Gene expression biomarkers as tools to interpret high-throughput transcriptomics data streams

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Disclaimer

- The views expressed are those of Dr. Chris Corton and do not reflect US-EPA policy or product endorsement by the US-EPA.

Outline

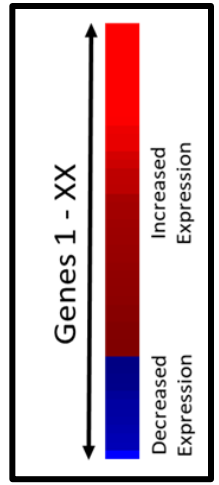
- Gene expression biomarkers
  - General information
  - Methods used for
    - Testing for predictive accuracy
    - Screening chemicals

- Biomarkers for screening transcript profiles generated in mice
  - Identification of mode of action

- Biomarkers for screening transcript profiles generated in rats to reduce 2-year bioassay
  - Identification of mode of action
  - Identification of chemical doses that would cause cancer

- Biomarkers for Tier 1 screening in high-throughput transcriptomics (HTTr) profiling
  - E.g., identification of estrogen receptor modulators

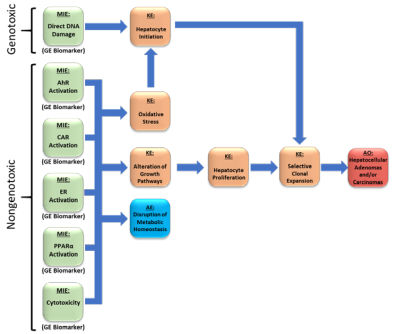
Gene expression biomarkers – moving towards regulatory acceptance

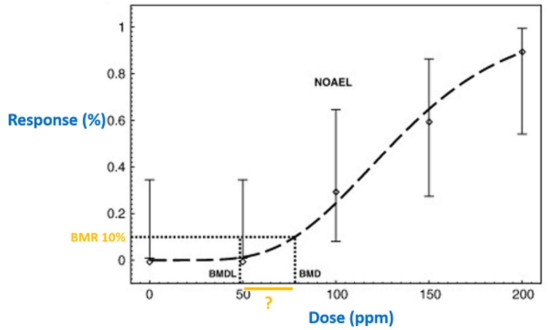


- Biomarker defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.” (1998, the National Institutes of Health Biomarkers Definitions Working Group)

- A gene expression biomarker is a short list of genes and associated fold-change values or ranks used to predict the activity of a factor important in mediating effects of chemicals or toxicity

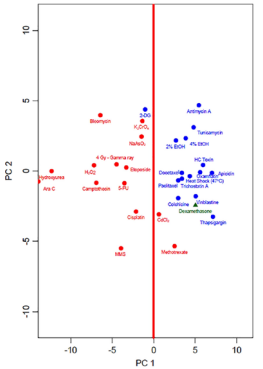
- Can be used to
  - Identify mode of action
  - Predict tumorigenic potential
  - (Determine a benchmark dose)



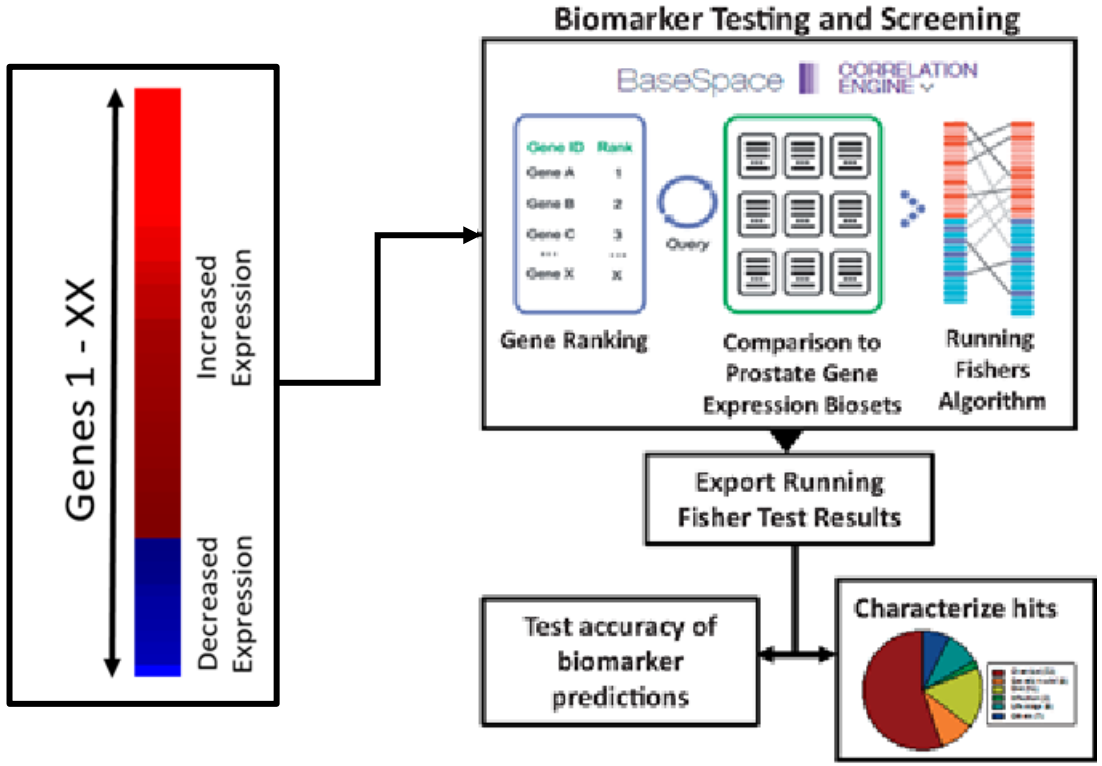


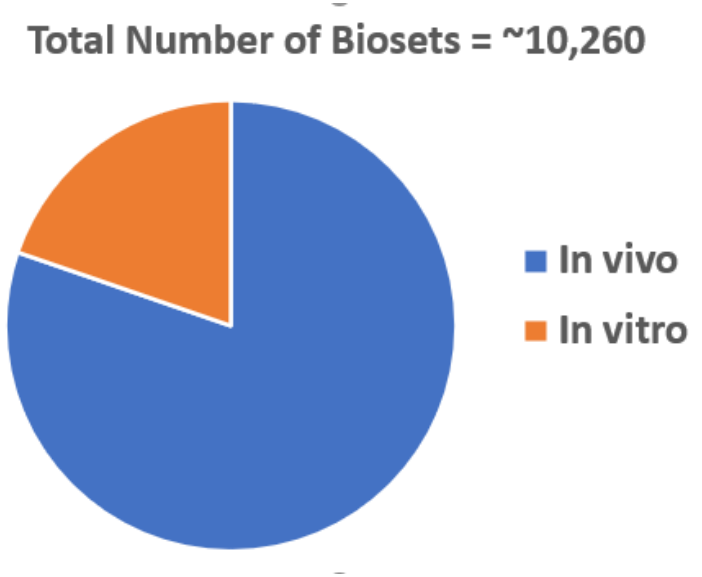
- Very few examples of well characterized gene expression biomarkers with known accuracies
  - Signature/pathway analysis often used as hypothesis generators

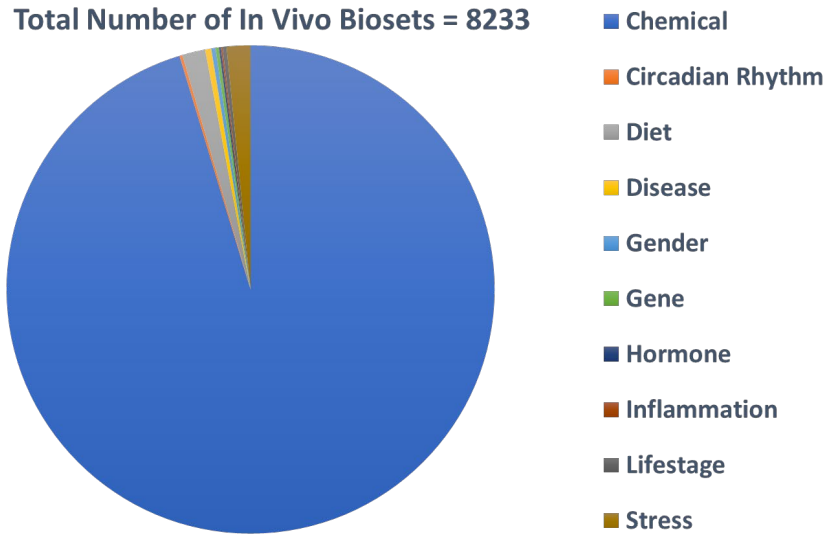
- Only two biomarkers have been considered for regulatory acceptance
  - GARDskin/GARDpotency – used to identify skin sensitizers in human myeloid dendritic-like cell line; accepted for regulatory studies (OECD TGP 4.106)
  - TGx-DDI biomarker – used to identify DNA damage-inducing chemicals in TK6 cells; under review by the FDA



Comparing gene lists in BaseSpace Correlation Engine





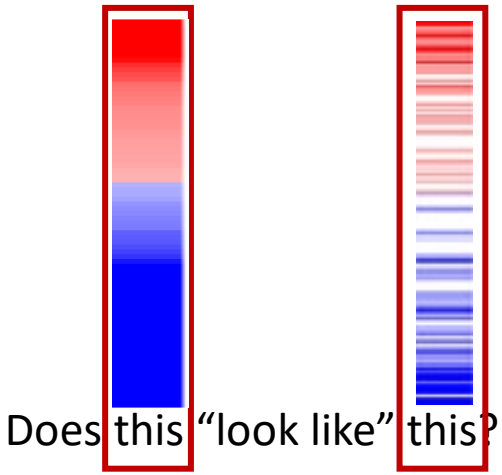


- Utilize Illumina's BaseSpace Correlation Engine
- Contains ~140,000 microarray lists of statistically significant genes
- Valuable computational tools
- Compares all microarray comparisons to each other in a pairwise fashion using a Running Fisher test
- For each pair-wise comparison: generates the number of overlapping genes, correlation direction and p-value

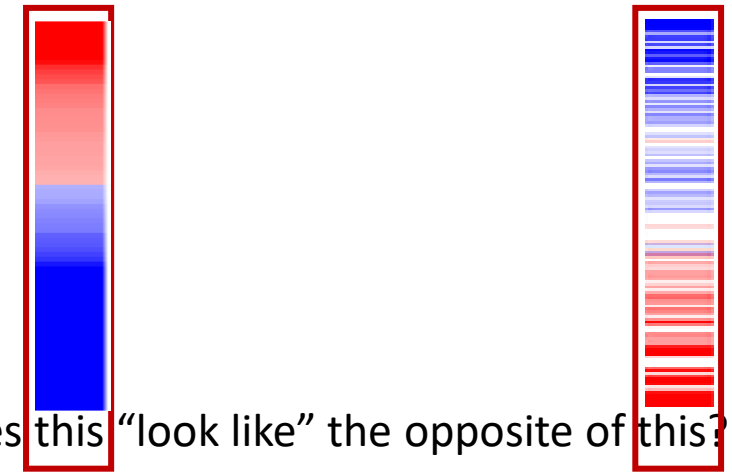
- Greatly accelerated construction and analysis of rat biomarkers

Correlation analysis using the Running Fisher Test

- Identification of factors (chemicals, hormones, diets, genes, etc.) that “look” like your gene list



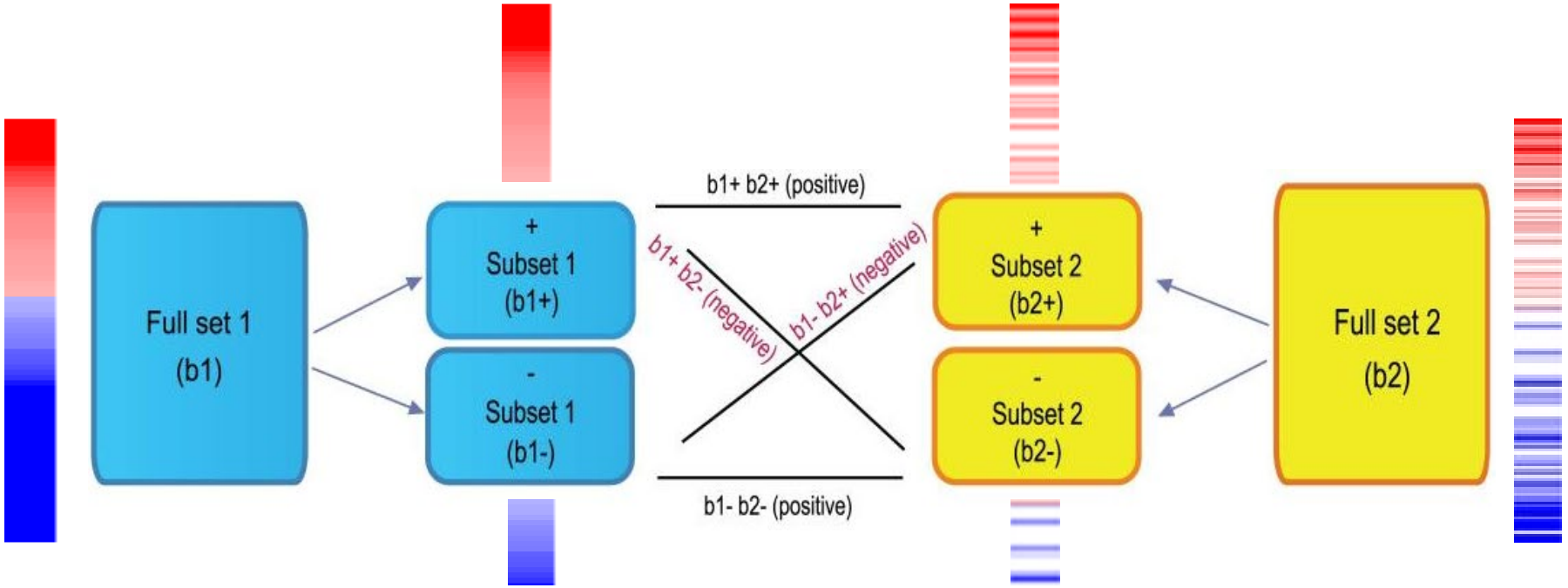
Does this “look like” this?



Does this “look like” the opposite of this?

- Correlation can be determined computationally using the Running Fisher test in BSCE

Computing directionality and final correlation scores between two gene lists



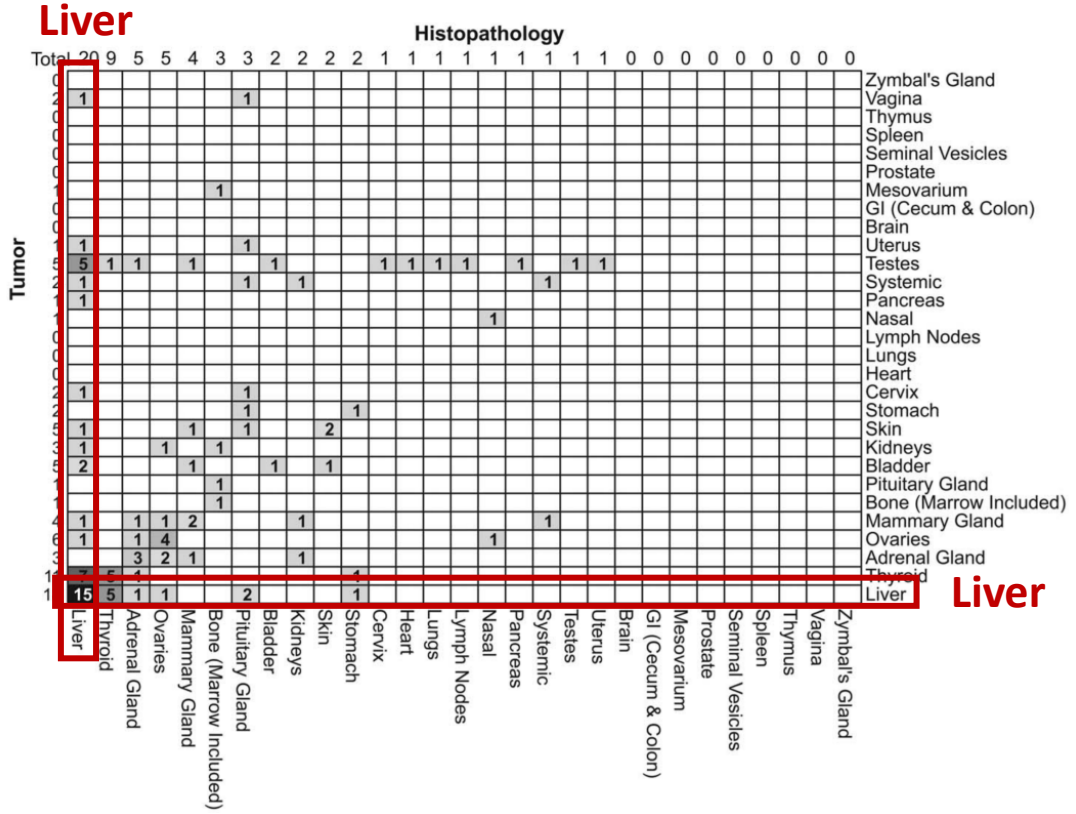
- Score(b1, b2) = sum(b1+b2+, b1+b2-, b1-b2+, b1-b2-)
- Running Fisher Test p-value
- Direction of the correlation

- **The Running Fisher test p-value is a useful metric of correlation between gene sets**

Adapted from Kuperschmidt et al. (2010) PLoS One

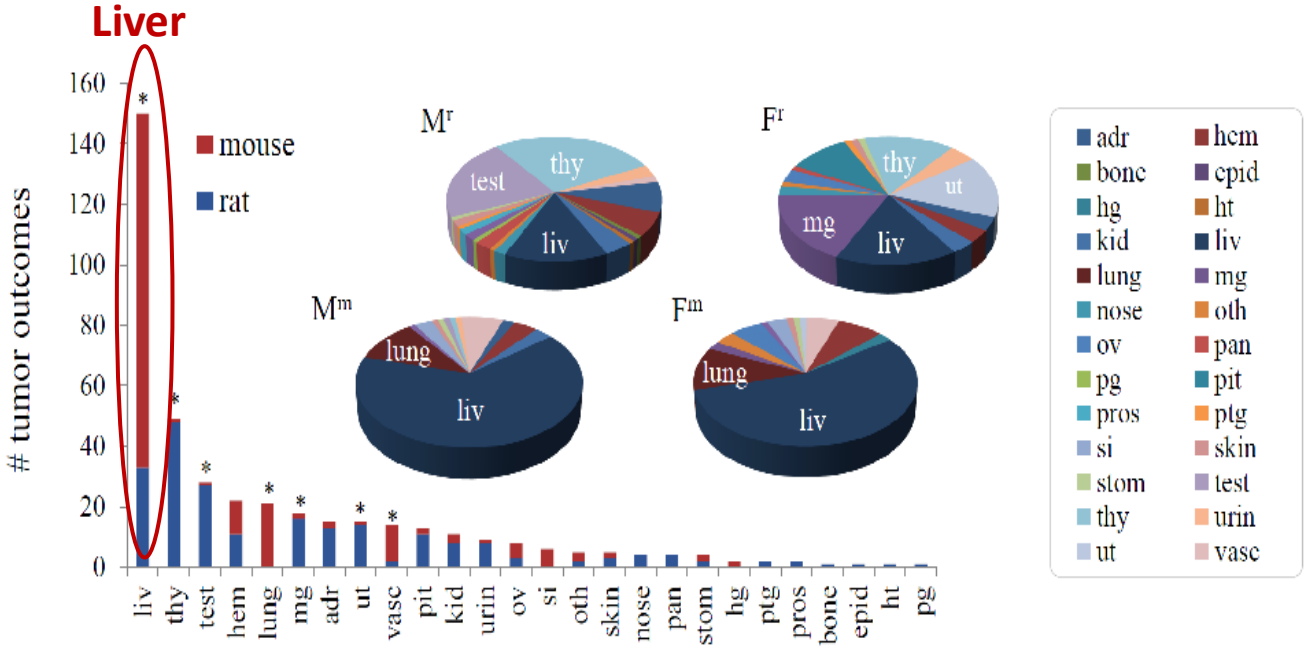
Liver is a major site for chemical-induced carcinogenesis in rodents

Marketed Pharmaceuticals in Rats



From Sistare et al. Toxicol Pathol. 2011 Jun; 39(4):716-44.

Environmental Chemicals in Mice and Rats

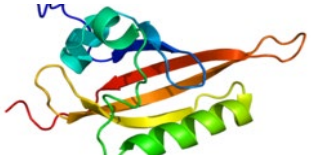


- Results of 628 two-sex carcinogenicity studies (n = 324 rat, n = 304 mouse) available in ToxRefDB
- Studies covered 336 unique compounds (n = 307 rat, n = 288 mouse), 259 of which were tested in both species

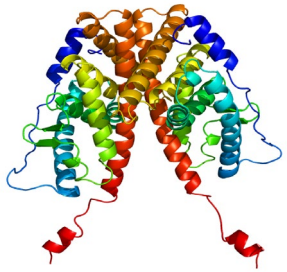
From Hill et al. Toxicol Sci. 2017 Jan; 155(1):157-169

Biomarkers that predict key events in the livers of mice and rats

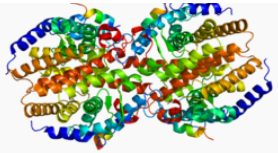
AhR



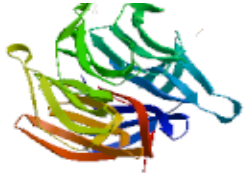
Estrogen Receptor α



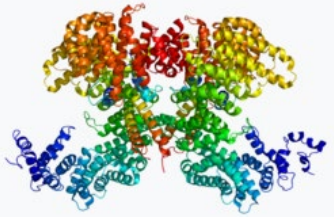
CAR



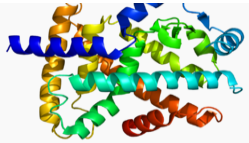
NRF2



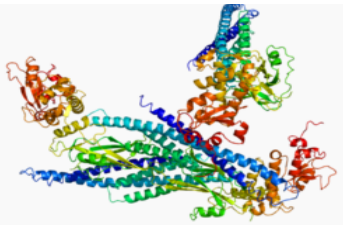
SREBP



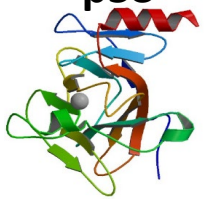
PPARα



STAT5b

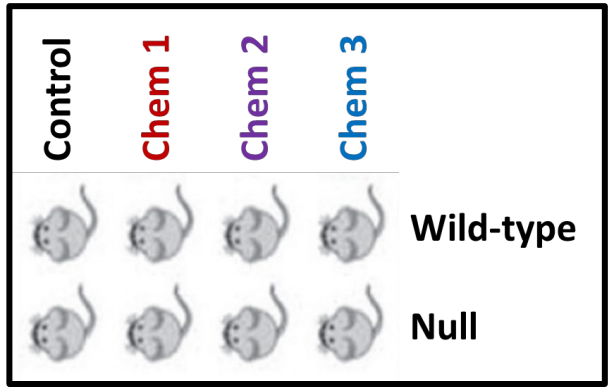


p53



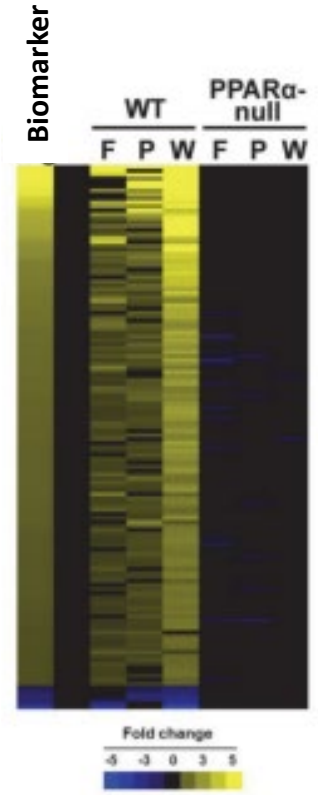
- Oshida et al. (2015). Identification of Modulators of the Nuclear Receptor Peroxisome Proliferator-Activated Receptor α (PPARα) in a Mouse Liver Gene Expression Compendium. PLoS One. 10(2):e0112655.
- Oshida et al. (2015). Identification of Chemical Modulators of the Constitutive Activated Receptor (CAR) in a Mouse Liver Gene Expression Compendium. Nuclear Receptor Signaling. 13:e002.
- Oshida et al. (2015). Screening a Mouse Liver Gene Expression Compendium Identifies Effectors of the Aryl Hydrocarbon Receptor (AhR). Toxicology. 336:99-112.
- Oshida et al. (2015). Disruption of STAT5b-Regulated Sexual Dimorphism of the Liver Transcriptome by Diverse Factors Is a Common Event. PLoS One. 11(3):e0148308.
- Oshida et al. (2015). Chemical and Hormonal Effects on STAT5b-Dependent Sexual Dimorphism of the Liver Transcriptome. PLoS One. 2016 11(3):e0150284.
- Rosen et al. (2017). PPARα-independent transcriptional targets of perfluoroalkyl acids revealed by transcript profiling. Toxicology. 387:95-107.
- Rooney et al. (2017). Genomic Effects of Androstenedione and Sex-Specific Liver Cancer Susceptibility in Mice. Toxicol Sci. 160(1):15-29.
- Rooney et al. (2018) Activation of Nrf2 in the liver is associated with stress resistance mediated by suppression of the growth hormone-regulated STAT5b transcription factor. PLoS One. 13(8):e0200004.
- Rooney et al. (2018). Activation of CAR leads to activation of the oxidant-induced Nrf2. Toxicol Sci. 167:172-189.
- Rooney et al. (2018). Adverse outcome pathway-driven identification of rat liver tumorigens in short-term assays. Toxicol Appl Pharmacol. 356:99-113.
- Corton (2019). Frequent Modulation of the Sterol Regulatory Element Binding Protein (SREBP) by Chemical Exposure in the Livers of Rats. Comput. Toxicol. 10:113-129.

Construction of mouse biomarkers using wild-type vs. nullizygous comparisons



- Identified genes that were regulated in wild-type mice but not null mice
- Genes had to be similarly regulated across the three chemicals (2 or 3 out of 3) in wild-type but not the same direction in null mice

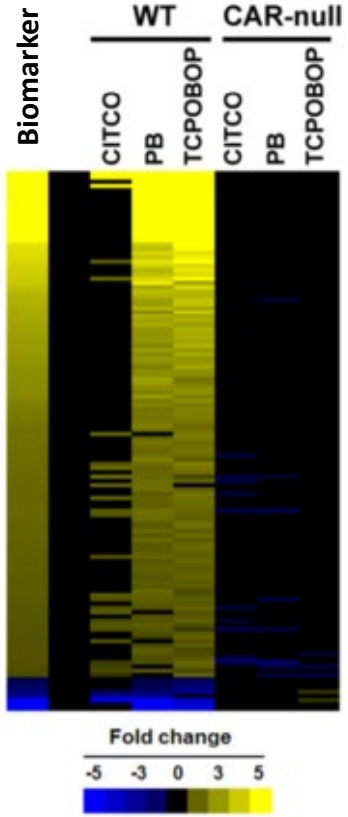
PPARα



F=fenofibrate
P=PFOA
W=Wy-14,643

Oshida et al. PLoS One. 2015 10(2):e0112655

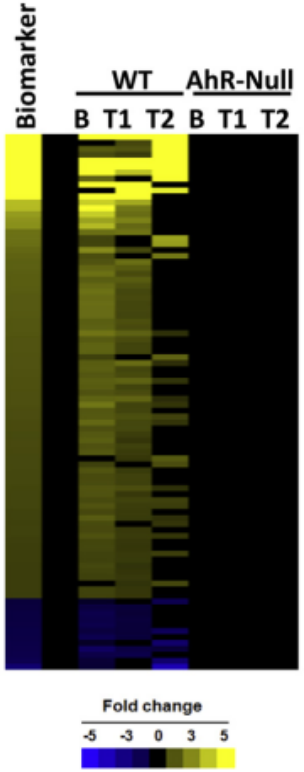
CAR



PB=phenobarbital

Oshida et al. Nucl Recept Signal. 2015 13:e002

AhR

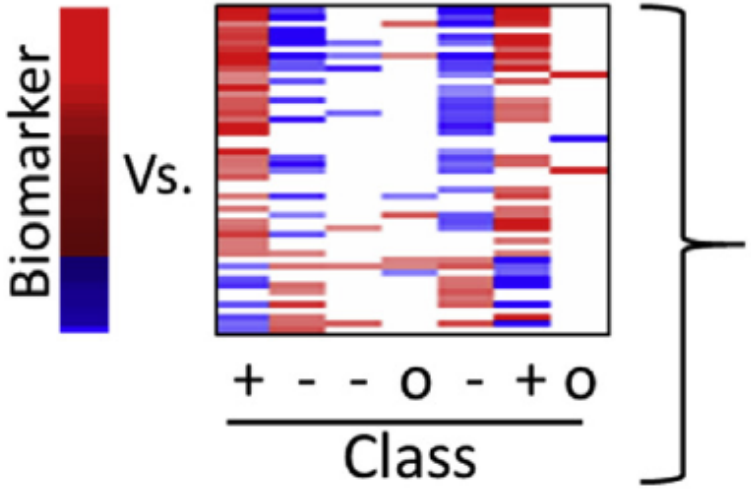


B=BaP
T1,T2=TCDD

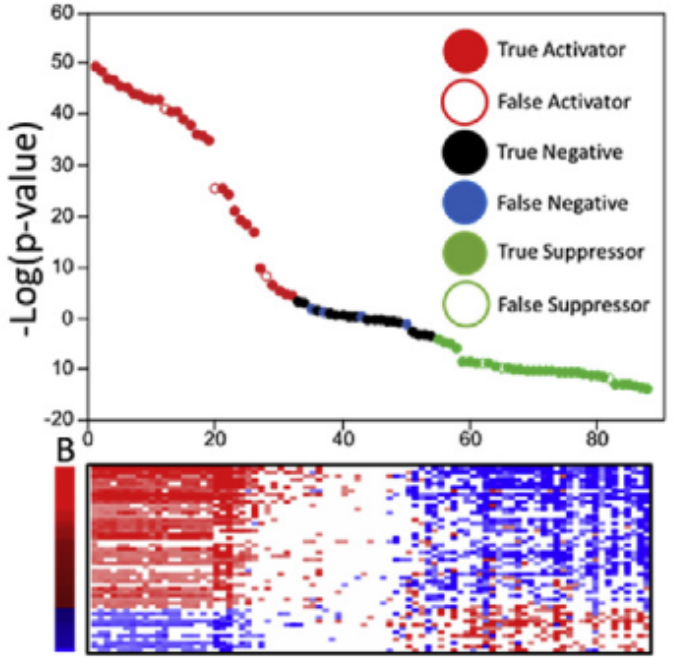
Oshida et al. Toxicology. 2015 336:99-112

Determination of biomarker accuracy using chemical-induced profiles

Comparison of biomarker to chemical profiles with known outcomes



Ranking by Correlation



Accuracy Determination

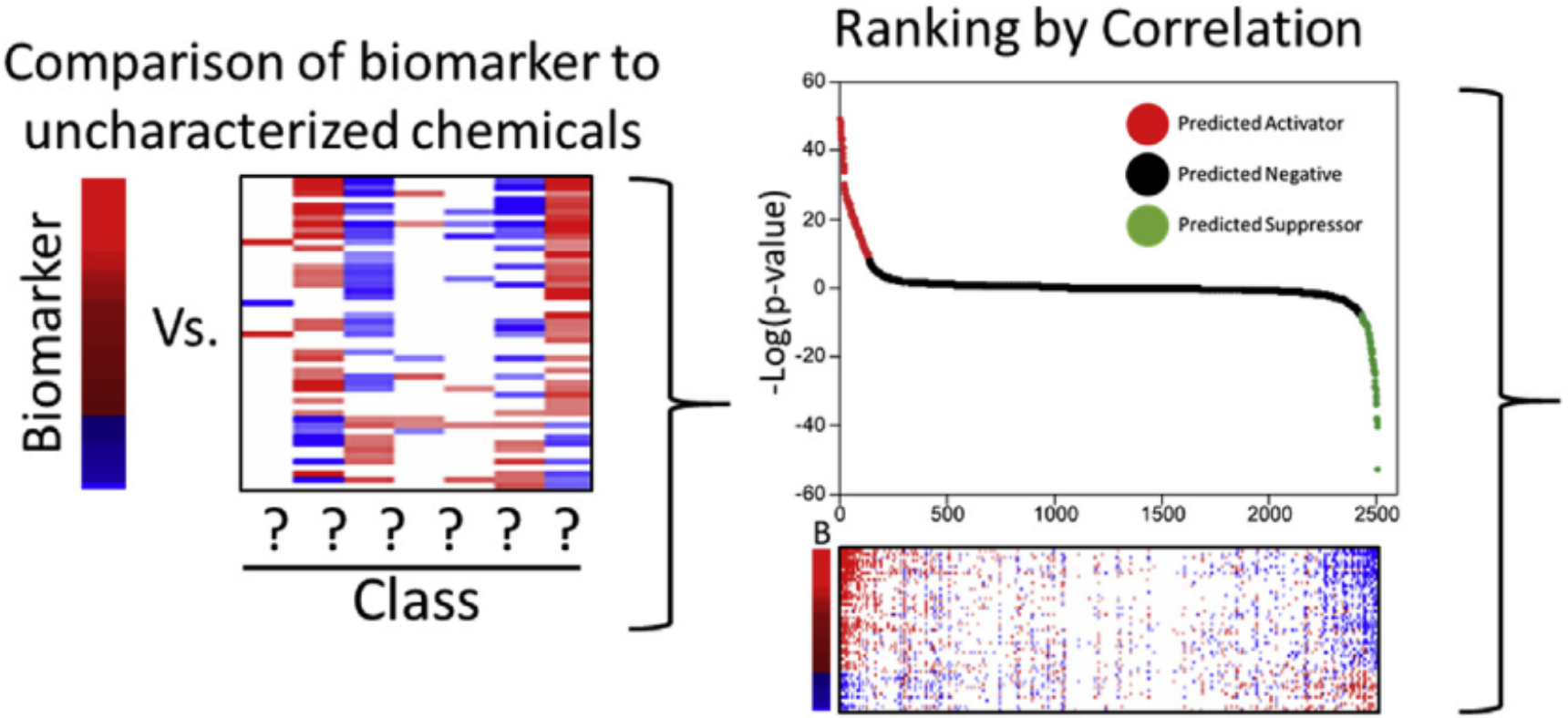
- Sensitivity
- Specificity
- Positive predictive value
- Negative predictive value
- Balanced accuracy

- Defining activation as –Log(p-value) ≥ 4 and suppression as –Log(p-value) ≤ -4

The mouse biomarkers have excellent predictive accuracy

Mouse Biomarker	Number of Genes	Mutant mice used	Predictive Accuracy for Activation	Publication
PPARalpha	131	*Ppara*	98%	PLoS One. 2015 10(2):e0112655
CAR	83	*Nr1i3*	97%	Nucl Recept Signal. 2015 13:e002
AhR	63	*Ahr*	95%	Toxicology. 2015 336:99-112
Nrf2	48	*Nfe2l2, Keap1*	96%	PLoS One 2018 13(8):e0200004
Stat5b	144	*Stat5b*	97%	PLoS One 2016 11(3):e0150284
Srebp	99	*Srebf1a, Srebf1c, Srebf2, Scap*	94%	Comp Tox 10 (2019) 63-77

Use of biomarkers in chemical screening

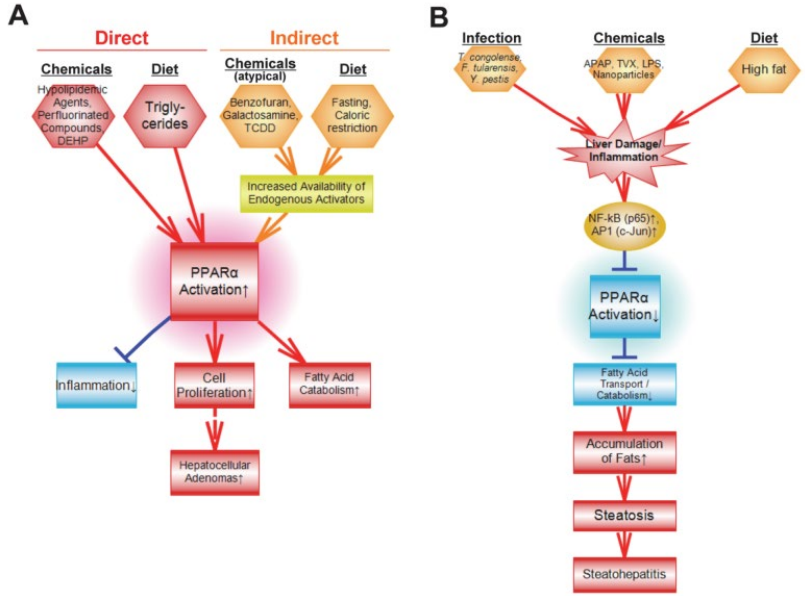


Characterize Hits

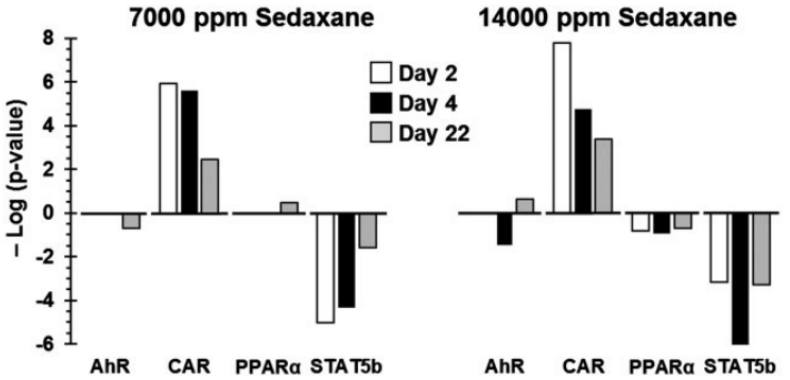
- Confirm positives
- Determine mechanism of modulation

Use of mouse biomarkers for screening

- Expanded and confirmed the factors that modulate PPARα
  - Oshida et al. PLoS One. 2015 10(2):e0112655.



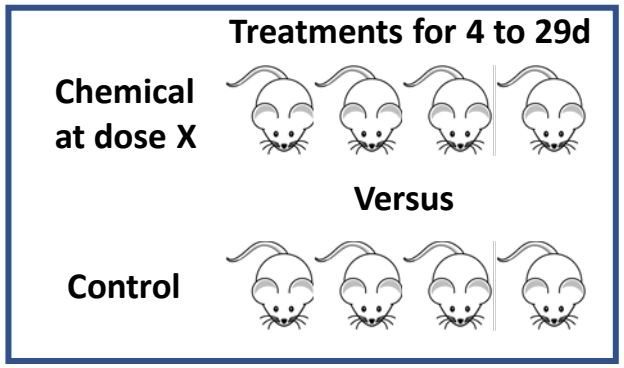
- Predict mode of action of a chemical (sedaxane) that causes mouse liver tumors
  - Peffer et al. Toxicol Sci. 2018 162(2):582-598.



- Database of mouse profiles was limited
  - No opportunity to make predictions of chemical-dose conditions that would lead to induction of cancer

NAM: Prediction of rat liver tumor induction using toxicogenomics analysis of short-term exposures

Would a chemical candidate at dose X cause increases in liver tumors in chronic studies?

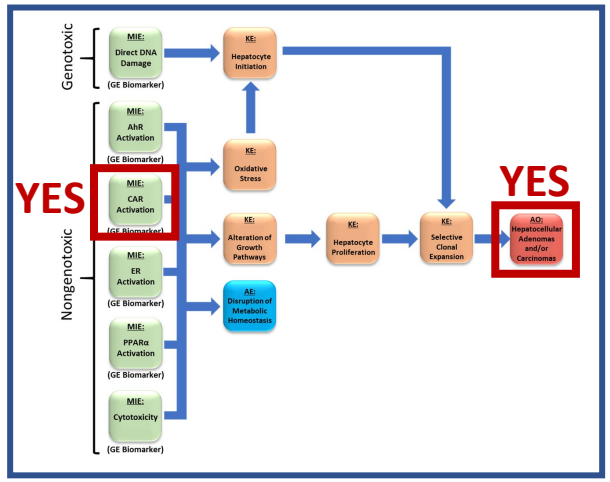


List of genes and fold-changes

Transcript Profiling

NAM Computational Model

Network of Liver Cancer AOPs



- Is the dose tumorigenic?
- Which mode(s) of action is activated?
- Is the mode(s) of action human irrelevant?
- Is a waiver for testing appropriate?

Data Used to Construct the Model

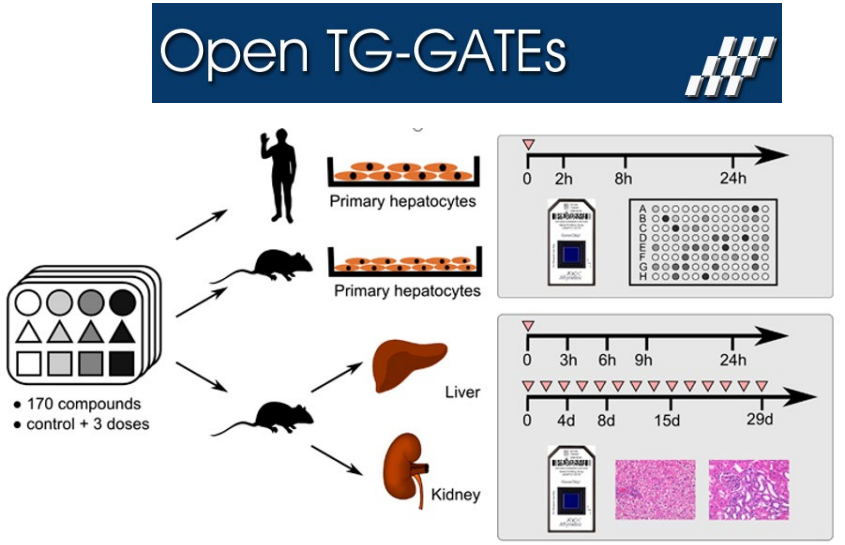
- Microarray data
  - TG-GATES
  - DrugMatrix
- 2-year cancer data
  - Lhasa carcinogenicity database

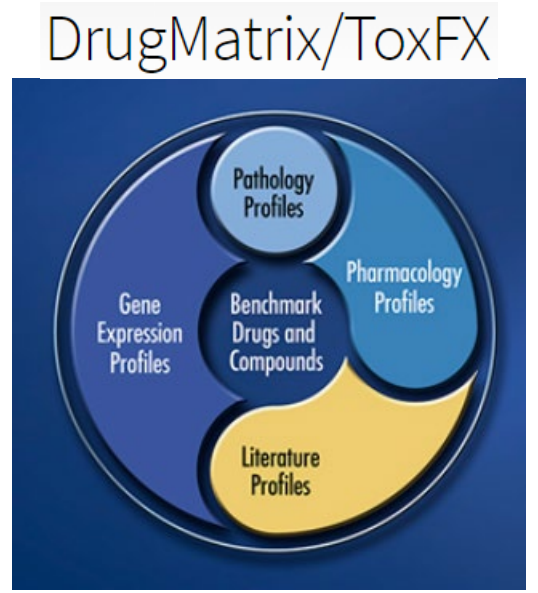
When to use the NAM:

- Screening chemicals in short-term exposures
- After a (sub)chronic study when liver is found to be a tissue with histopath findings of concern

Data Used to Construct the Model

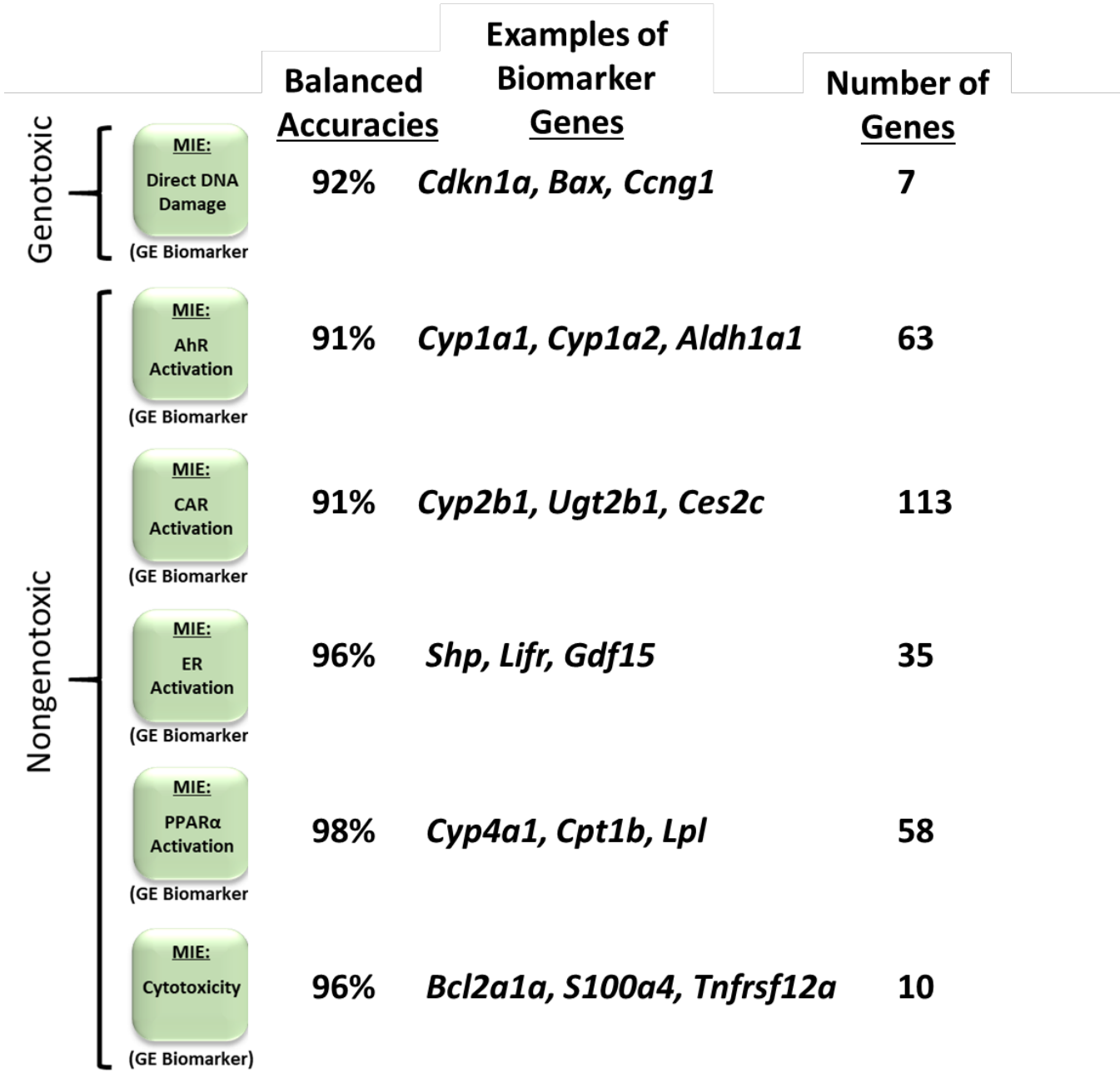
- TG-GATES microarray data
  - ~130 chemicals, 8 time points, 3 doses
- DrugMatrix microarray data
  - >600 chemicals, 4 time points, 2 doses
- Carcinogenicity Potency Database
  - Carcinogenicity data on >1500 chemicals in rats and mice
  - Used data to categorize the hepatotumorigenic potential of chemical-dose comparisons in TG-GATES and DrugMatrix
  - Used the data to identify thresholds for tumorigenicity





Predictive Accuracies of Six Gene Expression Biomarkers

- **Context of use: Male rat liver**
- **All biomarkers have balanced accuracies above 90%**
- **Genes identified are known to be regulated by the MIE**



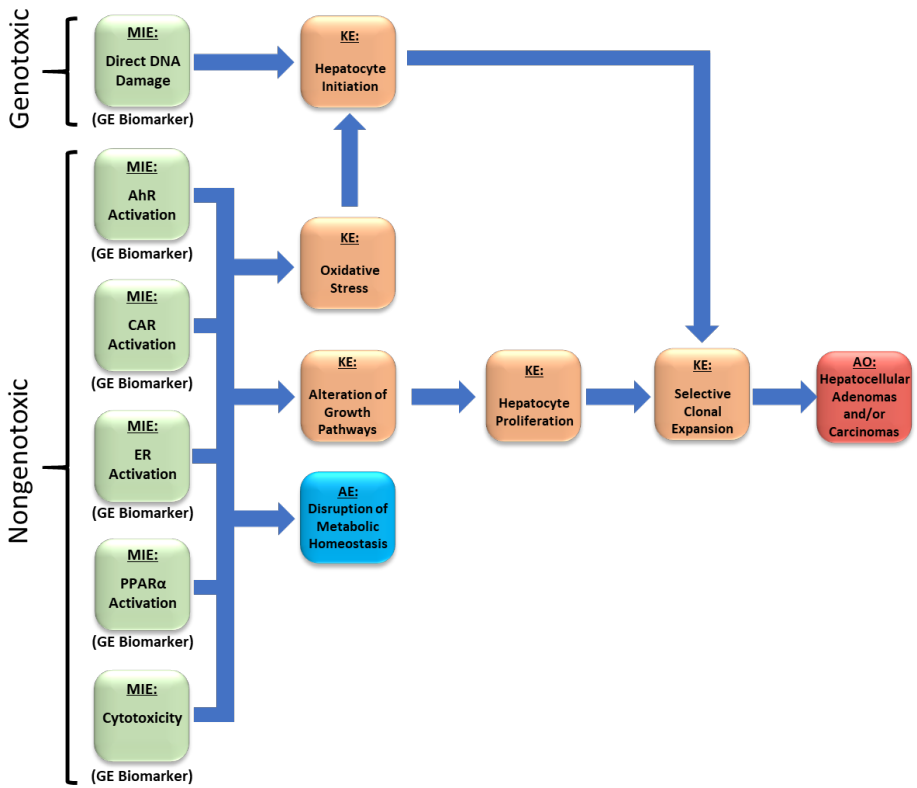
- Rooney et al., (2018) *Tox Appl Pharm* 356:99–113
- Corton et al. (2020). A Set of Gene Expression Biomarkers Identify Rat Liver Tumorigens in Short-Term Assays. *Tox Sci.* 177(1):11-26

Defining biological activation levels for liver cancer

- Central premise of AOP framework: Key events are necessary but not sufficient
  - Induction of an AO depends on the degree or amount of disruption of preceding key events

- Can we define activation levels associated with liver tumor induction for each of the MIEs?

- Defined the tumorigenic activation levels for the 6 biomarkers



http://www.silverdoctors.com

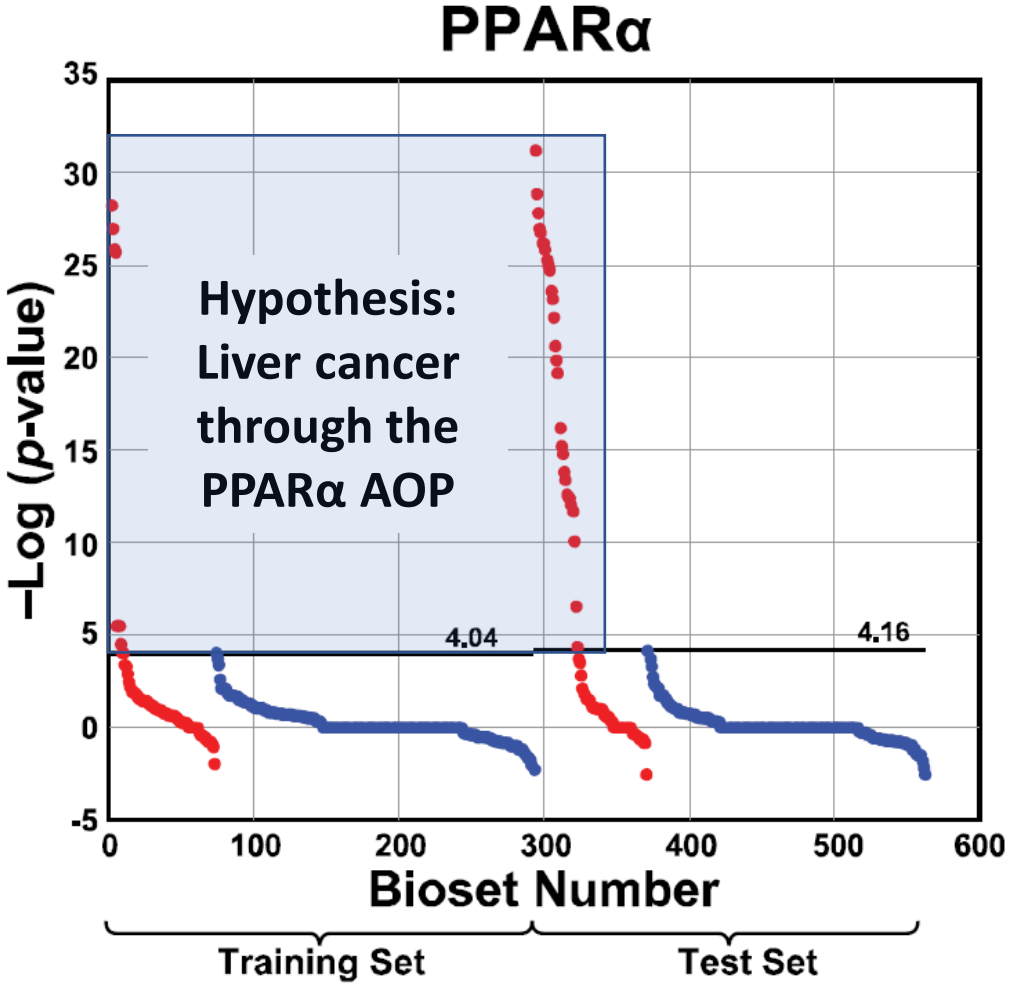
Identification of tumorigenic activation levels for gene expression biomarkers

- Divided the chemical-dose conditions
  - Tumorigenic and nontumorigenic groups
  - Training and test sets

- Thresholds defined as the maximum value in the nontumorigenic group
  - Reach an upper limit for activation that would not cause liver cancer

- Generated tumorigenic activation levels for all 6 MIEs

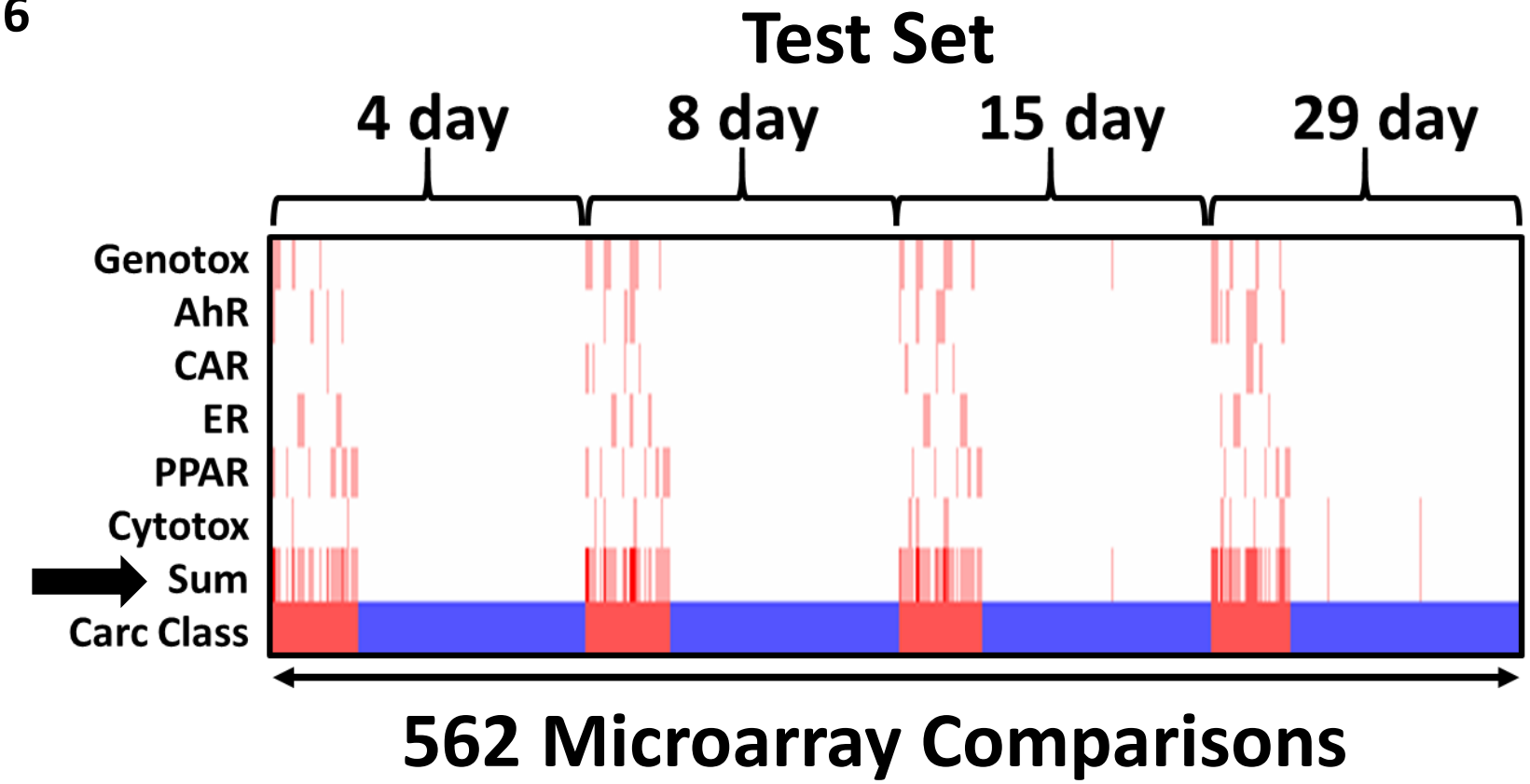
- Levels were similar between the training and test sets



From Hill et al. (2020) ToxSci 177(1):41-59

Biomarker Activation Levels Accurately Predict Liver Tumors

- Identified activation levels for the 6 biomarkers associated with tumor induction from the TG-GATES training set and then applied to a test set
- Each red line is a chem-dose condition in which the biomarker tumorigenic level is surpassed
- Almost all of the tumorigenic conditions exceeded one or more of the 6 activation levels
- Tumorigenic activation levels were rarely exceeded in any of the nontumorigenic conditions

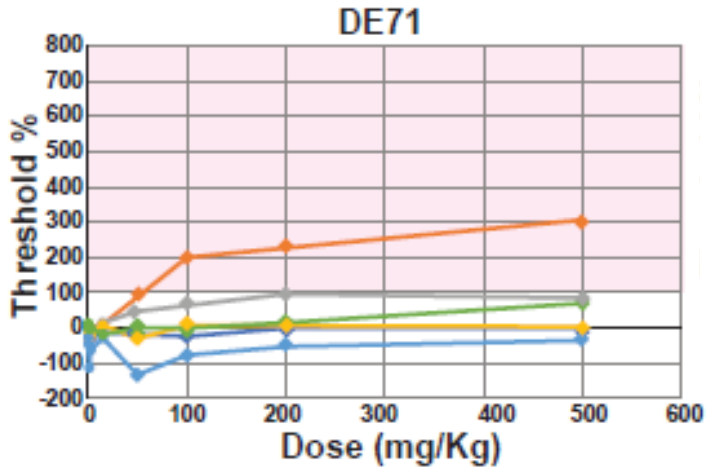


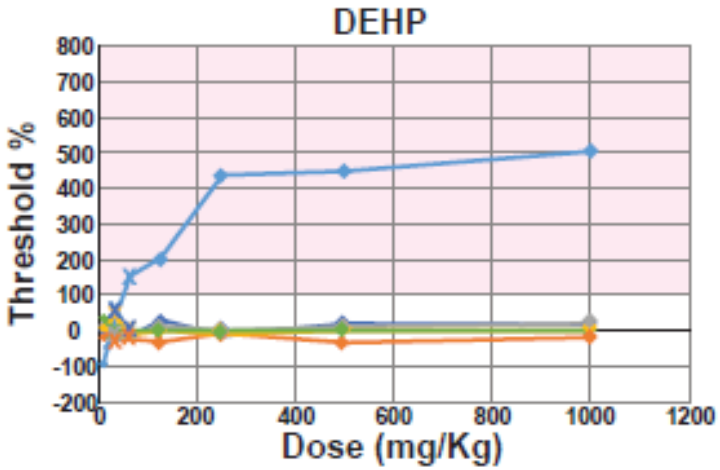
- Test set: 100% sensitivity, 93% specificity, and a balanced accuracy of 97%

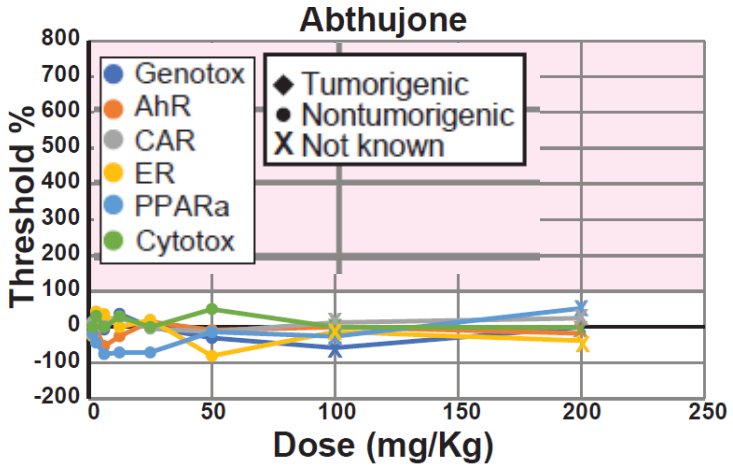
From Hill et al. (2020) ToxSci 177(1):41-59

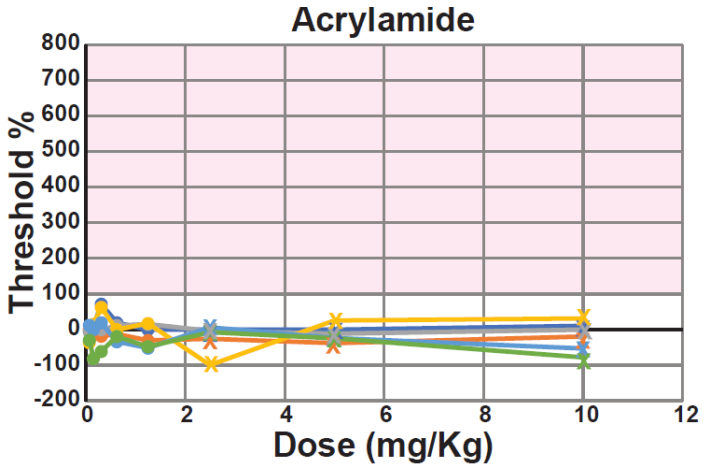
NAM identifies chemical-dose pairs that are tumorigenic in the liver using TempO-Seq

- Examined 16 chemicals at up to 10 doses; 5d exposures (Gwinn et al., 2021 ToxSci)
- Liver gene expression analyzed using full genome TempO-Seq
- Model correctly identified all tumorigenic chemicals
- Balanced accuracies = 74-91% depending on the tumorigenic activation level used and whether individual chem-doses were considered or all doses for a chemical





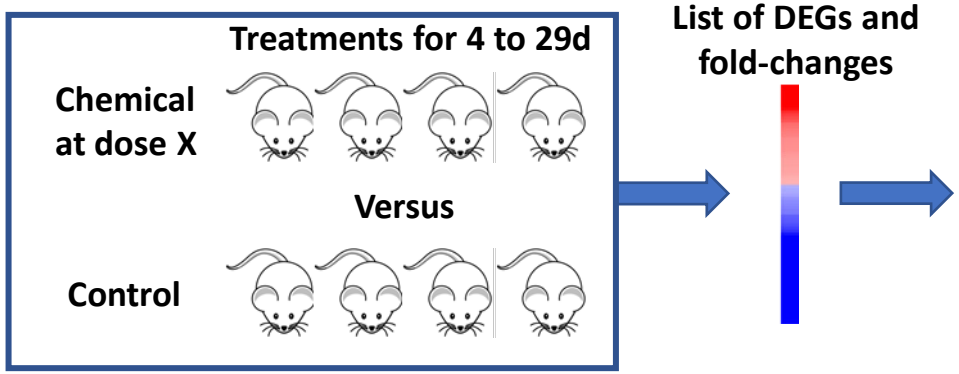




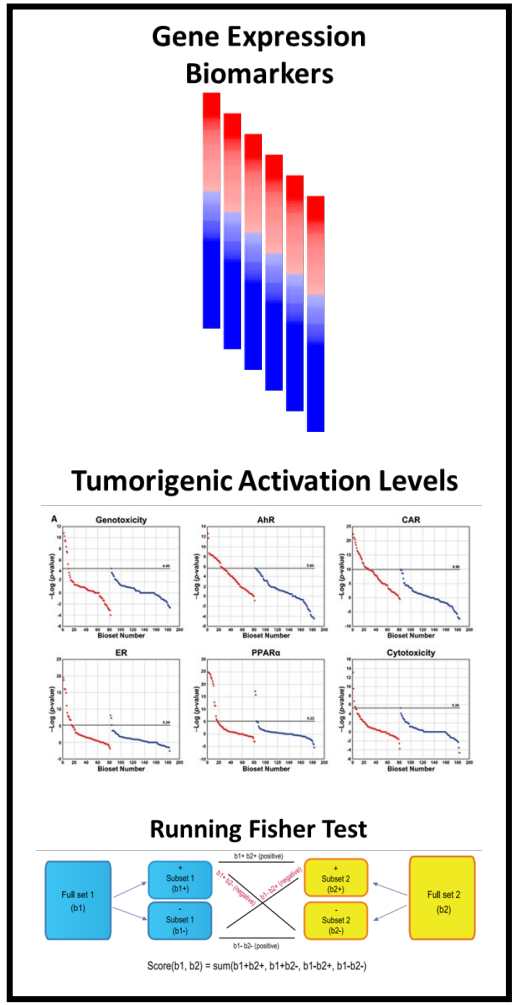
Ledbetter et al., submitted to management

NAM: Prediction of rat liver tumor induction using toxicogenomics analysis of short-term exposures

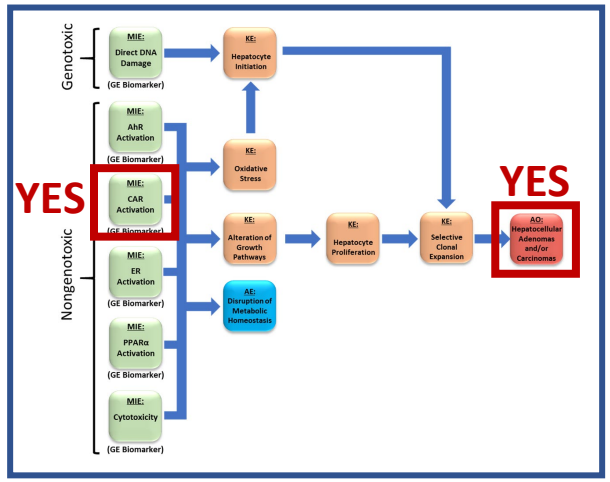
Will a chemical candidate at dose X cause increases in liver tumors in chronic studies?



NAM Computational Model



Network of Liver Cancer AOPs



- Is the dose tumorigenic?
- Which mode of action is activated?
- Is the mode of action human irrelevant?
- Is a waiver for testing appropriate?

Questions still to be addressed:

- Can we improve accuracy by incorporating
  - More data?
  - A greater diversity of chemicals?
  - Wild-type and null rat comparisons?

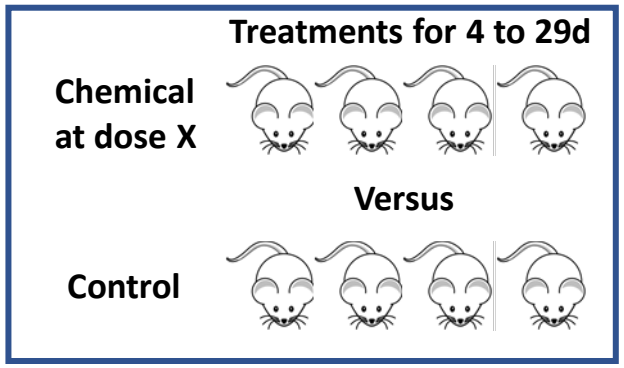
Future Studies:

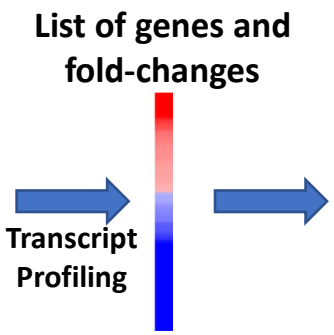
- Studies conducted through the HESI eSTAR Carcinogenomics Workgroup

Emerging Systems Toxicology for the Assessment of Risk (eSTAR) Committee

NAM: Prediction of rat liver tumor induction using toxicogenomics analysis of short-term exposures

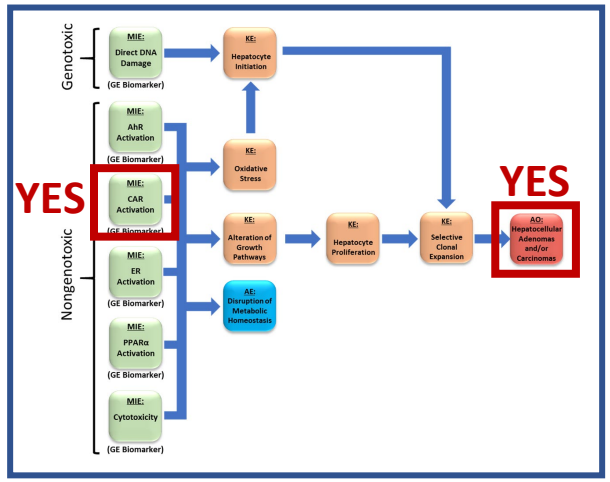
Will a chemical candidate at dose X cause increases in liver tumors in chronic studies?





NAM Computational Model

Network of Liver Cancer AOPs



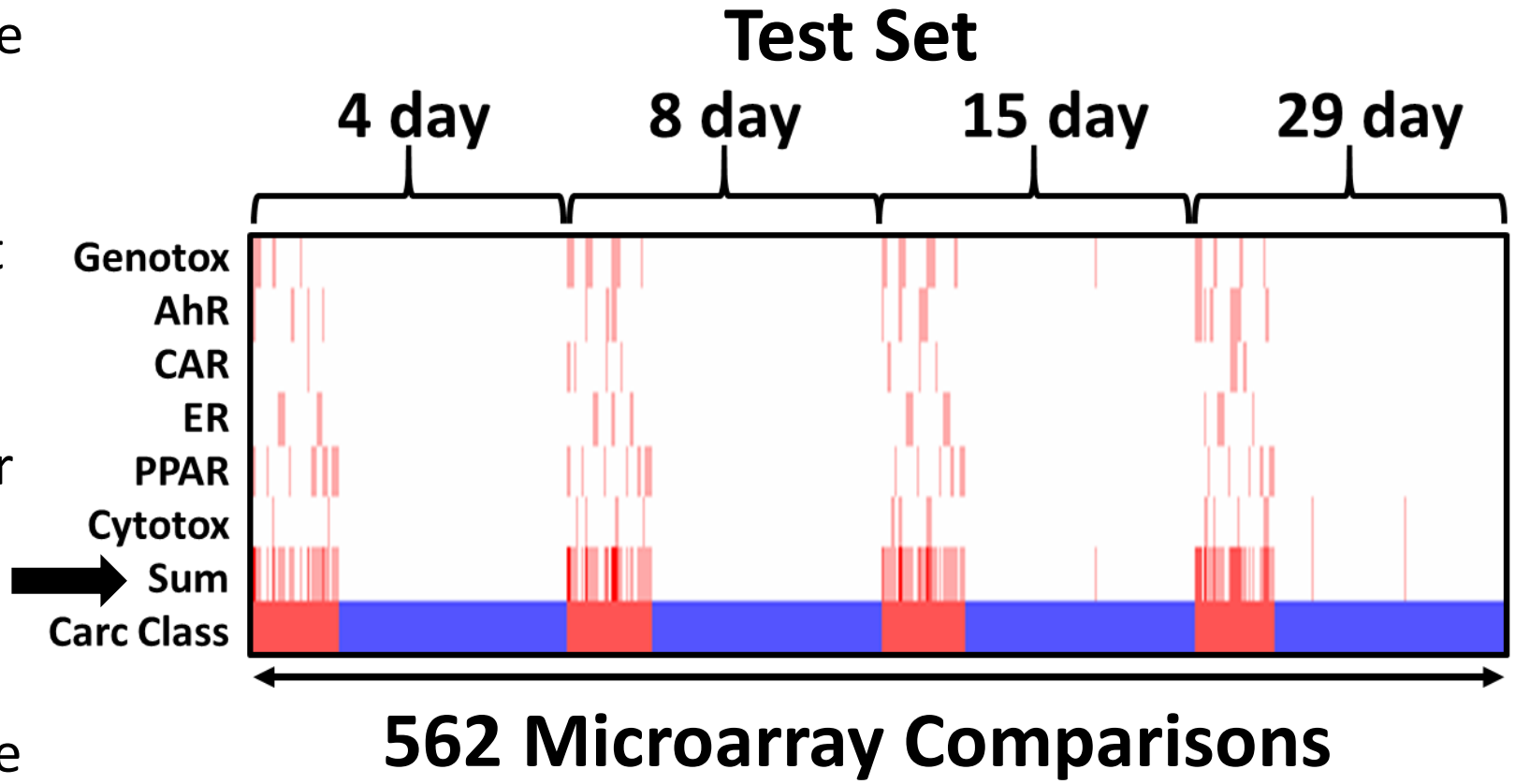
- Is the dose tumorigenic?
- Which mode(s) of action is activated?
- Is the mode(s) of action human irrelevant?
- Is a waiver for testing appropriate?

<u>When to use the NAM:</u>

- Screening chemicals in short-term exposures
- After a chronic study when liver is found to be a tissue with histopath findings of concern

Biomarker Activation Levels Accurately Predict Liver Tumors

- Identified activation levels for the 6 biomarkers associated with tumor induction from a training set and then applied to a test set
- Each red line is a chem-dose condition in which the biomarker tumorigenic level is surpassed
- Almost all of the tumorigenic conditions exceeded one or more of the 6 activation levels
- Tumorigenic activation levels rarely exceeded in any of the nontumorigenic conditions

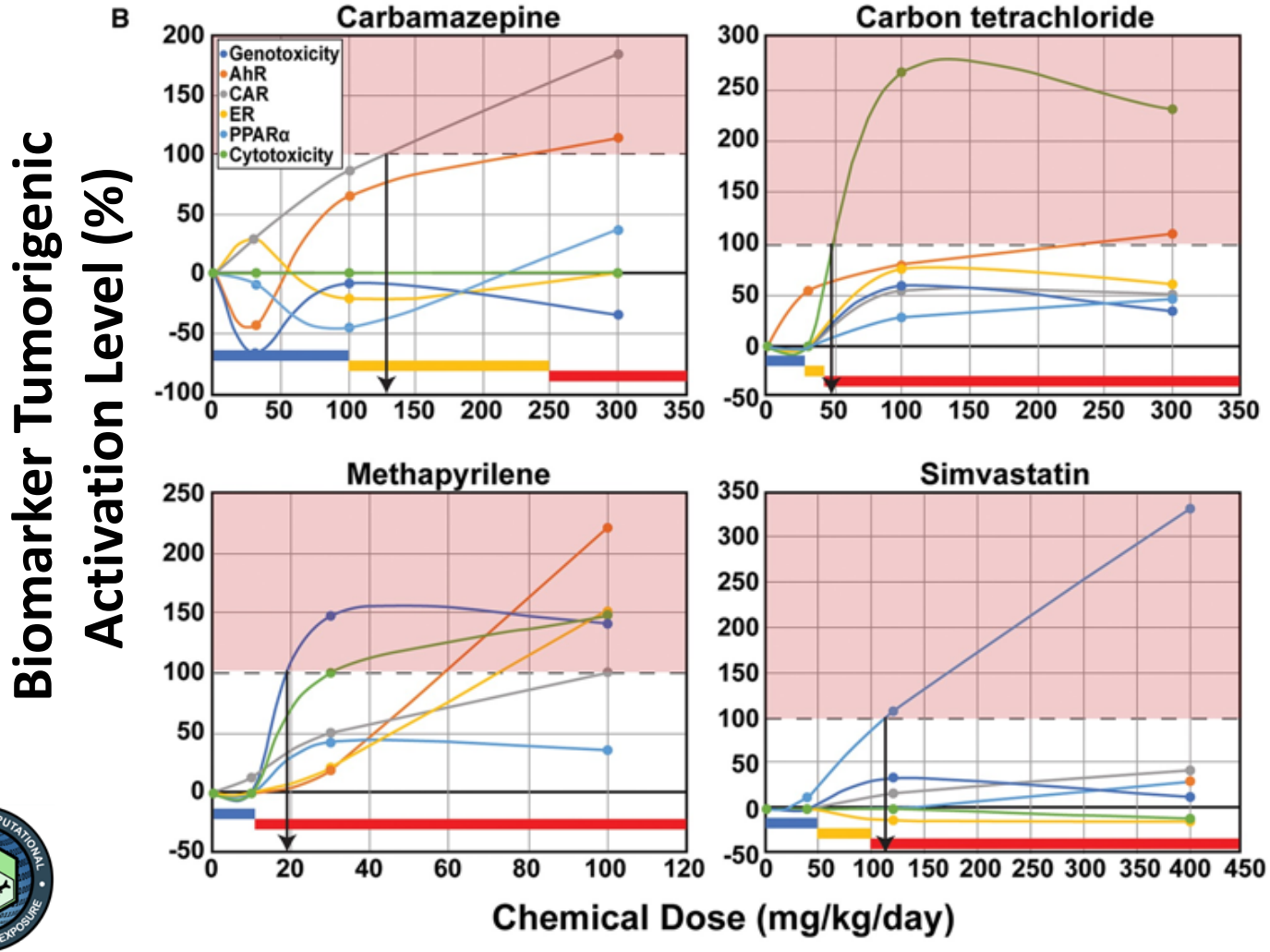


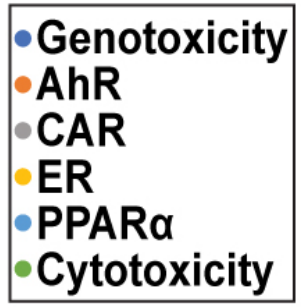
- **<u>Test set</u>: 100% sensitivity, 93% specificity, and a balanced accuracy of 97%**

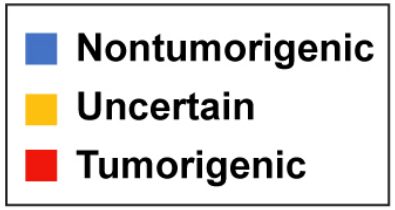
From Hill et al. (2020) ToxSci 177(1):41-59

Application of Biomarkers and Activation Levels to Liver Tumorigens

- Chemicals examined in the TG-GATES study in male rats for 15d at 3 doses







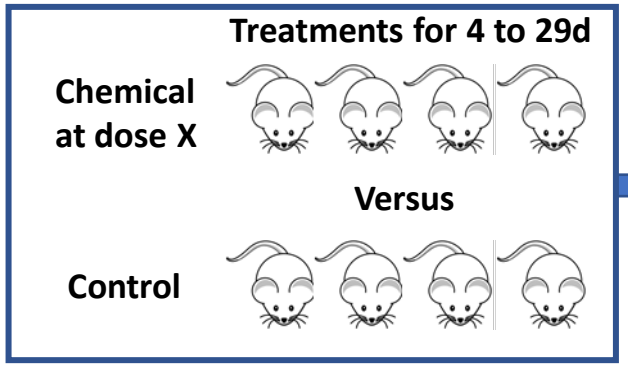
Pink = conditions predicted to be tumorigenic

- Approach identifies the MOA and the lowest tumorigenic dose
- Confidence would increase with greater numbers of doses examined

From Hill et al. (2020) ToxSci 177(1):41-59

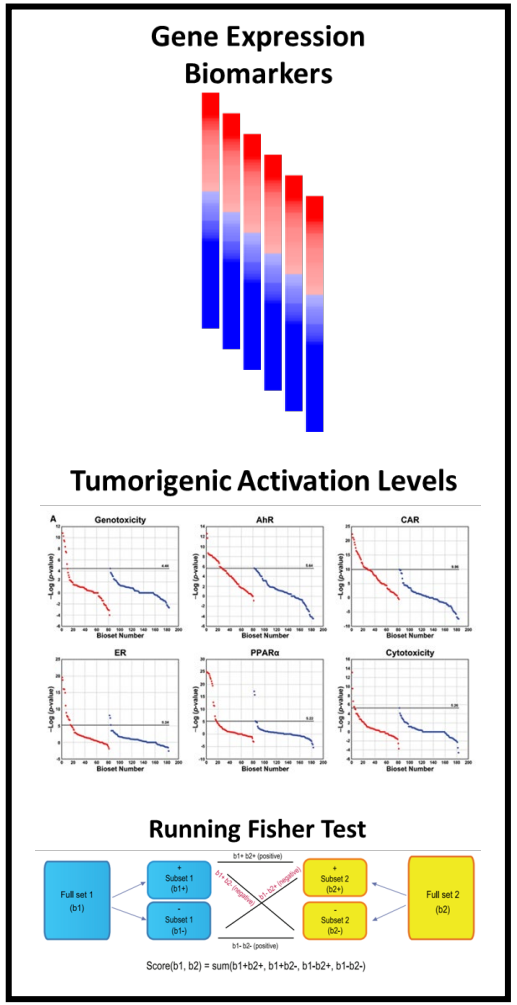
NAM: Prediction of rat liver tumor induction using toxicogenomics analysis of short-term exposures

Will a chemical candidate at dose X cause increases in liver tumors in chronic studies?

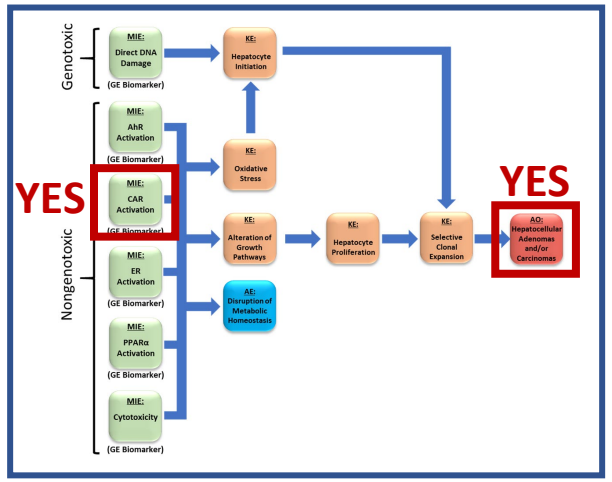


List of DEGs and fold-changes

NAM Computational Model



Network of Liver Cancer AOPs



- Is the dose tumorigenic?
- Which mode of action is activated?
- Is the mode of action human irrelevant?
- Is a waiver for testing appropriate?

Questions still to be addressed:

- Can the methods be used for (targeted) RNA-Seq?
- Can we make predictions using in vitro models?

Future Studies:

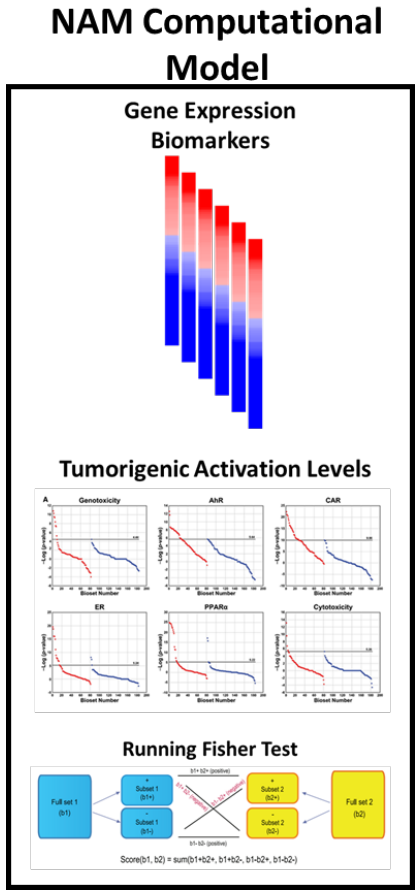
- Studies conducted through the HESI eSTAR Carcinogenomics Workgroup

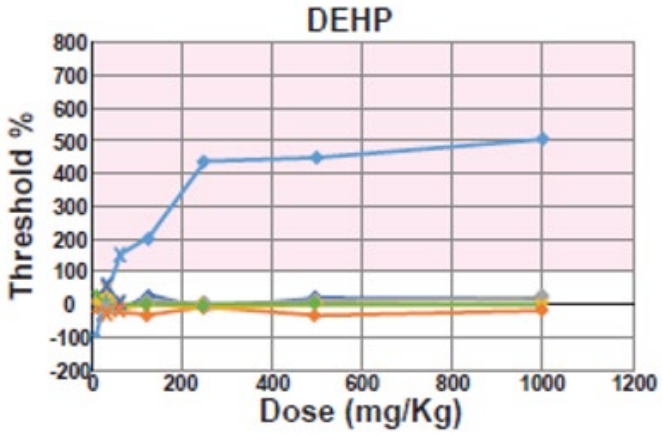
Emerging Systems Toxicology for the Assessment of Risk (eSTAR) Committee

Summary (First Part)

- The NAM can be used to identify liver tumorigens
  - Identification of mode of action
  - Identification of chemical doses that would cause cancer

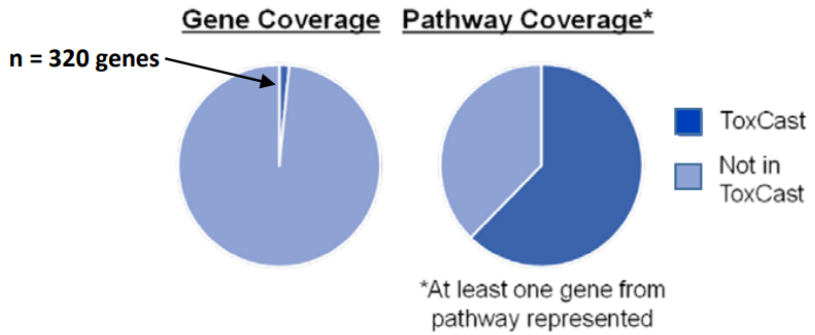
- In multiple studies have examined ~250 chemicals (~50 caused liver tumors)
  - Accuracy was ~75-95% depending on the dataset used
  - Accuracy is independent of platform used to assess gene expression
  - Missed only two positives
    - Acetamide
    - Ethionine
  - Provides opportunities to build additional biomarkers for prediction





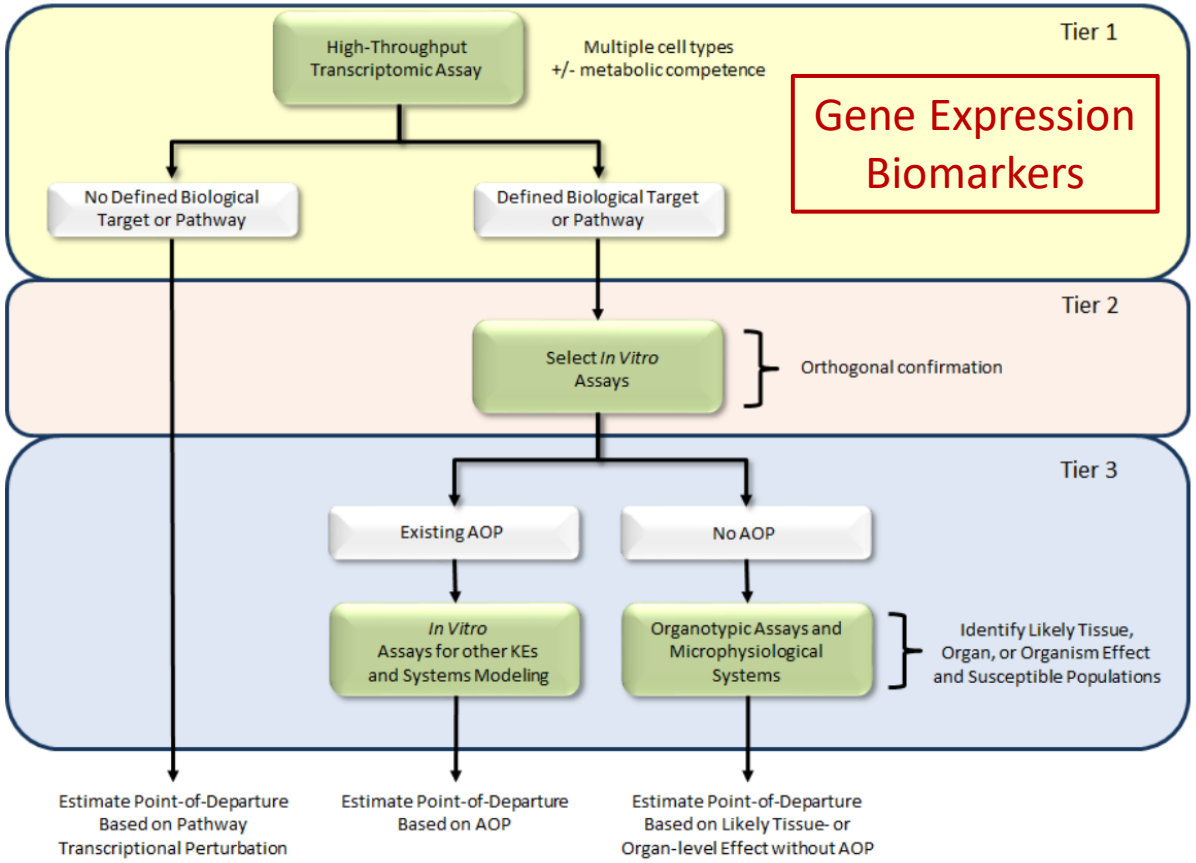
High-throughput toxicity testing

- ToxCast assays cover many genes and pathways, but do not provide complete coverage of biological space.



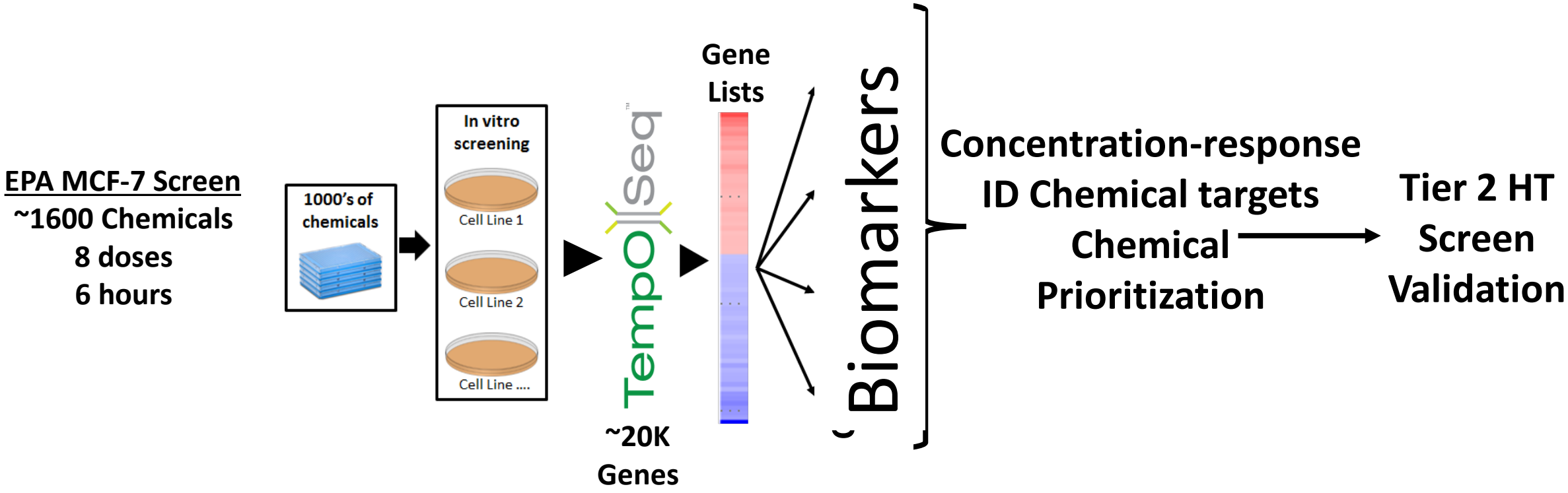
- **USEPA Strategic Vision and Operational Roadmap:**
  - Tier 1 strategy must cast the broadest net possible for capturing hazards associated with chemical exposure.
  - Global gene expression provides a robust and comprehensive evaluation of chemically induced changes in biological processes.
  - Increasing efficiency and declining cost of generating whole transcriptome profiles has made high-throughput transcriptomics (HTTr) a practical option for determining bioactivity thresholds in *in vitro* models.

A strategic vision and operational road map for computational toxicology at the U.S. Environmental Protection Agency



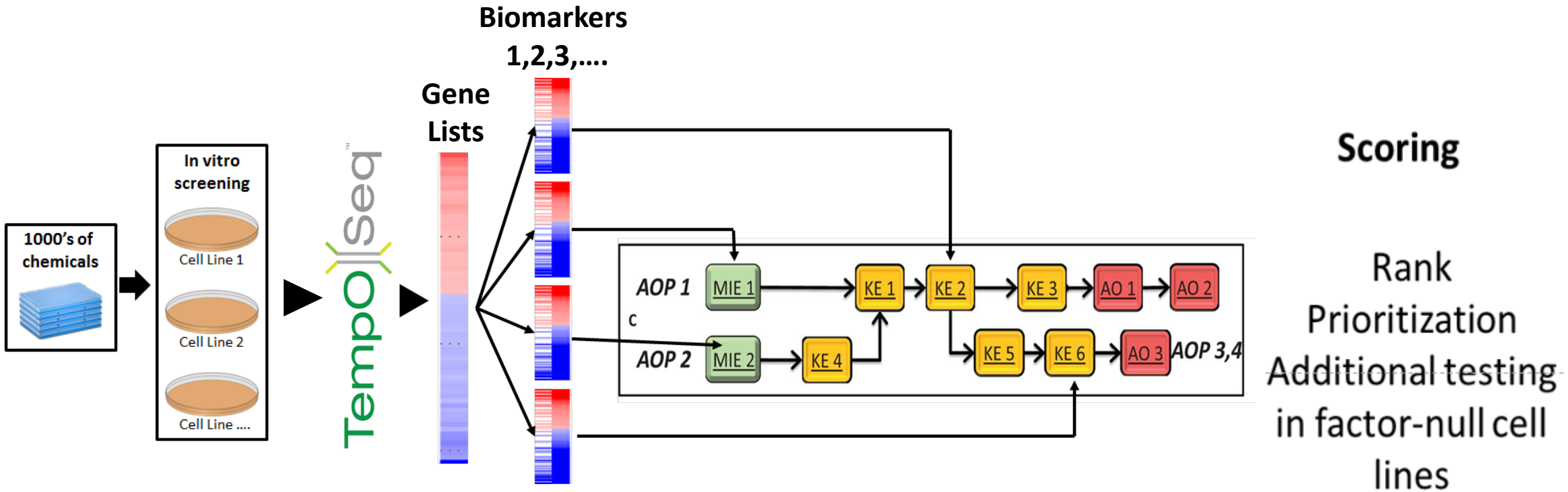
Joshua Harrill, EPA

Using gene expression biomarkers to identify molecular targets of chemicals in transcriptomic studies



- Use predictions for
  - Chemical prioritization as part of Tier 1 screening
- Followed up with short-term tests in organotypic cultures or animals

Using gene expression biomarkers to identify molecular targets of chemicals in transcriptomic studies



- Use predictions for
  - Chemical prioritization as part of Tier 1 screening
  - Predict molecular initiating events and key event perturbations in adverse outcome pathways
- Followed up with short-term tests in knockout/knockdown cell lines, organotypic cultures or animals
- **Ultimate Goal: Move from hypothesis generation to final predictions to minimize further testing**

Biomarkers that predict key events in human cells in vitro

Used genetic profiles to develop the biomarker

Endocrine disruption

- Ryan et al. (2016). Moving Toward Integrating Gene Expression Profiling Into High-Throughput Testing: A Gene Expression Biomarker Accurately Predicts Estrogen Receptor α Modulation in a Microarray Compendium. Toxicol Sci. 151(1):88-103.
- Androgen receptor: Rooney et al. (2018). Identification of Androgen Receptor Modulators in a Prostate Cancer Cell Line Microarray Compendium. Toxicol Sci. 166:146-162.
- Robarts et al. (2023). Characterization of a 50-gene estrogen receptor biomarker. In preparation.

DNA Damage Response – TGx-DDI Biomarker

- Corton et al. (2018). Using a gene expression biomarker to identify DNA damage-inducing agents in microarray profiles. Environ Mol Mutagen. 59:772-784.
- Cho et al. (2019). Assessment of the performance of the TGx-DDI biomarker to detect DNA damage-inducing agents using quantitative RT-PCR in TK6 cells. Environ Mol Mutagen. 60:122-133.
- Corton JC, Witt KL, Yauk CL. (2019). Identification of p53 Activators in a Human Microarray Compendium. Chem Res Toxicol. 32(9):1748-1759.

Epigenetic effects – HDACi and BRDi

- Corton et al. A Gene Expression Biomarker Identifies Inhibitors of Two Classes of Epigenome Effectors in a Human Microarray Compendium. Chemico-Biological Interactions. 365:110032.

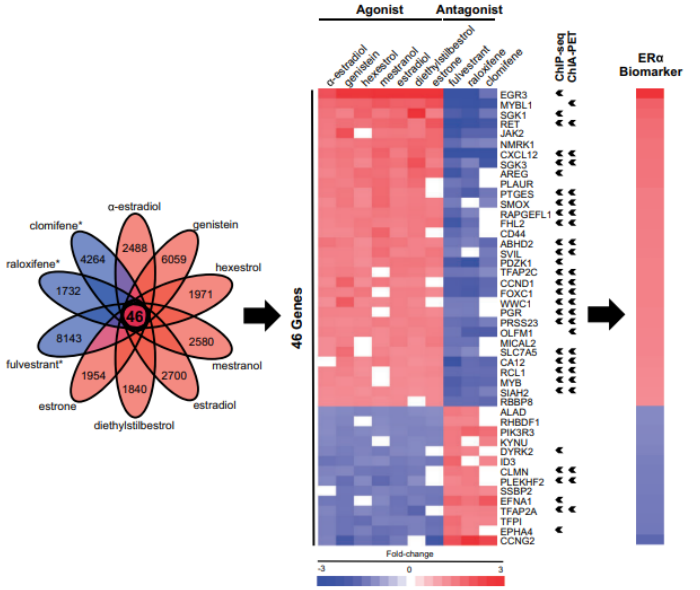
Stress factors

- Cervantes PW, Corton JC. (2021). A Gene Expression Biomarker Predicts Heat Shock Factor 1 Activation in a Gene Expression Compendium. Chem Res Toxicol. 2021 34(7):1721-1737.
- Jackson AC, Liu J, Vallanat B, Jones C, Nelms MD, Patlewicz G, Corton JC. (2020). Identification of novel activators of the metal responsive transcription factor (MTF-1) using a gene expression biomarker in a microarray compendium. Metallomics. 12(9):1400-1415.
- Korunes KL, Liu J, Huang R, Xia M, Houck KA, Corton JC. (2022). A gene expression biomarker for predictive toxicology to identify chemical modulators of NF-κB. PLoS One. 17(2):e0261854.
- Rooney JP, Chorley B, Hiemstra S, Wink S, Wang X, Bell DA, van de Water B, Corton JC. (2020). Mining a human transcriptome database for chemical modulators of NRF2. PLoS One. 15(9):e0239367.

In progress

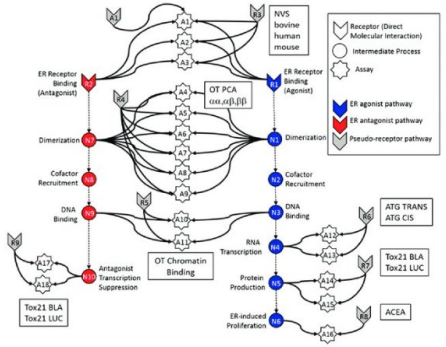
- HIF1a, Unfolded Protein Response (ATF4, ATF6, XBP1), Cell Proliferation, AhR, Epigenome Effectors

Use of an estrogen receptor biomarker to identify ER modulators in human cells in vitro

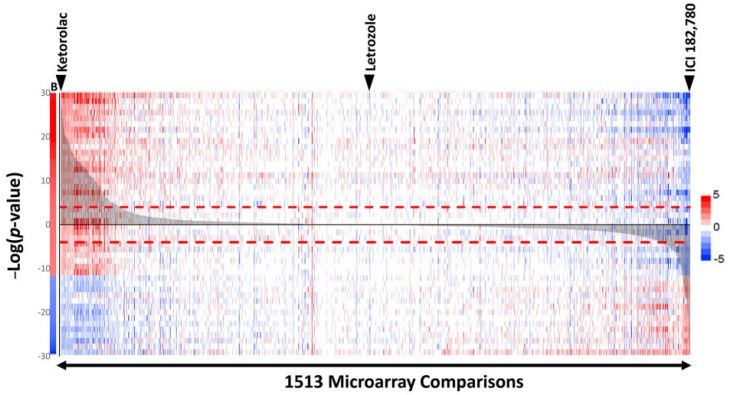


- Used 7 agonists and 3 antagonist to identify predictive genes
- Used profiles generated in MCF-7 cells
- 46 gene biomarker

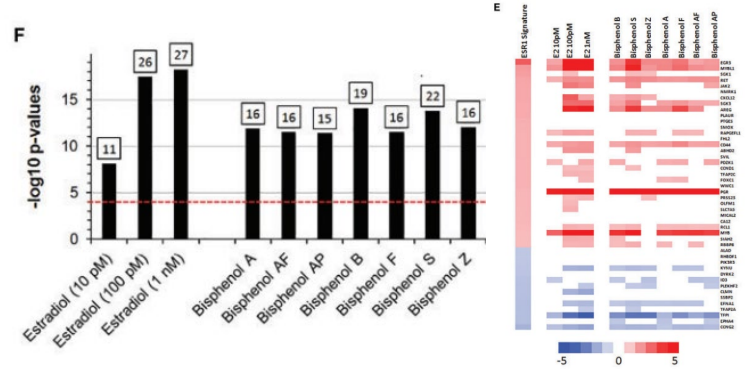
- Ryan et al., 2016 ToxSci



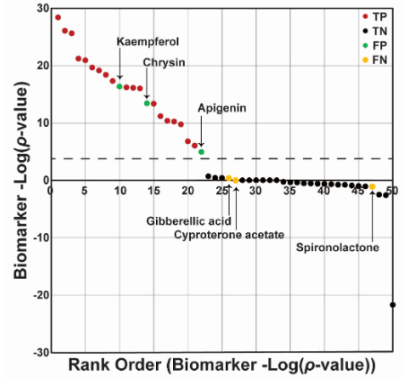
- Accurately replicates the predictions of the ToxCast ER Model based on 18 HTS assays (Ryan et al. Toxicol Sci. 2016 151(1):88-103)



- Used in screening in an MCF-7 compendium (Rooney et al. Chem Res Toxicol. 2021 34(2):313-329)

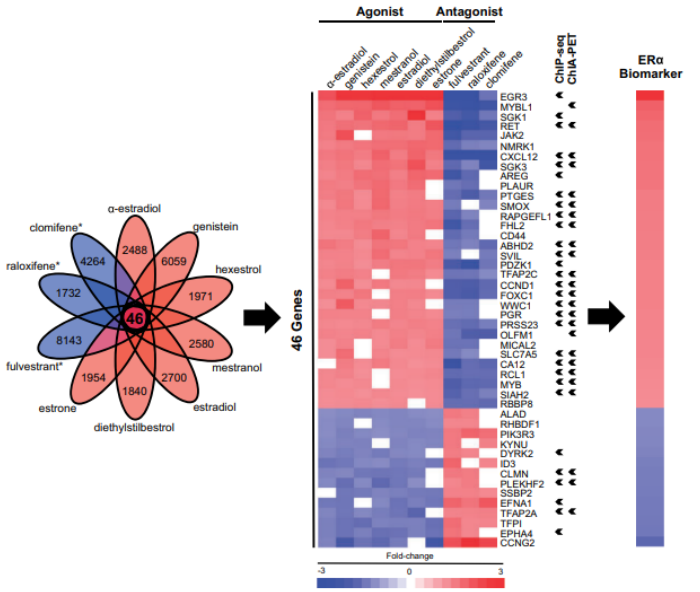


- Used to identify BPA alternatives with estrogenic activity (Mesnage et al. Toxicol Sci. 2017 158(2):431-443)



- Methods could be used to identify positives in the rodent uterotrophic assay (Corton et al., Chem Biol Interact. 2022 363:109995)

Use of an estrogen receptor biomarker to identify ER modulators in human cells in vitro



- Used 7 agonists and 3 antagonist to identify predictive genes
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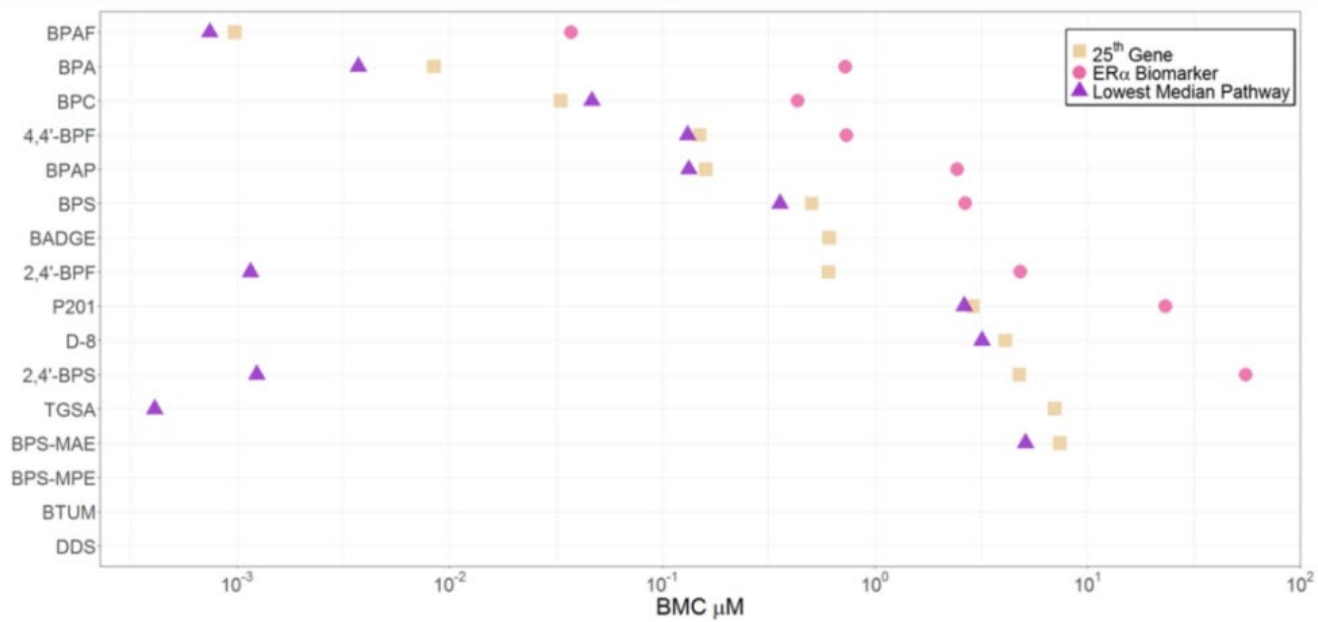
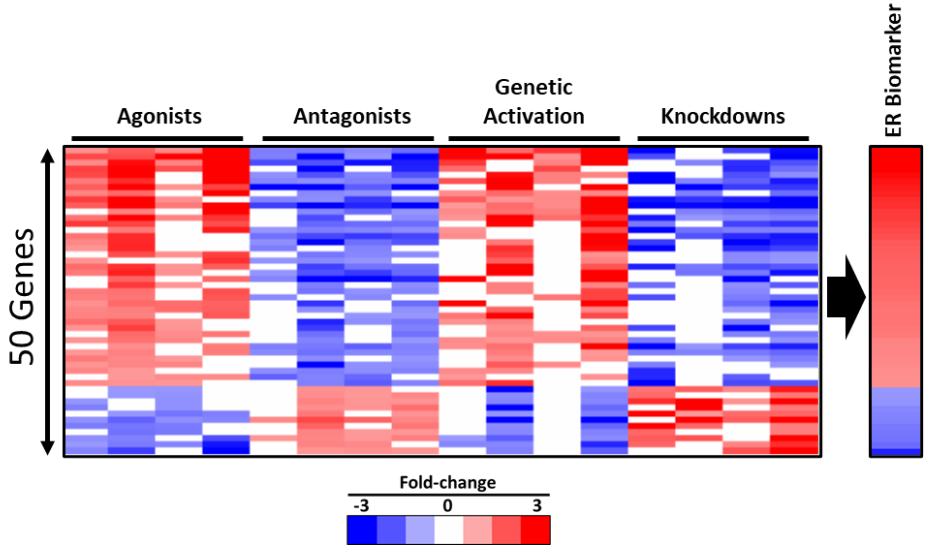
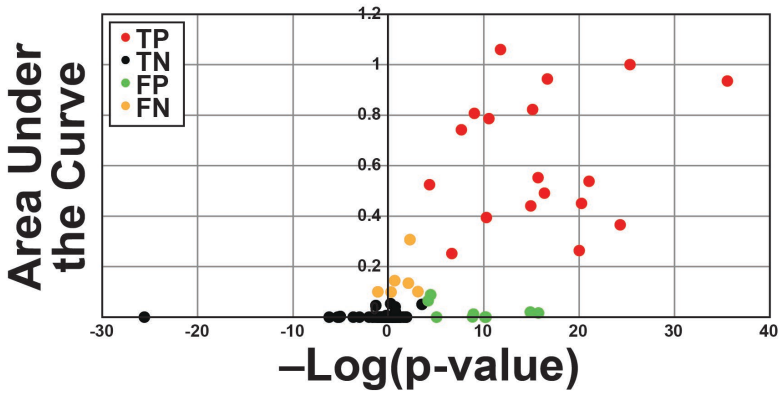


- Used the ER biomarker to derive potencies for data-poor BPA alternatives (Matteo et al., ToxSci. 2023. In press.

Use of an estrogen receptor biomarker to identify ER modulators by high-throughput transcriptomics (HTTr) screening

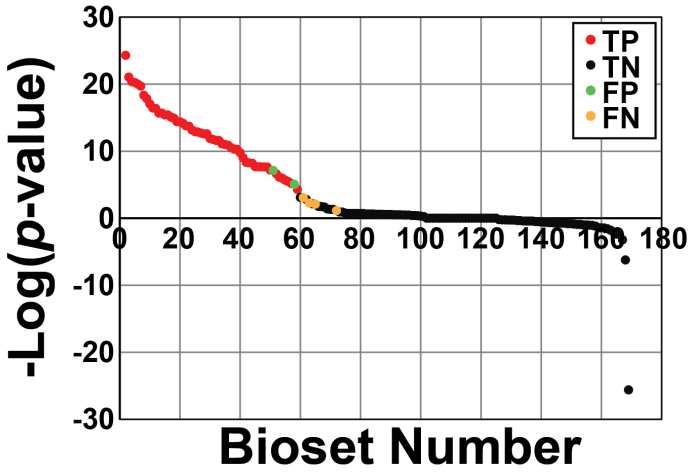


50-gene biomarker built from profiles of
- 4 ER agonists
- 4 ER antagonists
- 4 constitutively active ER mutants
- 4 knockdowns of *ESR1* expression



Using the ToxCast ER model as the reference data set:
- Sensitivity = 75%
- Specificity = 90%
- Balanced accuracy = 82%

- Replicates the predictions of the ToxCast ER Model based on 18 HTS assays

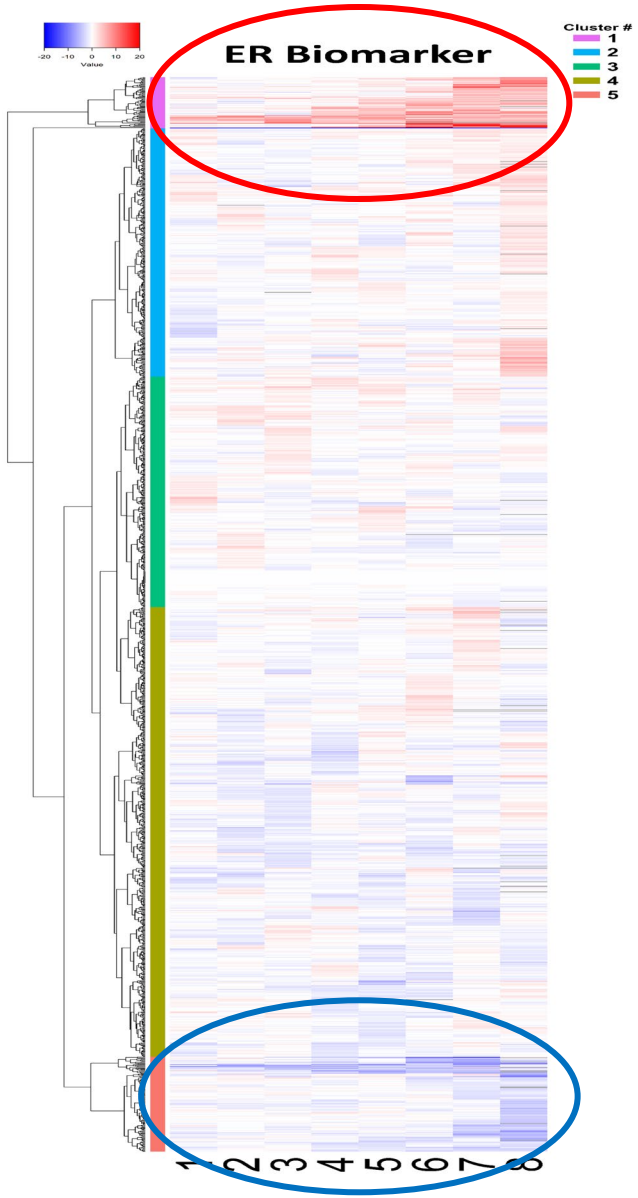


Using the NCATS Tox21 ER trans-activation assays as the reference data set:
- Sensitivity = 93%
- Specificity = 98%
- Balanced accuracy = 96%

- Excellent predictive accuracy with HTTr TempO-Seq data (Robarts et al., in prep)

Identification of ER modulators using an estrogen receptor biomarker in MCF-7 cells

- Examined transcript changes in MCF-7 cells treated with ~1600 chemicals at 8 concentrations (~12,800 comparisons)
- Compared the profiles to the 50-gene estrogen receptor (ER) biomarker
- 2D hierarchical clustering of chemicals across 8 concentrations

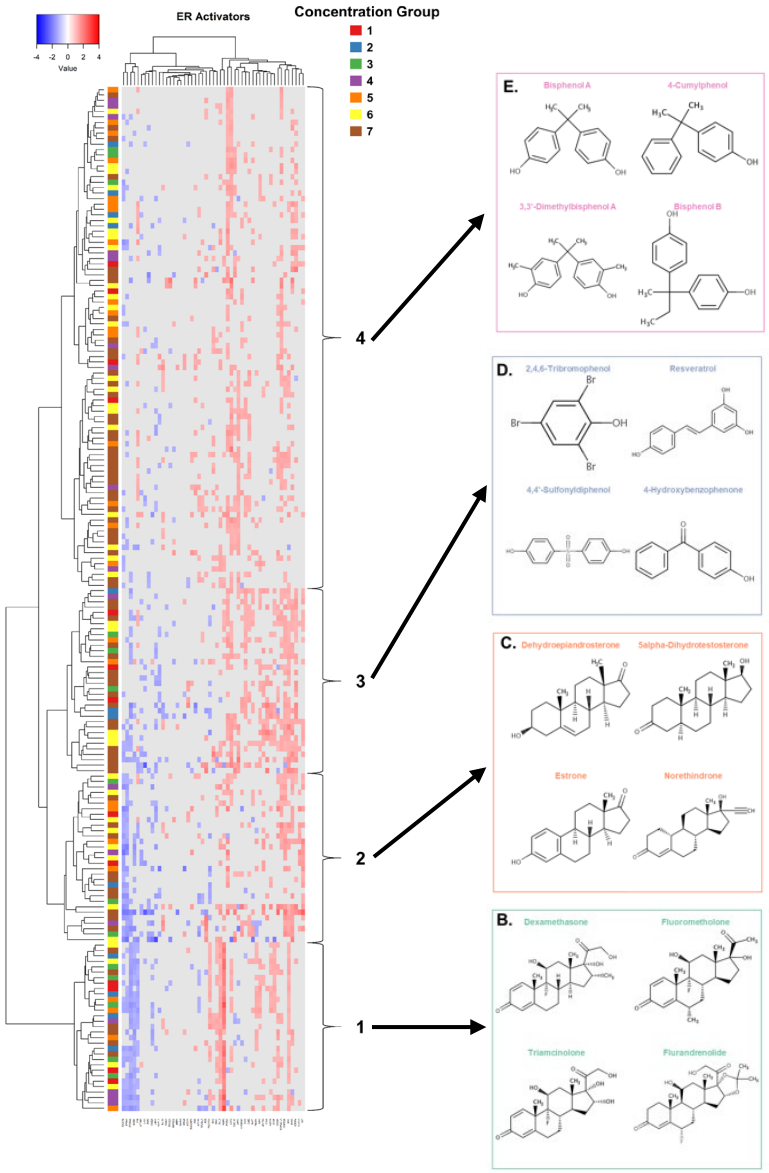


ER Activators

ER Suppressors

ER activators regulate ER biomarker genes in a structure-dependent manner

- **Examined transcript changes in MCF-7 cells treated with ~1600 chemicals at 8 concentrations (~12,800 comparisons)**
- **Compared the profiles to the 50-gene estrogen receptor (ER) biomarker**
- **2D hierarchical clustering of ~120 chem-concentration pairs that activated ER**

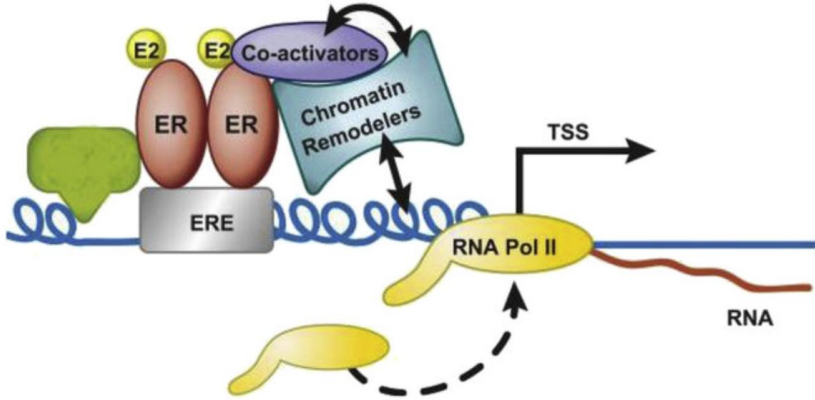


Bisphenols

Misc activators

Classical estrogens

GR and PR agonists



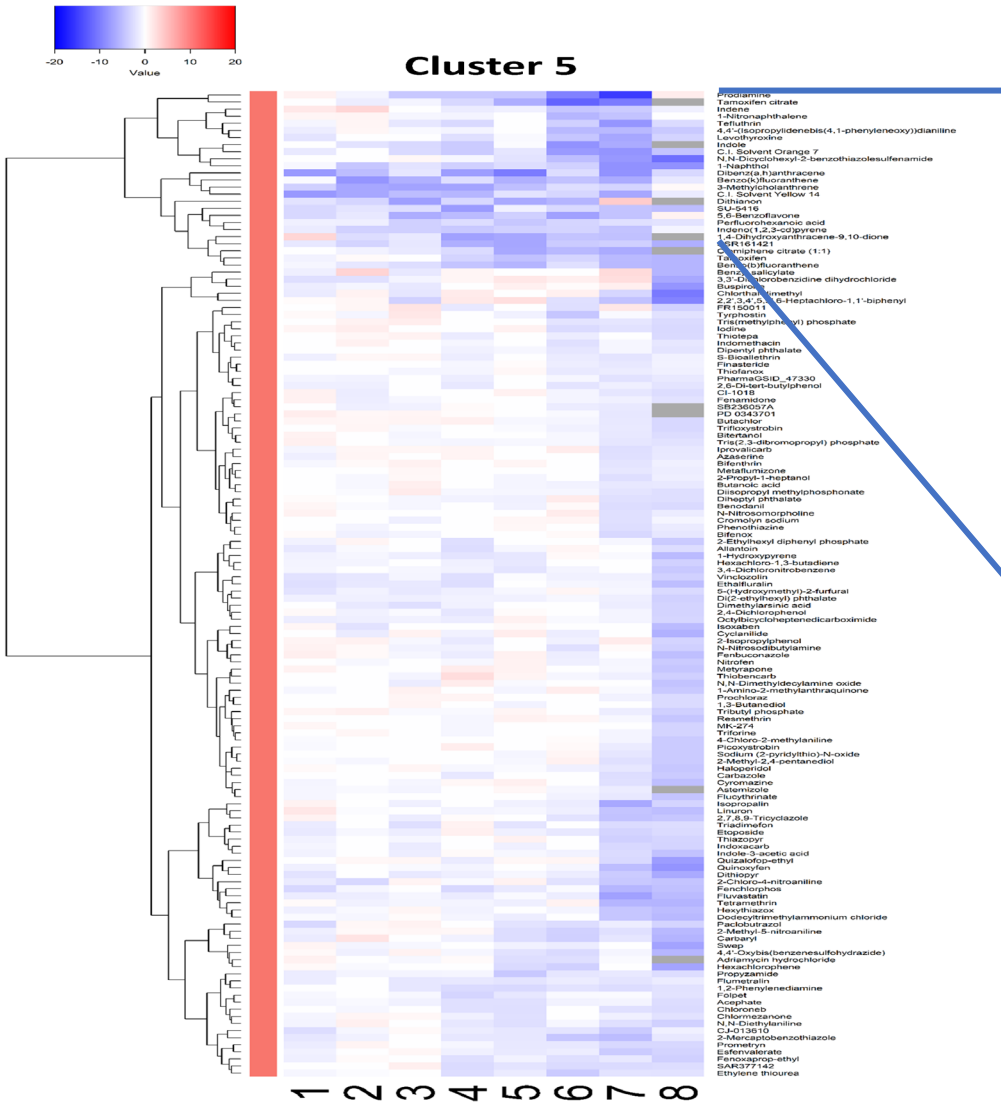
Results consistent with

- Agonists induce different conformations of the receptor
- ER conformation determines which co-activators interact
- ER-co-activator complexes determine which genes are activated

Robarts et al., in preparation

Many ER suppressors appear to be AhR activators

- Examined transcript changes in MCF-7 cells treated with ~1600 chemicals at 8 concentrations (~12,800 comparisons)
- Compared the profiles to the 50-gene estrogen receptor (ER) biomarker
- 2D hierarchical clustering of chemicals across 8 concentrations



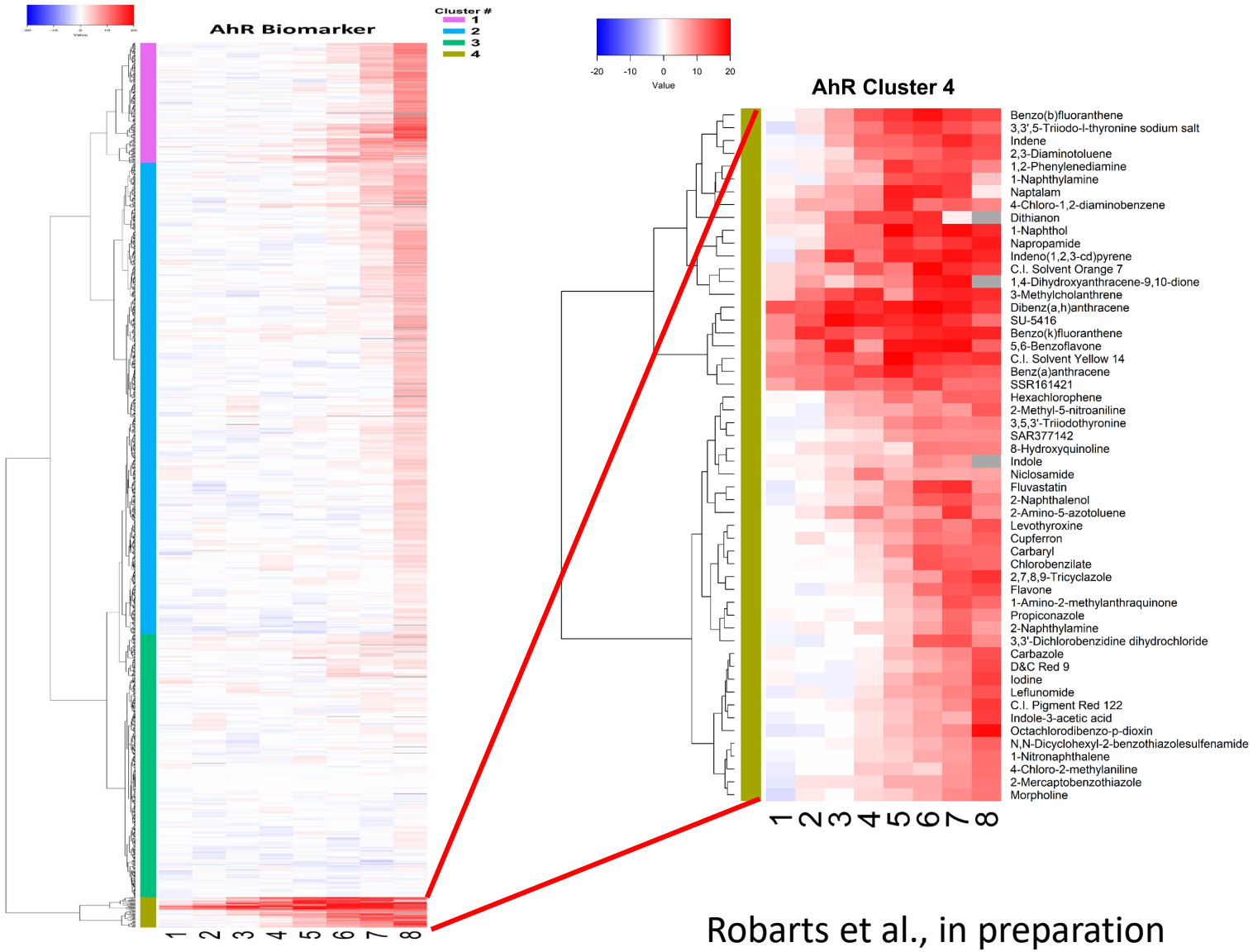
Prodiamine
Tamoxifen citrate
Indene
1-Nitronaphthalene
Tefluthrin
4,4'-(Isopropylidenebis(4,1-phenyleneoxy))dianiline
Levothyroxine
Indole
C.I. Solvent Orange 7
N,N-Dicyclohexyl-2-benzothiazolesulfenamide
1-Naphthol
Dibenz(a,h)anthracene
Benzo(k)fluoranthene
3-Methylcholanthrene
C.I. Solvent Yellow 14
Dithianon
SU-5416
5,6-Benzoflavone
Perfluorohexanoic acid
Indeno(1,2,3-cd)pyrene
1,4-Dihydroxyanthracene-9,10-dione

Robarts et al., in preparation

Identification of AhR activators in an HTTr screen in MCF-7 cells

- Built and characterized a gene expression biomarker to identify AhR activators in MCF-7 cells
- 16 genes consistently regulated by 12 AhR activators and in the opposite direction by knockdown of AhR using gene-specific siRNA
- Compared predictions to NCATS Tox21 AhR transactivation assay carried out in HepG2 cells
  - Sensitivity = 73%
  - Specificity = 59%
  - Balanced accuracy = 66%
- 7 out of the 29 were positive in the ToxCast ATG_Ahr-Cis_up assay carried out in HepG2 cells.

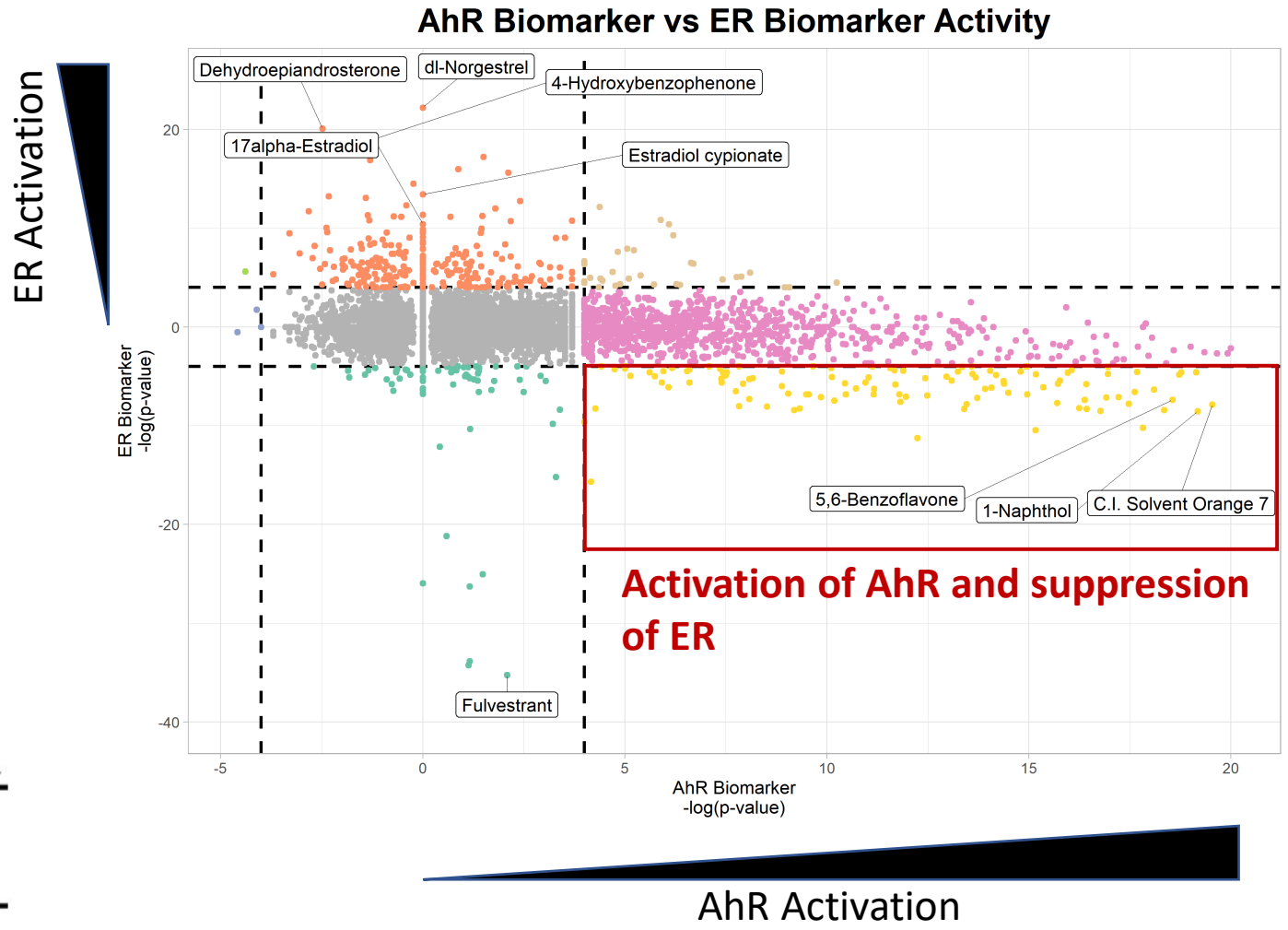
- Compared the ~12,800 profiles to the AhR biomarker

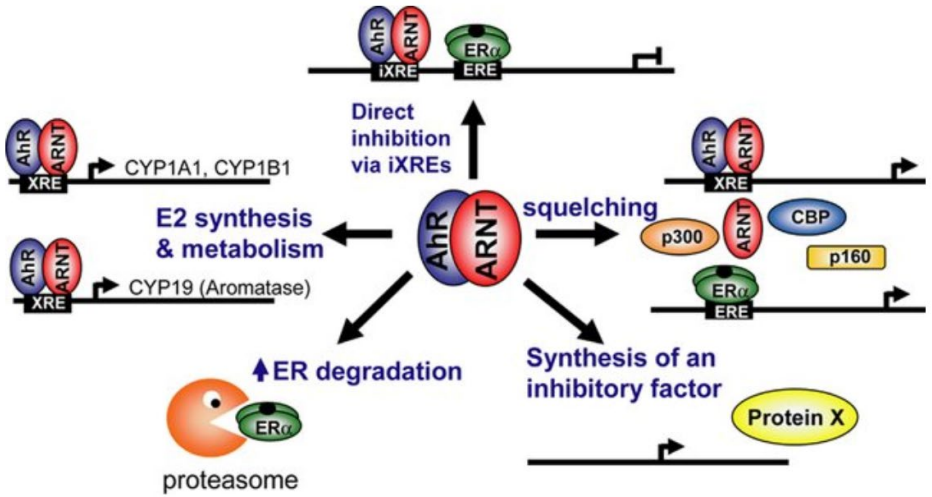


Robarts et al., in preparation

AhR activators suppress ER responses

- **Examined transcript changes in MCF-7 cells treated with ~1600 chemicals at 8 concentrations**
- **Compared the profiles to the estrogen receptor (ER) and aryl hydrocarbon receptor (AhR) biomarkers**





From Nuclear Receptor Signaling 4(1):e016

Robarts et al., in preparation

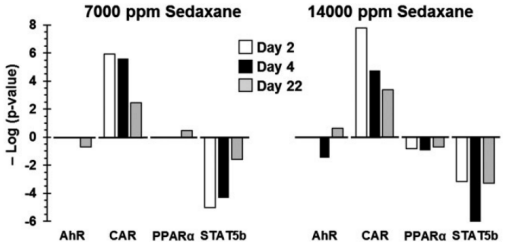
Summary

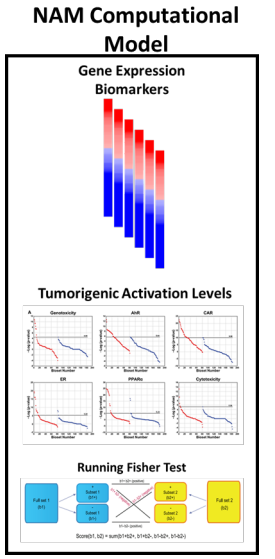
- Gene expression biomarkers have multiple uses

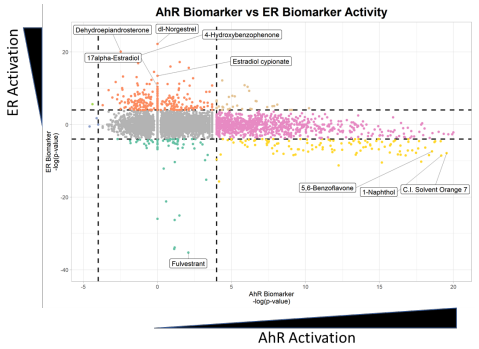
- Biomarkers for screening in mice
  - Identification of mode of action

- Biomarkers for screening in rats to reduce unnecessary testing
  - Identification of mode of action
  - Identification of chemical doses that would cause cancer

- Biomarkers for Tier 1 screening in high throughput transcript profiling
  - Estrogen receptor biomarker
    - Used to identify MIE modulation
    - Potential for replacing HTS assays
    - Potential for replacing the uterotrophic assay
    - Uncovers interesting biology
      - Biomarker gene expression pattern determined by chemical structure
      - Identified AhR-ER interactions







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