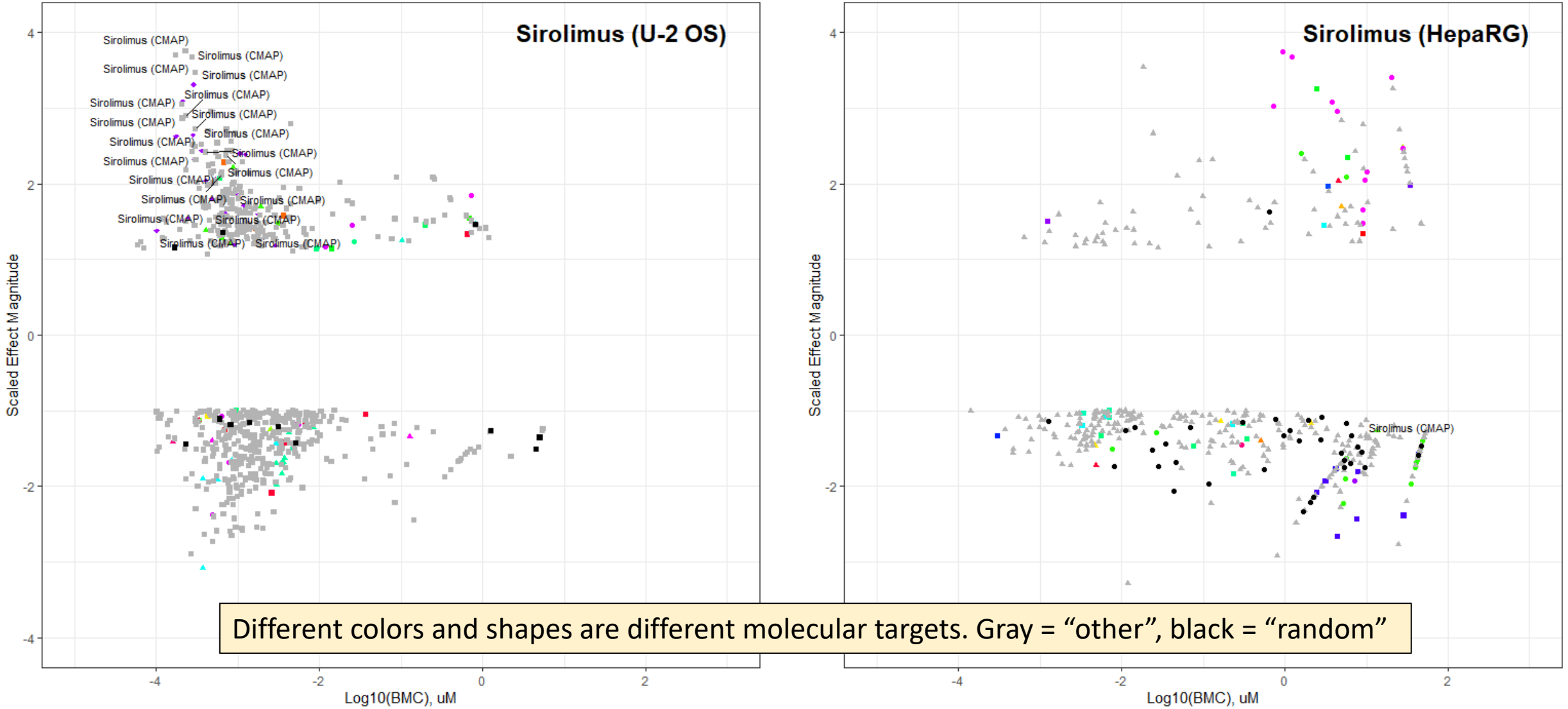
Example Chemical, AM580 (RAR – Positive)



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

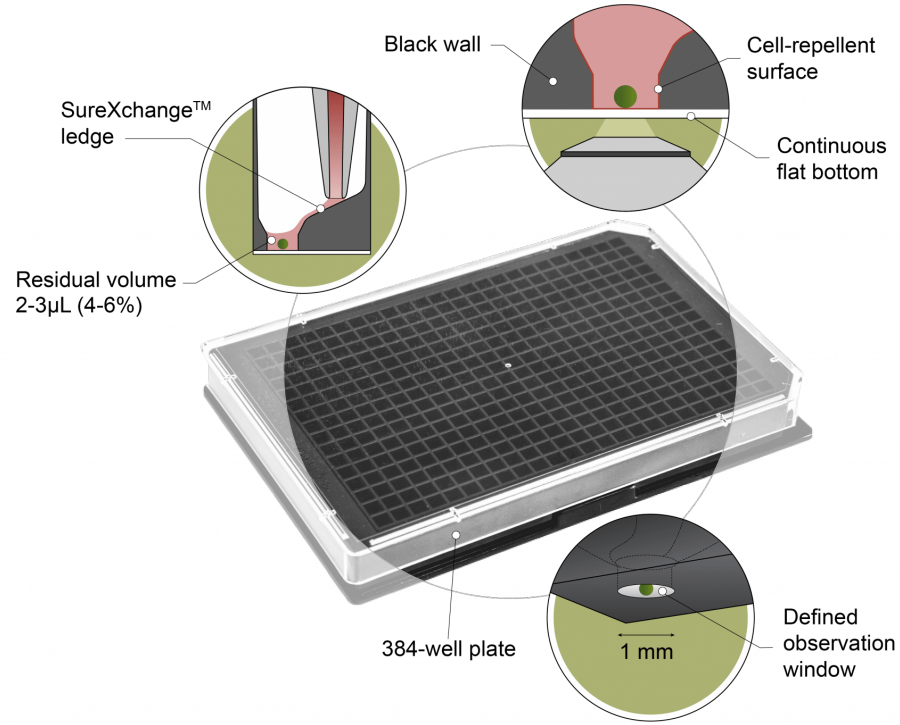
- **Continue analysis of U-2 OS and HepaRG_2D data**
  - Gene level HTTr analysis.
  - Build reference profiles.
  - Connectivity Mapping within the dataset.
  - Comparison to and interpretation of other TempO-Seq datasets.
  - Analyze HTPP data.

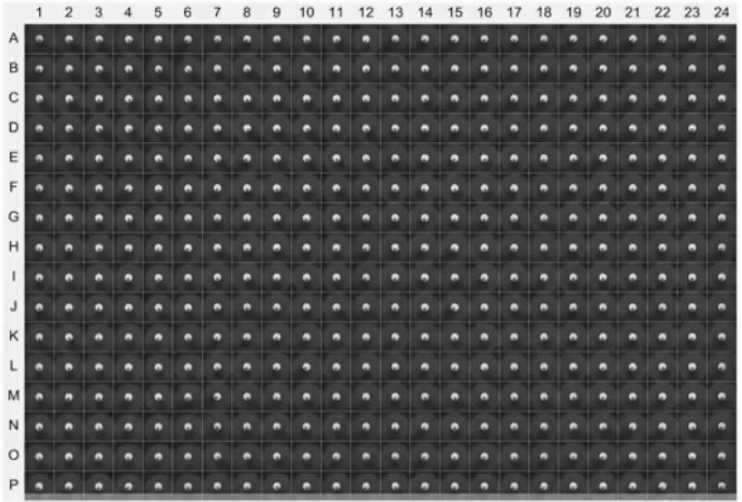
- **Continue curation of:**
  - Target_mode associations.
  - Potencies from HTS assays.
  - Signature catalog.

- **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)





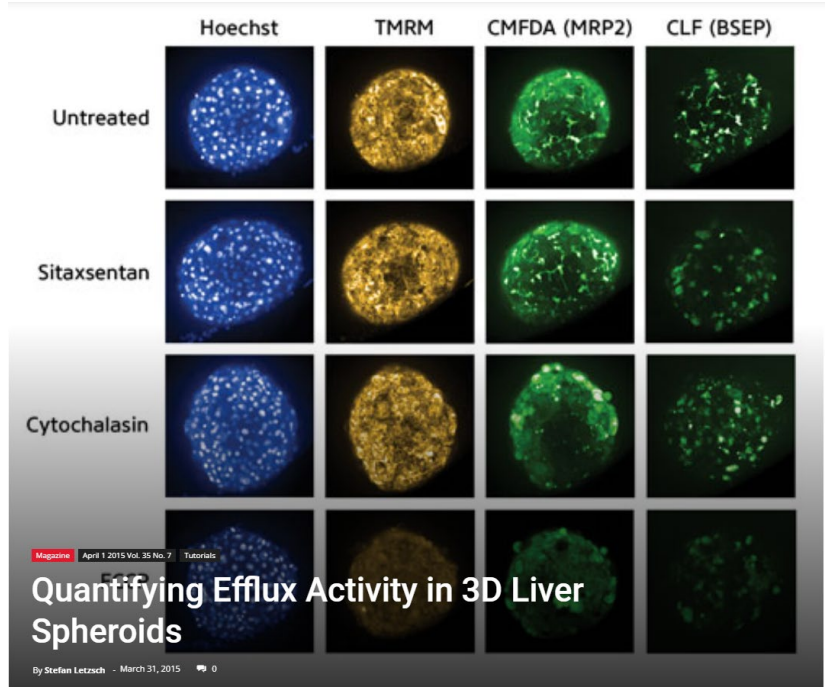
National Institute of Environmental Health Sciences
Division of the National Toxicology Program

Tox21 Cross-partner Project #5

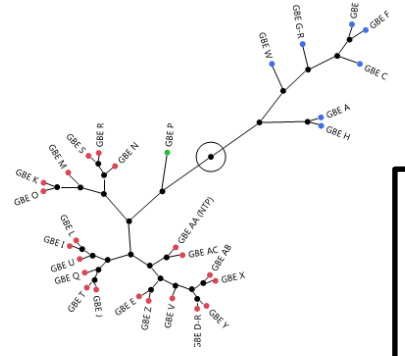
Project Status

- 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
    Response
    Analysis
    Visualization
    Oasis

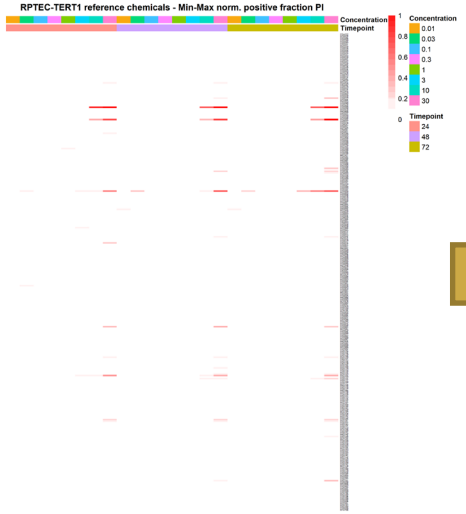


BRAVO Report

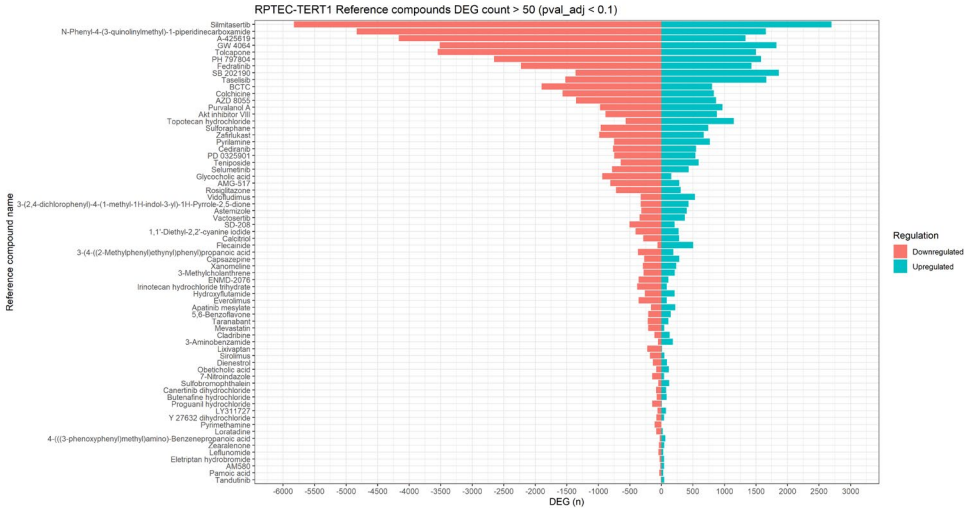
Reference compound panel in RISK-HUNT3R: RPTEC

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

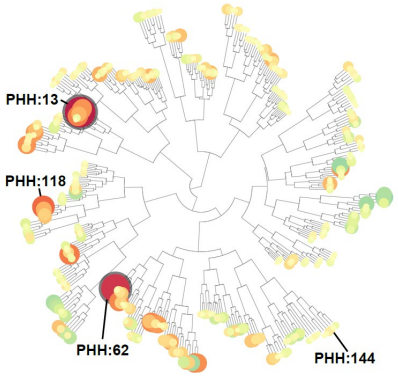


Toxicity profiling HCI



Highest conc WT TempOseq

Different concentrations for relevant compounds for WT TempOseq



TXG-MAPr projection

Other test systems (e.g. LUHMES)

Bob van de Water
Univ. Leiden

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
- Ferguson (NTP), Harrill (EPA), Xia (NCATS) – develop the appropriate human liver cellular model system
- 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
- Harris Ioannidis (EMBL-EBI)
- Bob van de Water (Universiteit Leiden)