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Transcriptomic thresholds from short-term assays predict rat liver tumorigens

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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.

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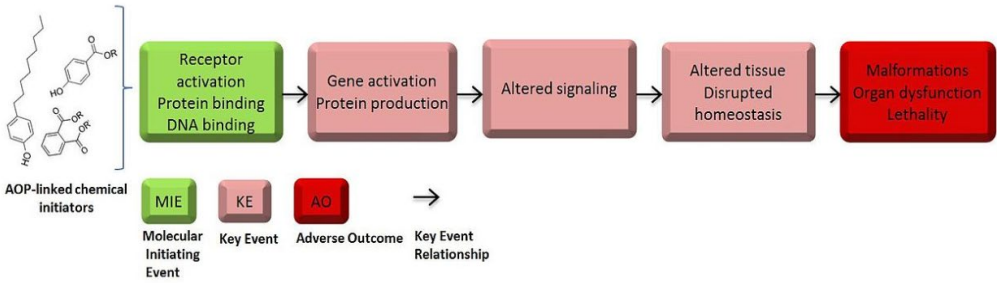
Outline

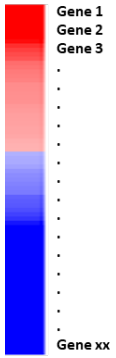
- Methods used to create and determine predictive accuracy of gene expression biomarkers

- Stratification of carcinogenic risk using gene expression biomarkers in short-term animal studies
  - Prediction of liver cancer

- Identification of biological thresholds predictive of liver cancer
  - Gene expression biomarkers
  - Individual genes
  - Liver weight and clinical chemistry endpoints

Definitions





Treated vs. Control

Adverse Outcome Pathway

- Structured representation of biological events leading to adverse effects; relevant to risk assessment
- A series of causally connected key events (KE) between two points — a molecular initiating event (MIE) and an adverse outcome (AO) that occur at a level of biological organization relevant to risk assessment

Gene Expression Biomarker

- List of genes and associated fold-change values or ranks
- Measures a molecular initiating event or key event in an adverse outcome pathway using transcript profiling

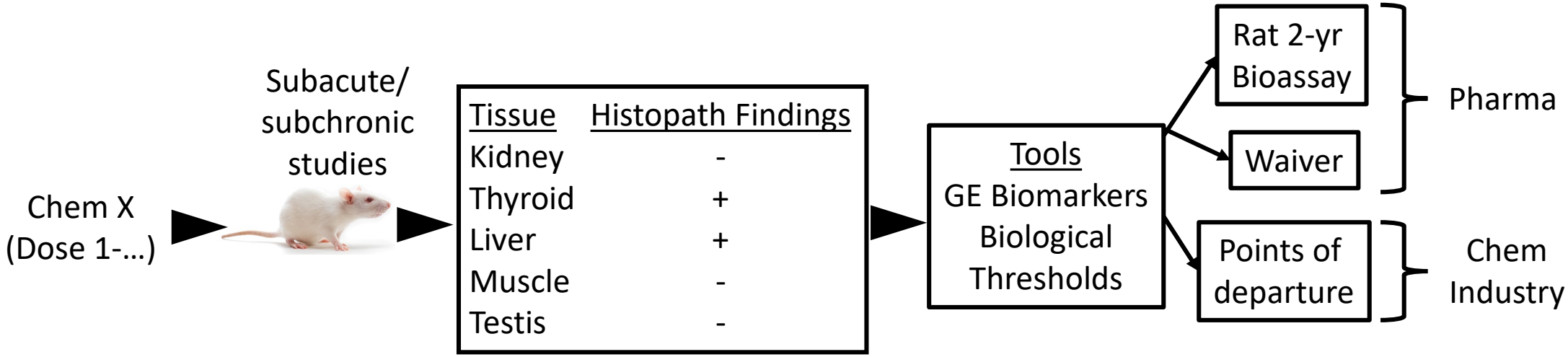
Biological Thresholds

- Empirically-derived by comparing exposure conditions that lead to toxic responses vs. those that do not
- Chemical-independent
- Derived for biomarkers, genes and traditional measures of toxicity

Bioset

- List of statistically-filtered genes derived from a comparison between treated and control groups

Use of biomarkers and thresholds to inform carcinogenic risk and mode of action



Problem: how can we better use 21st century tools in a prospective manner to avoid unnecessary 2-year bioassays?

Can we predict from short-term studies:

- Chemical-dose combinations that will cause tumors?
- Mode of action by which the tumors would arise?
- Whether the mechanism is human-relevant?

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

Gene Signature Subcommittee

Co-chairs: Chris Corton, EPA

Keith Tanis, Merck

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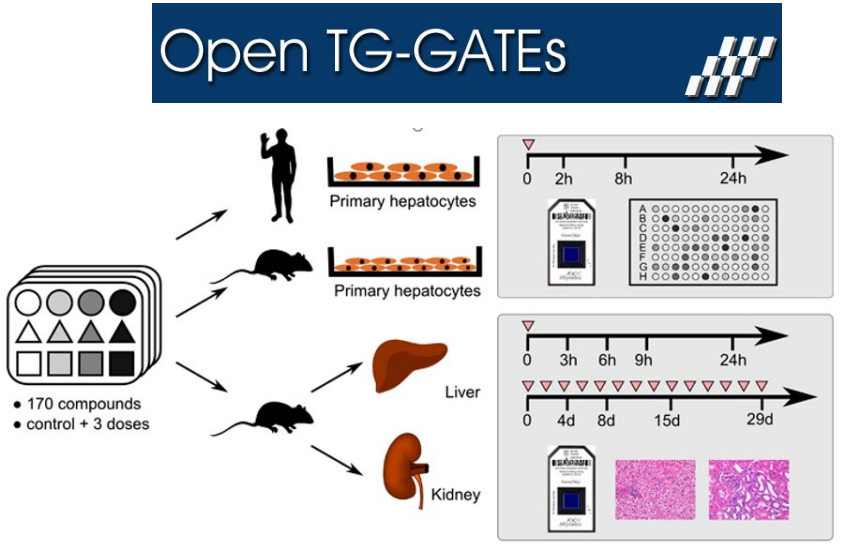
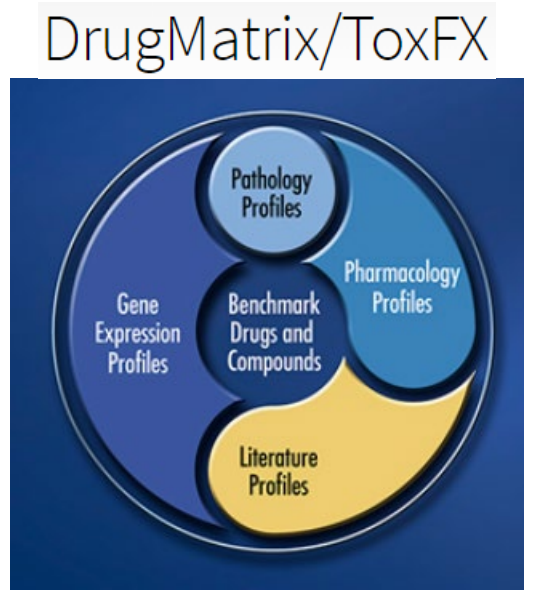
Sources of Rat Liver Tumorigenicity and Microarray Data

- TG-GATES microarray data
  - ~130 chemicals, 8 time points, 3 doses

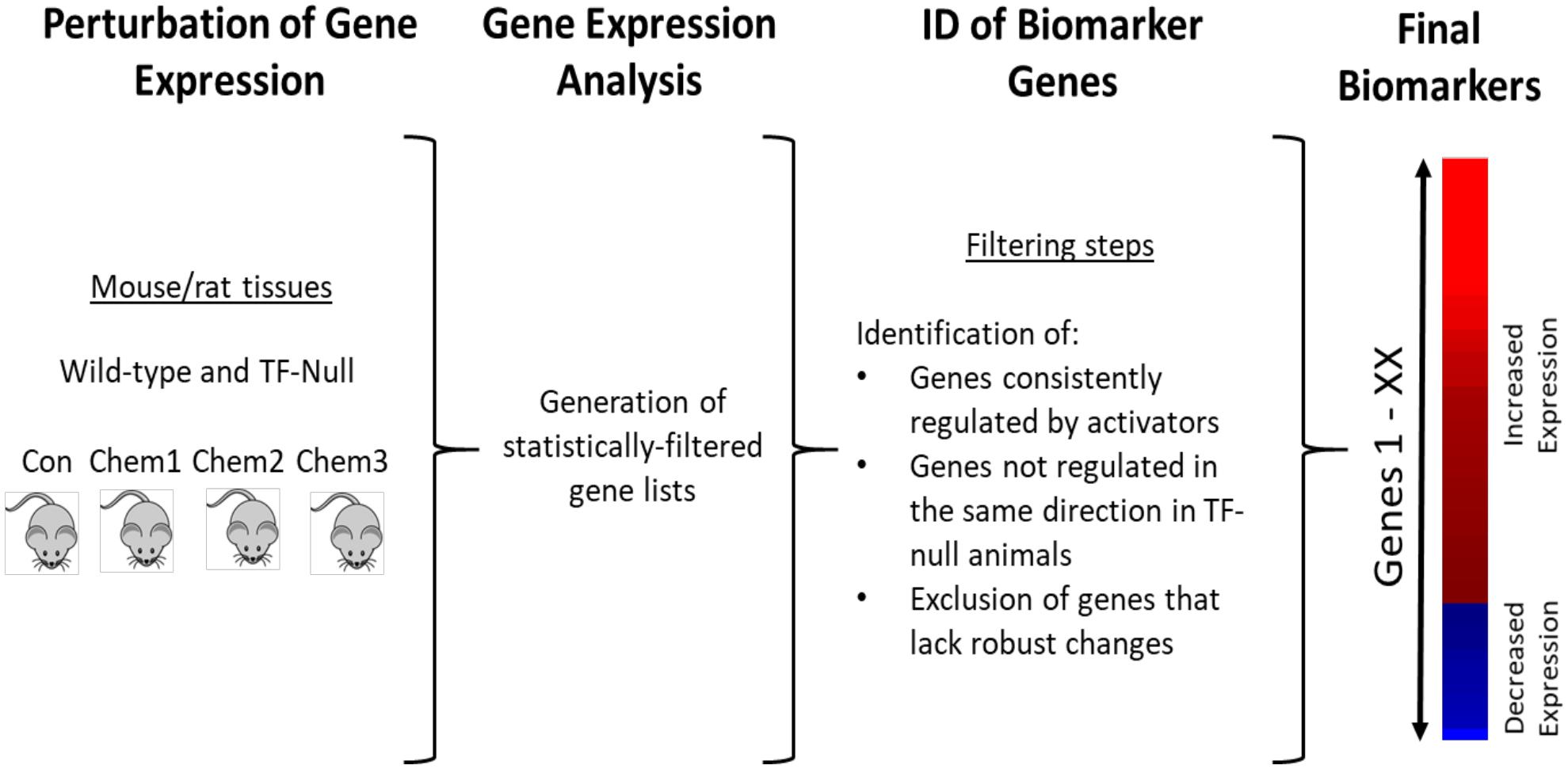
- DrugMatrix microarray data
- >600 chemicals, 4 time points, 2 doses

- Carcinogenicity Potency Database
  - Carcinogenicity data on >1500 chemicals in rats and mice
  - Categorized the hepatotumorigenic potential of 1182 chemical-dose-time comparisons representing ~130 chemicals



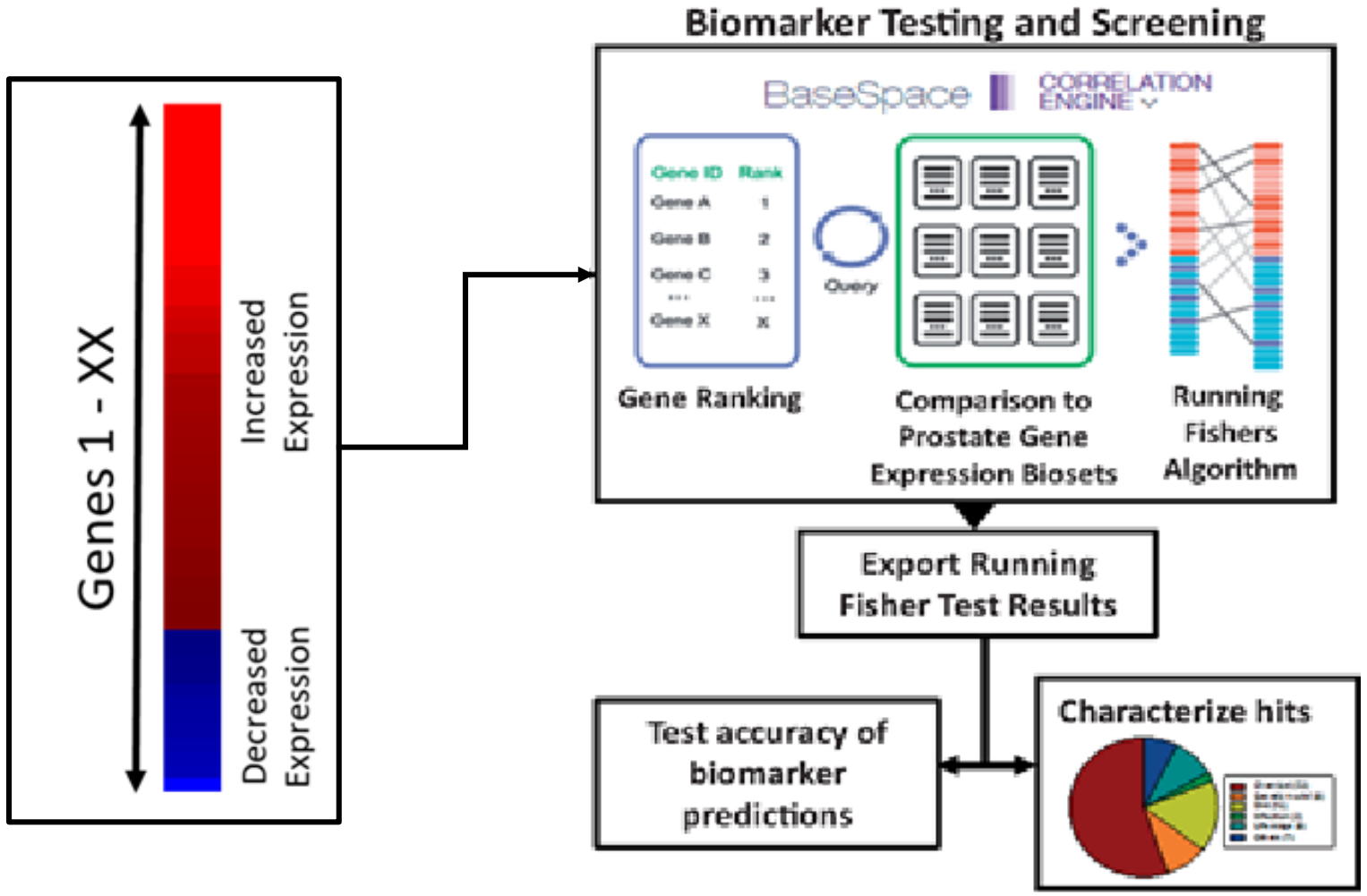


Construction of biomarkers from microarray data generated in animal tissues



From Corton (2019) Current Opinion in Toxicol 18:54

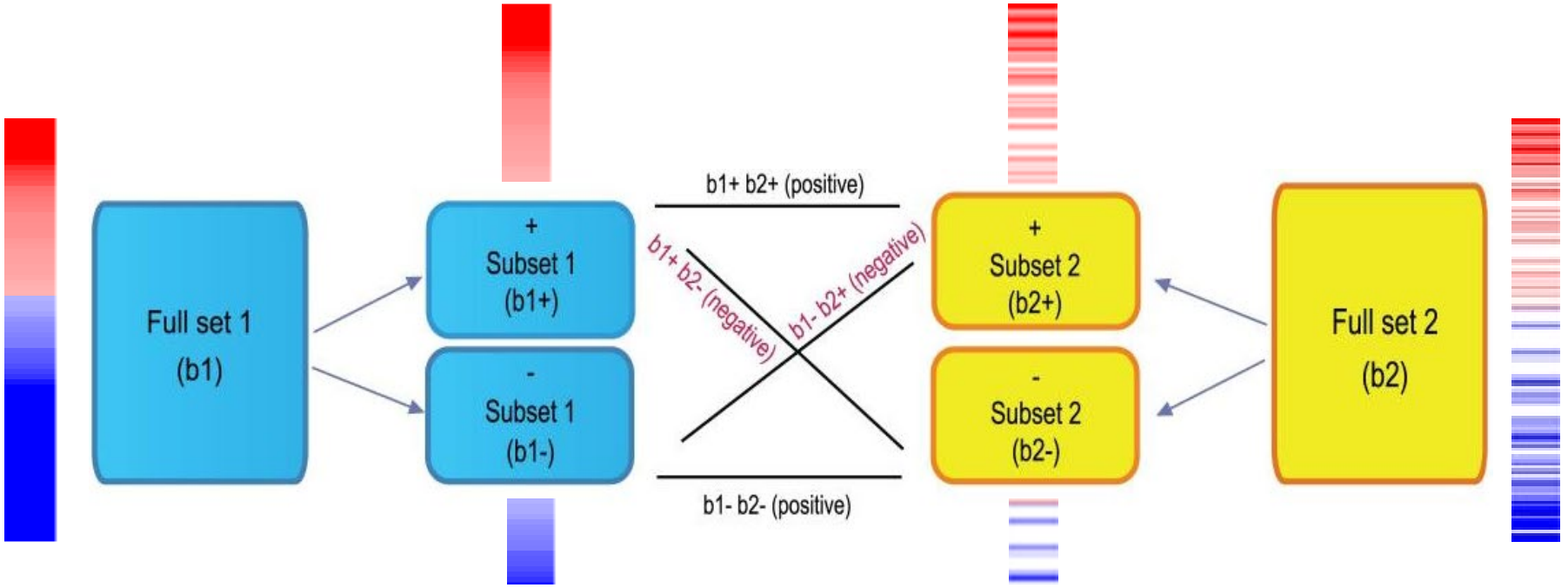
Comparing gene lists in BaseSpace Correlation Engine



>130,000 statistically filtered gene lists from > 25,000 studies

Derived from Rooney et al. Toxicol Sci. 166:146-162

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

Adapted from Kuperschmidt et al. (2010) PLoS One

Adverse Outcome Pathways that Lead to Liver Cancer

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/taap

Adverse outcome pathway-driven identification of rat liver tumorigens in short-term assays

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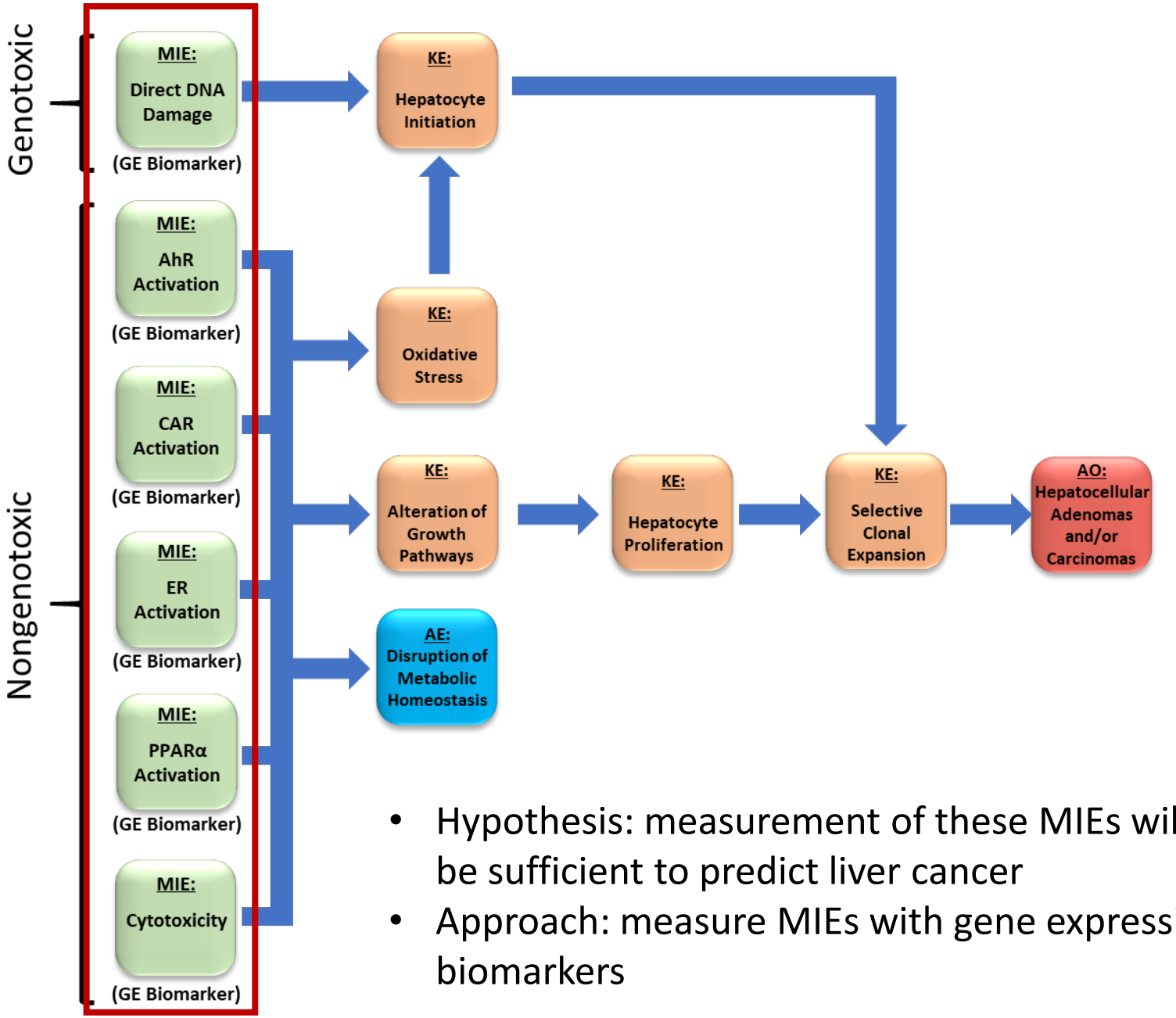

- Hypothesis: measurement of these MIEs will be sufficient to predict liver cancer
- Approach: measure MIEs with gene expression biomarkers

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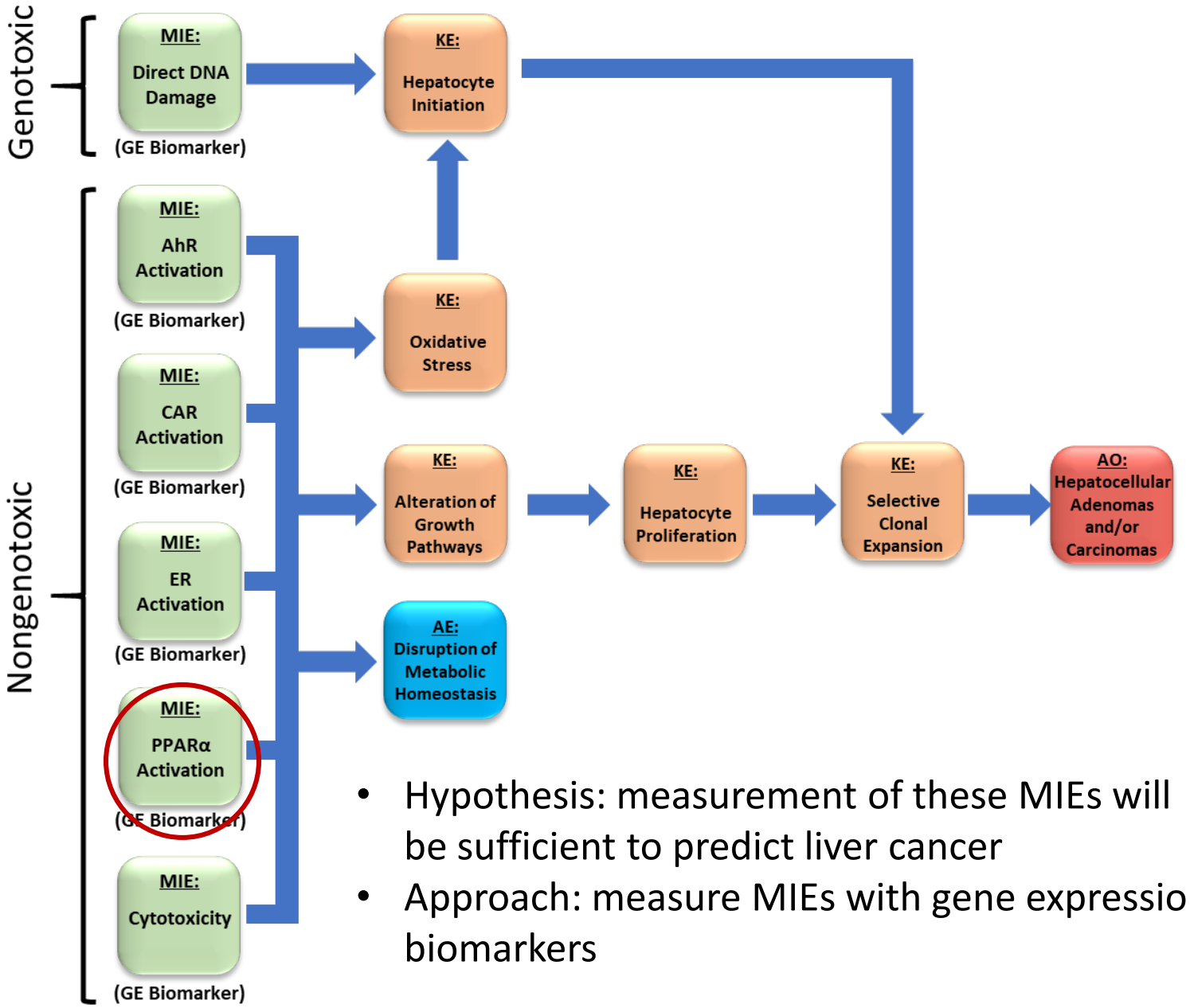

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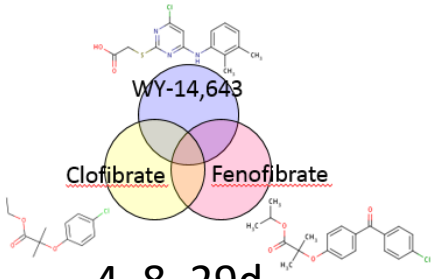
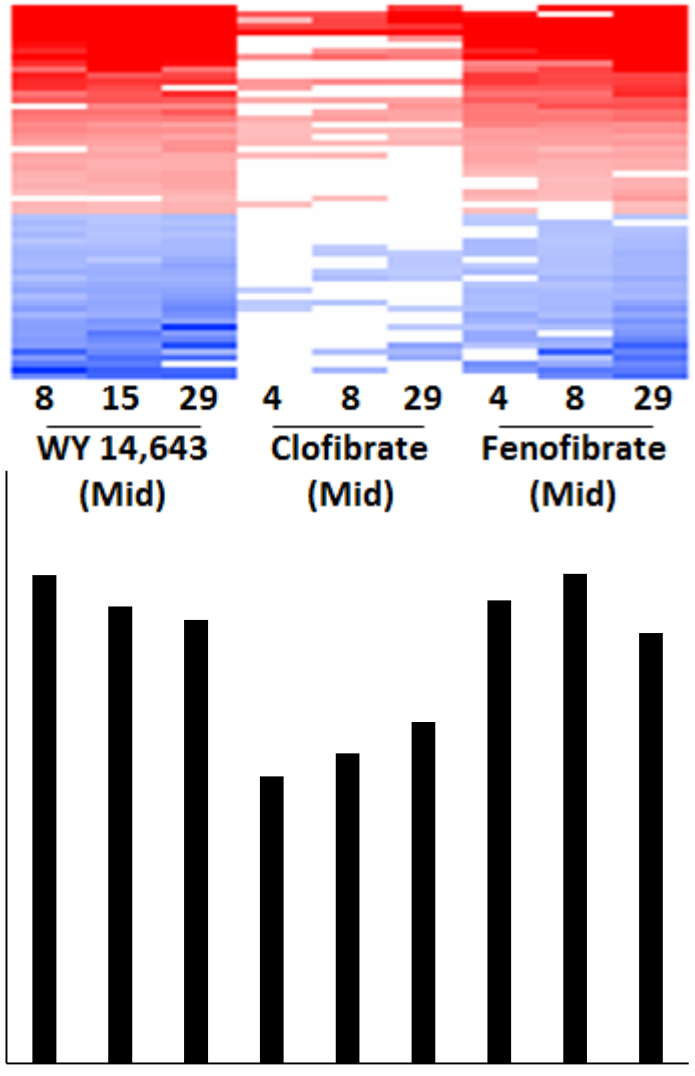
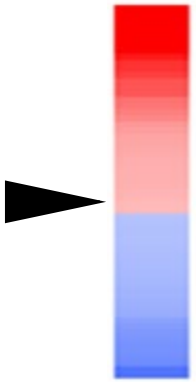
Using weight of evidence to build a rat liver PPARα biomarker

- Microarray data sets from TG-GATES study

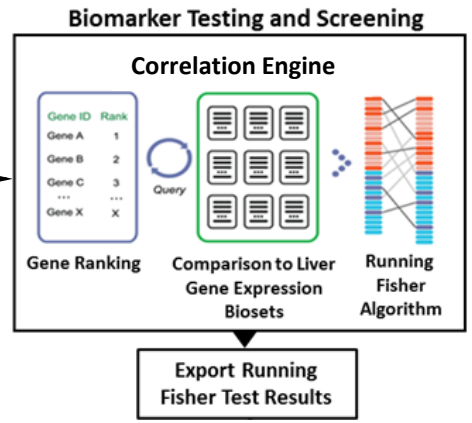


4, 8, 29d
4, 15, 29d





PPARα Biomarker (58 genes)



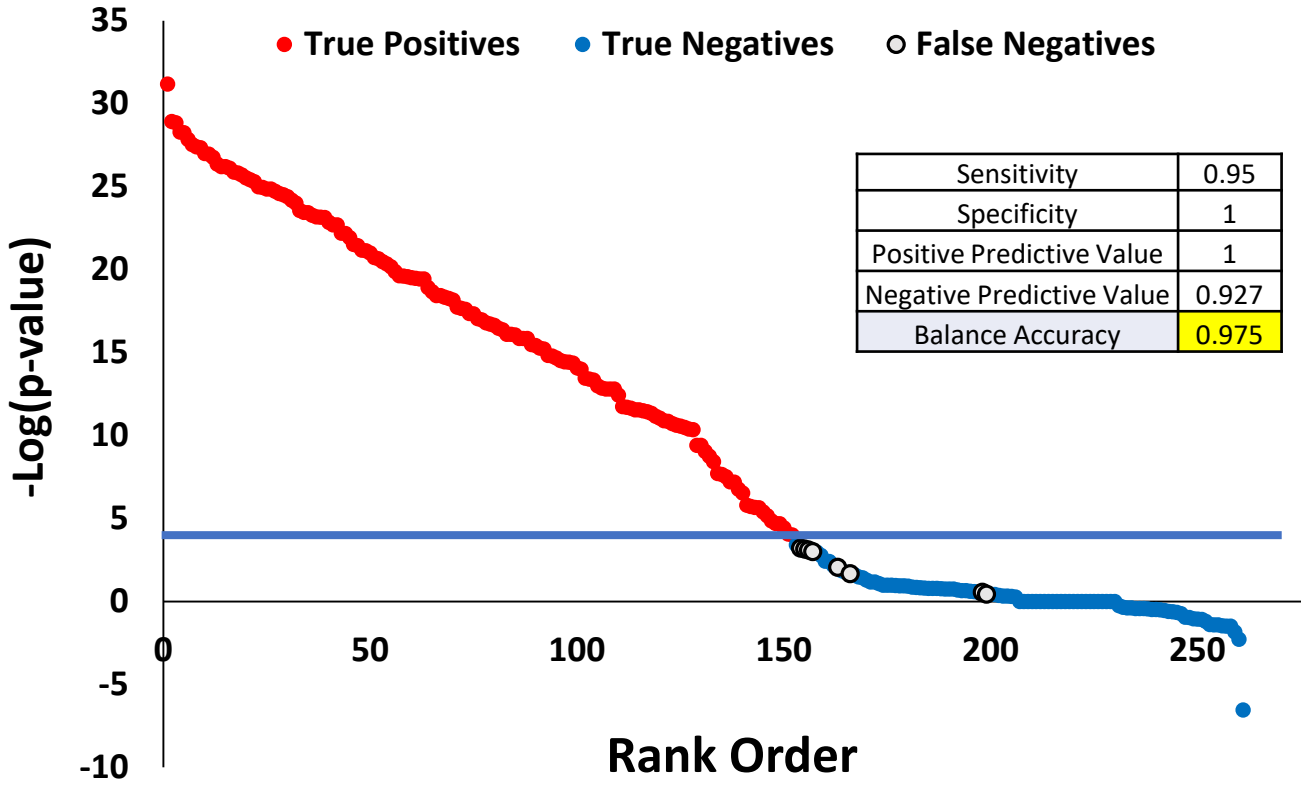
Weight of evidence gene selection

- Same direction of change
- Regulated in the majority of comparisons
- Ave absolute fold-changes ≥ 1.5
- Not found in other biomarkers

Rooney et al. (2018) TAAP

Testing the rat liver PPARα biomarker for predictive accuracy

- Examined 261 comparisons with known PPARα activity in rat liver (261 comparisons)
- A cutoff of –Log(p-value) = 4 was used as in prior studies
- Excluded comparisons used to create the biomarker



Sensitivity	0.95
Specificity	1
Positive Predictive Value	1
Negative Predictive Value	0.927
Balance Accuracy	0.975

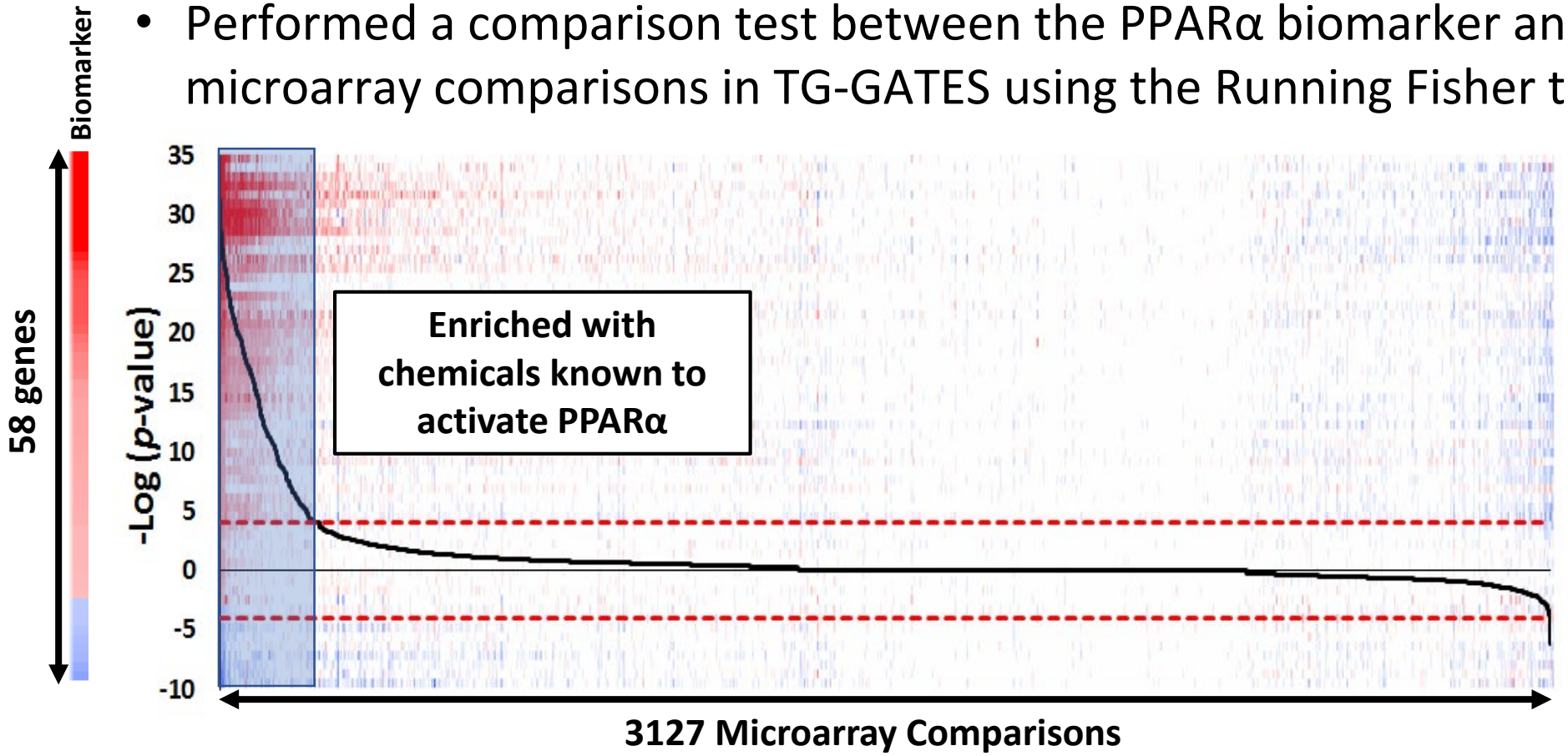
Balanced Accuracy = 98%

–Log(p-value) = 4

Rooney et al. (2018) TAAP

Identification of chemicals with PPARα activity

- Performed a comparison test between the PPARα biomarker and ~3100 microarray comparisons in TG-GATES using the Running Fisher test



- Heat map shows the relationship between expression of biomarker genes and –Log(p-value)s
- Positively correlated comparisons on the left and negatively correlated comparisons on the right

Rooney et al. (2018) TAAP

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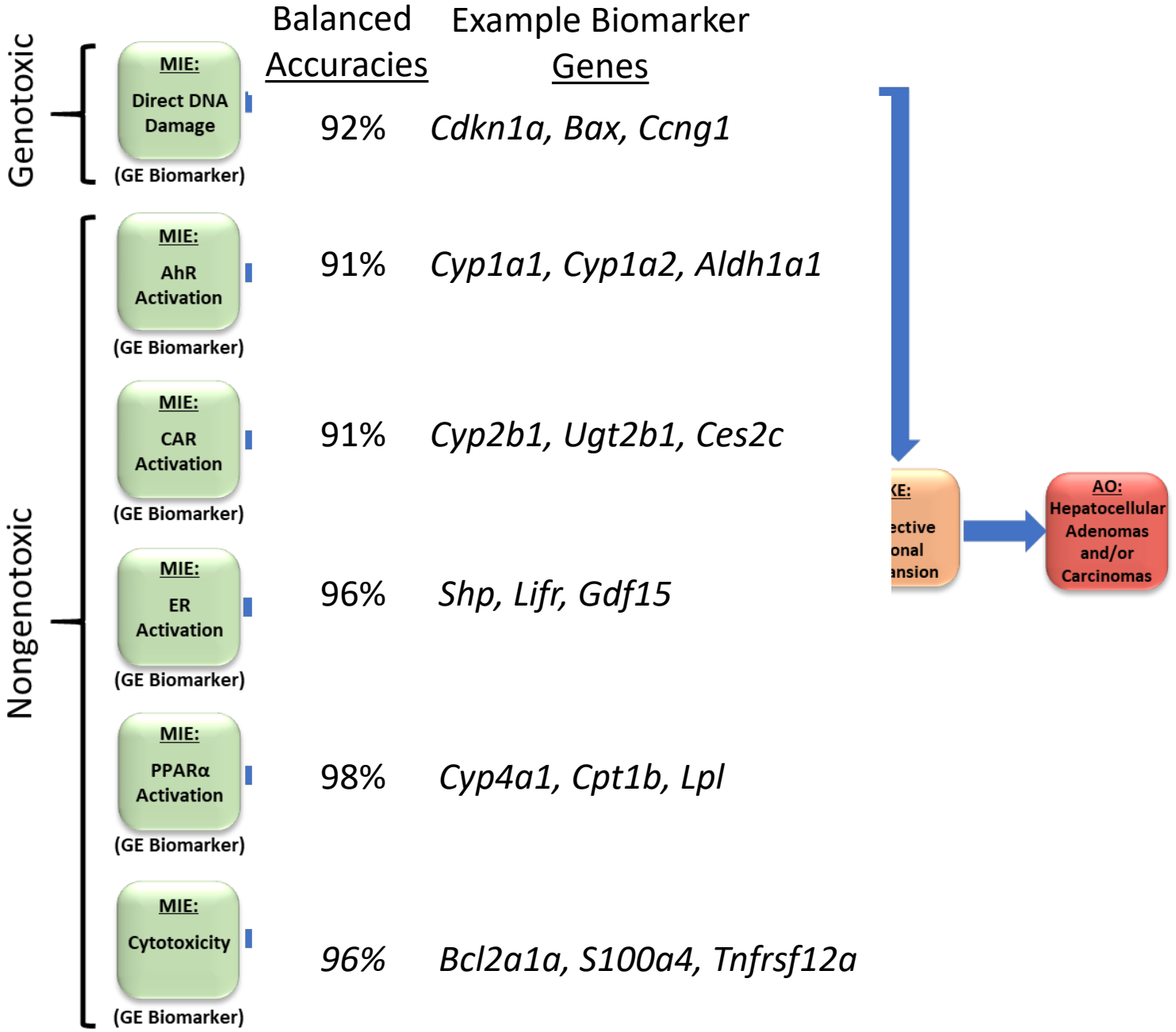

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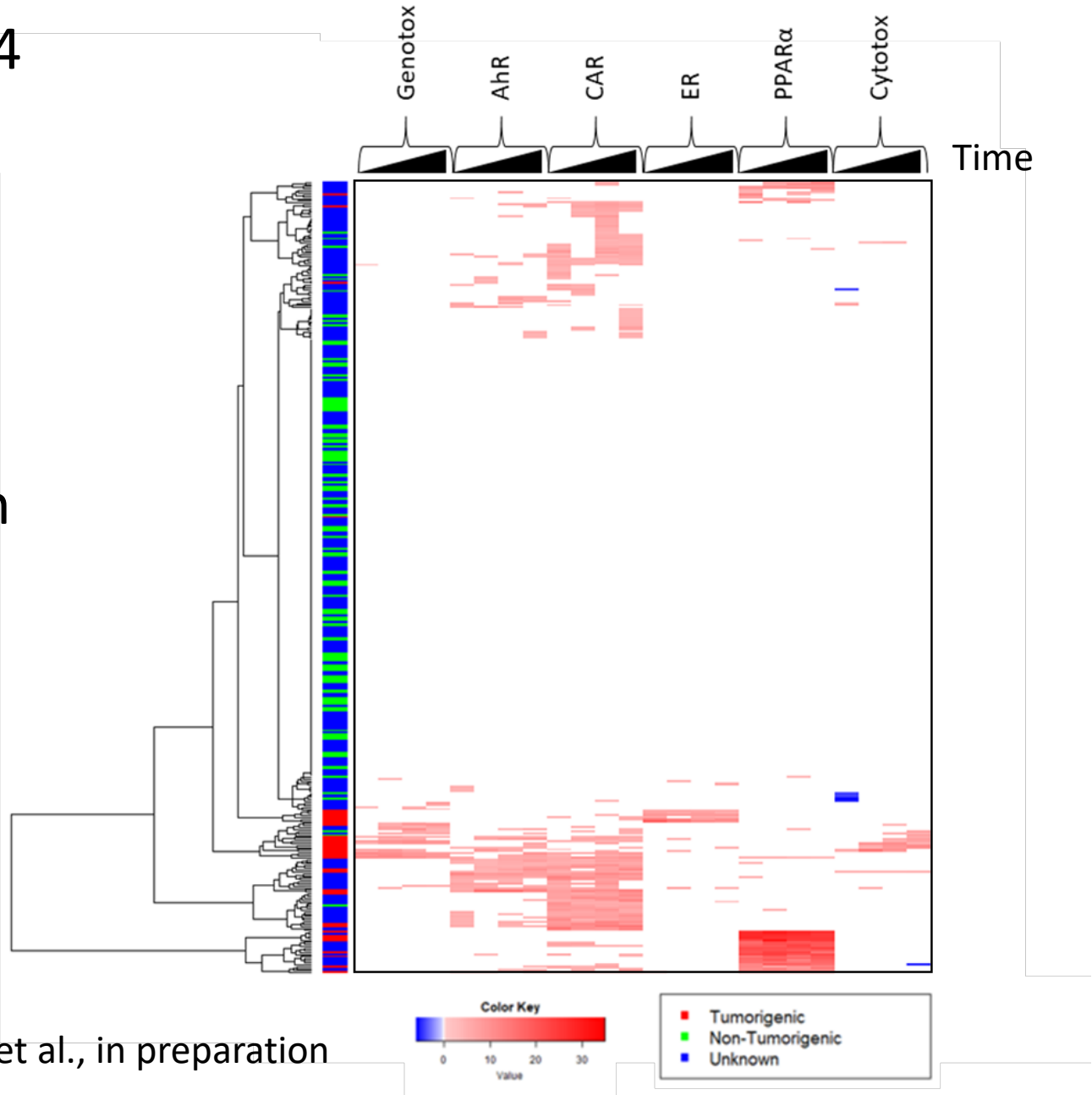
Biomarker scores across the TG-GATES study

- Activation of the 6 MIEs across the 4 time points (4d-29d); one-dimensional clustering; -Log(p-value)s being shown

- Most of the chemical-dose-time conditions did not lead to activation of any of the MIEs and those were associated with nontumorigenic doses

- Activation of one or more of the MIEs were associated with tumorigenic doses

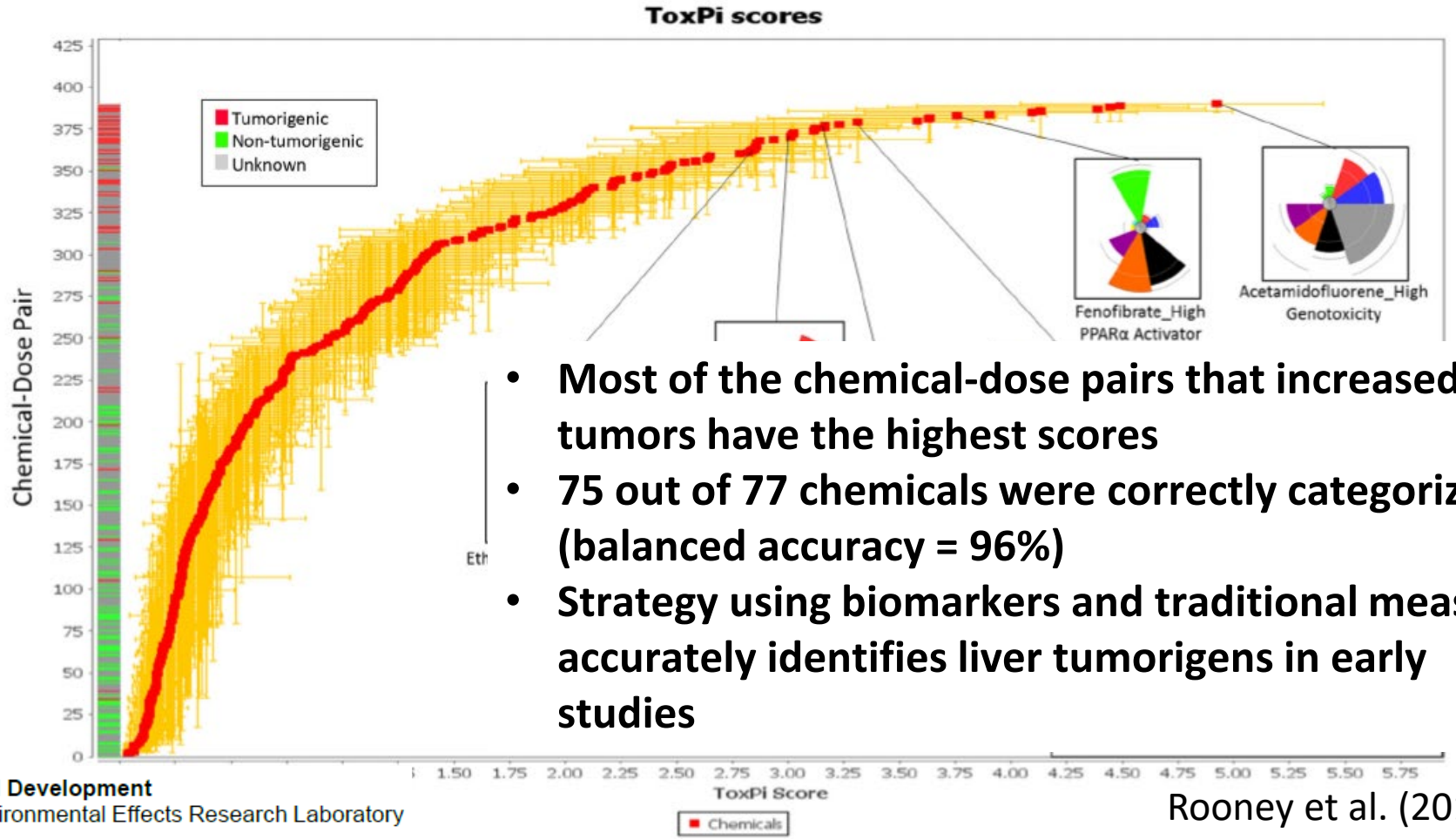


From Corton et al., in preparation

Using ToxPi to stratify chemicals based on key event modulation

- All chemical-dose comparisons ranked by ToxPi scores; dimensionless score, i.e., weighted combination of all data
- 390 chemical-dose combinations across 77 chems

- Red dots – chemical scores
- Orange bars – confidence intervals
- Distance from the origin is proportional to the normalized value of the component data points

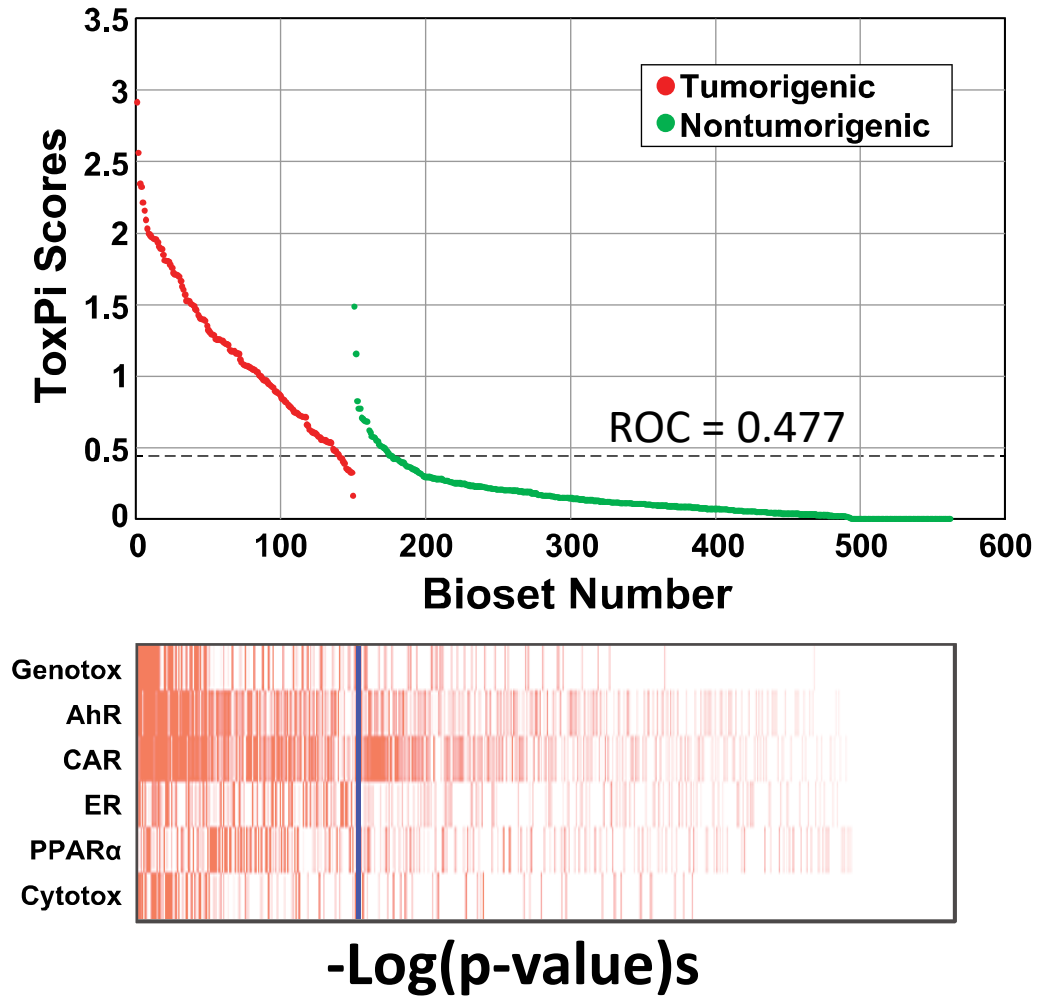


- **Most of the chemical-dose pairs that increased liver tumors have the highest scores**
- **75 out of 77 chemicals were correctly categorized (balanced accuracy = 96%)**
- **Strategy using biomarkers and traditional measures accurately identifies liver tumorigens in early studies**

Rooney et al. (2018) TAAP

Assessment of the 6 MIEs predicts liver cancer

- Determined ToxPi scores for each bioset using the 6 –Log(p-value)s
- Divided the TG-GATES study into training and test sets.
- DeLong, DeLong and Clarke-Pearson receiver operating curve (ROC) analysis to determine the optimal threshold in the training set; ROC=0.477
- Applied to the test set: 90% sensitivity, 97% specificity, and a balanced accuracy of 93%
- Out of 44 rat liver tumorigens, only two (5%) were missed (acetamide, ethionine)



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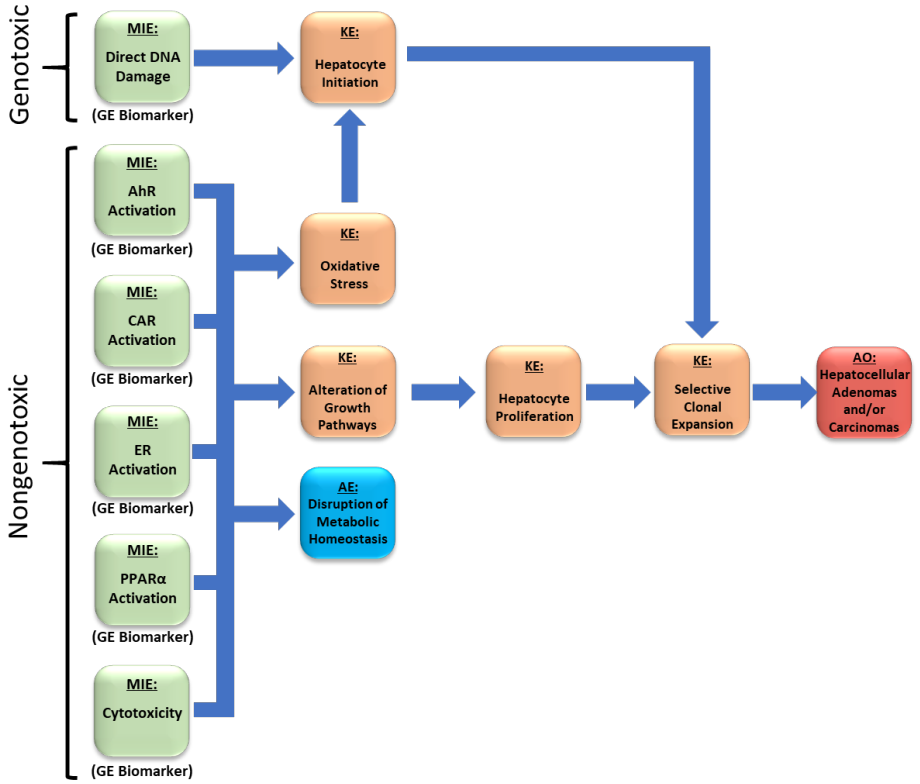
Defining biological thresholds for liver cancer

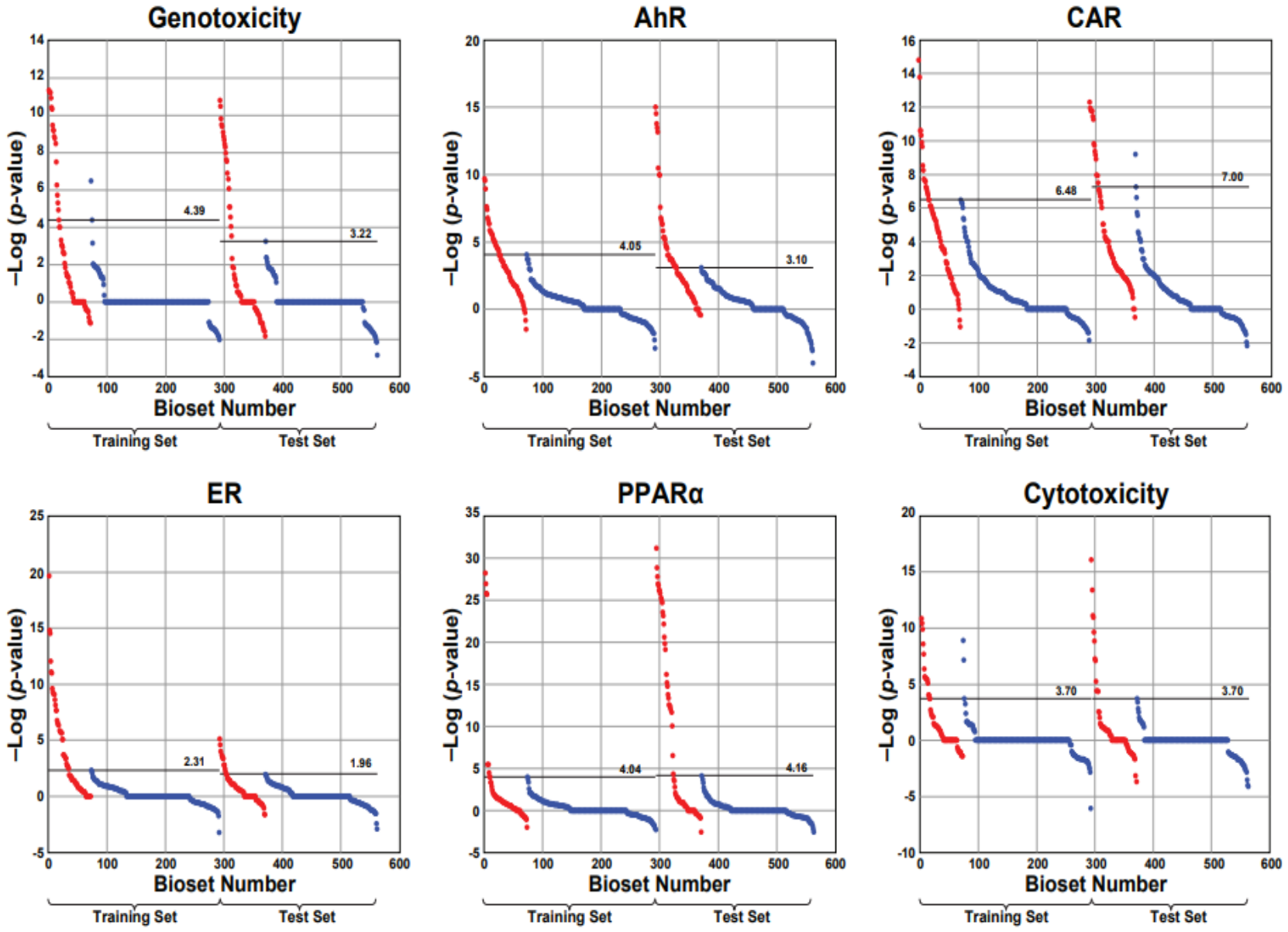
- Central premise of AOP framework: key events are necessary but not sufficient
- An early key event may not necessarily lead to an adverse outcome but depends on the degree or amount of disruption to the particular pathway
- Can we define thresholds “tipping points” for each of the key events?
- To define a threshold need to compare the range of values between conditions that cause cancer and those that do not



http://www.silverdoctors.com

Identification of thresholds for gene expression biomarkers

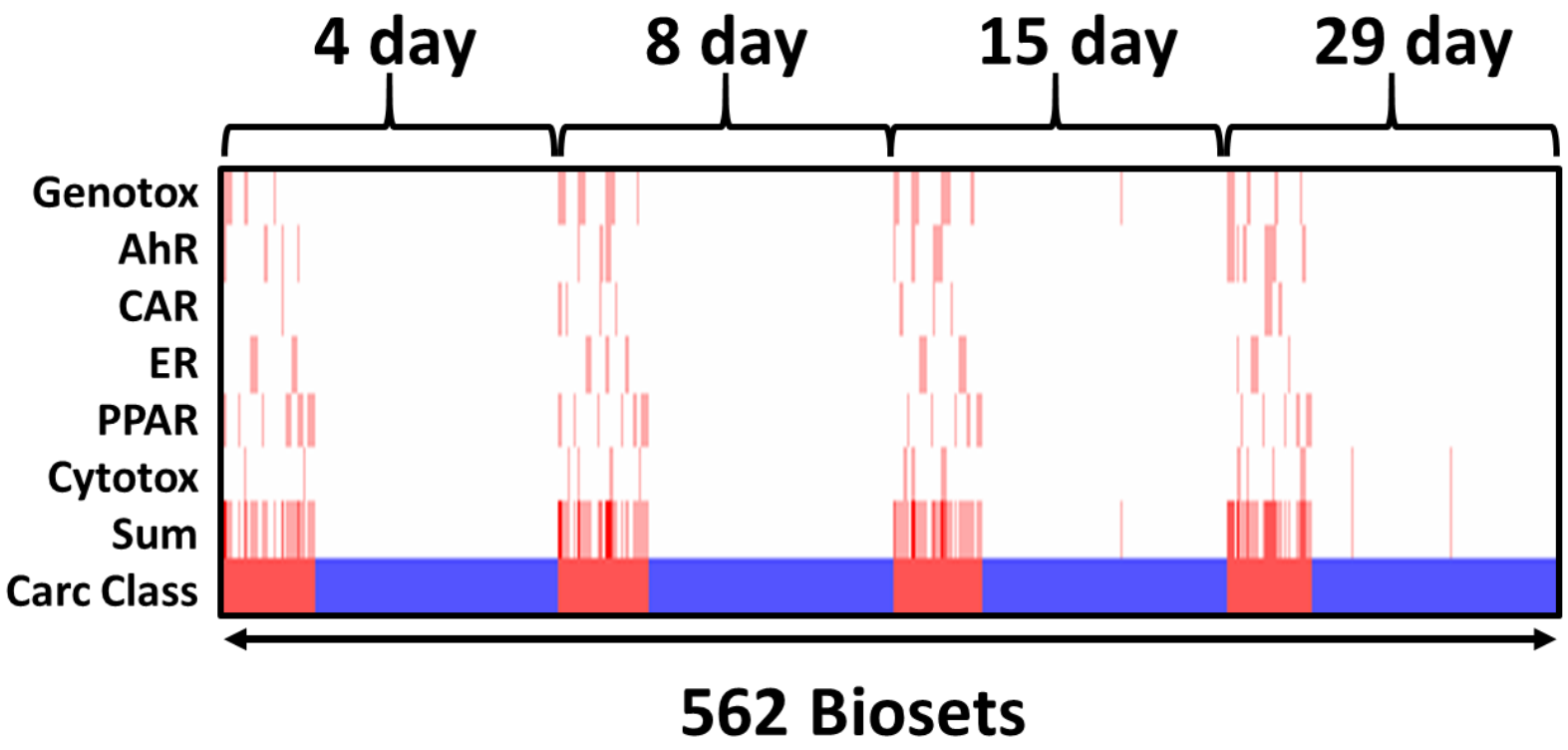
- Divided the chemical-dose conditions into tumorigenic and nontumorigenic and training and test sets
- Thresholds defined as the maximum value in the nontumorigenic group
- Outliers removed if they were ≥ 2 –Log(p-value) units
- Thresholds were similar between the training and test sets



From Hill et al., in preparation

Biomarker thresholds accurately predict liver cancer

- Derived thresholds from the TG-GATES training set and then applied to the entire dataset
- Each red line is a condition in which the biomarker –Log(p-value) exceeds the threshold
- Most of the tumorigenic conditions exceed one or more of the 6 thresholds
- Thresholds rarely exceeded in any of the nontumorigenic conditions

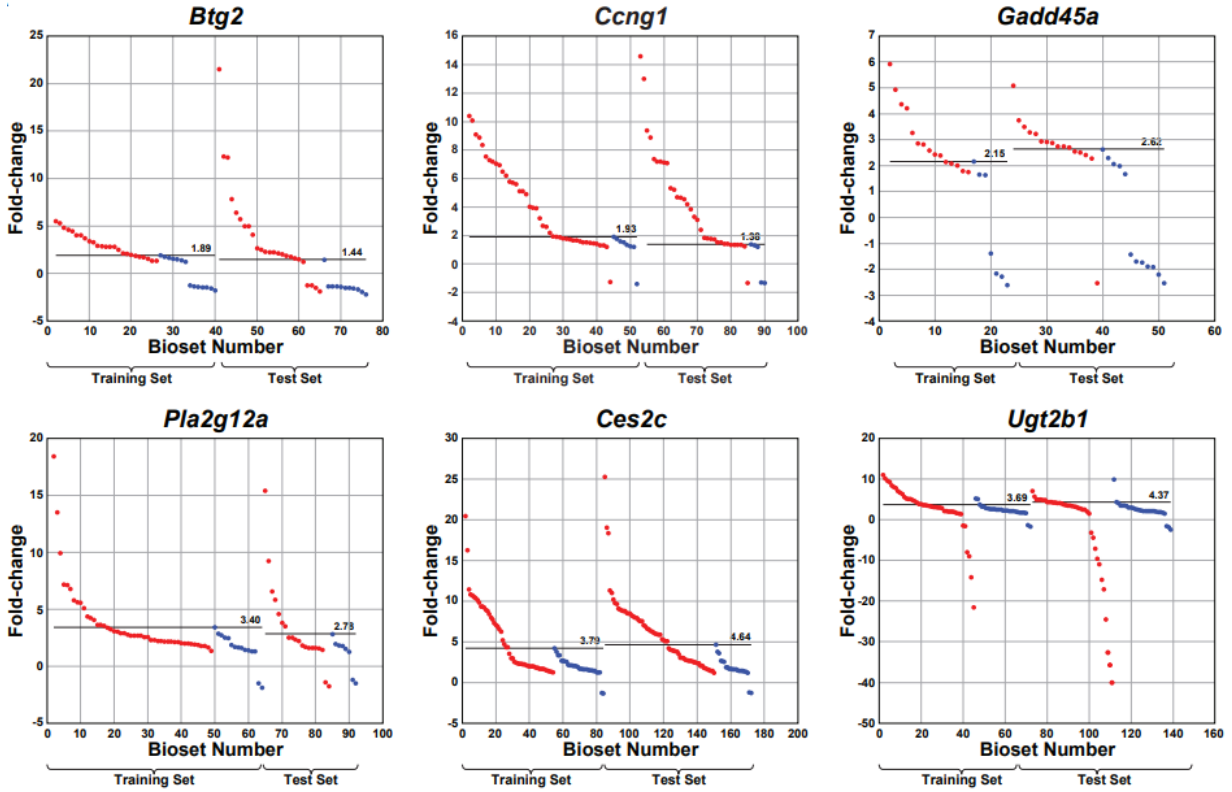


- **Test set: 100% sensitivity, 94% specificity, and a balanced accuracy of 97%**

From Hill et al., in preparation

Thresholds for individual genes or liver weights and clinical chemistry endpoints are predictive of liver cancer

- Using thresholds for 6 individual genes
  - 100% sensitivity, 80% specificity, and a **balanced accuracy of 90%**
- Using thresholds for liver weight and clinical chemistry endpoints
  - 88% sensitivity, 100% specificity, and a **balanced accuracy of 94%**



From Hill et al., in preparation and Corton et al. in preparation

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Summary

- An AOP-guided computational approach can be used to identify liver tumorigens in future prospective studies
  - 95% of the tumorigenic chemicals evaluated fell into one or more of the six AOP categories
  - ToxPi/ROC analysis could identify the top ranked chemical-dose combinations that caused liver cancer

- Identified clear thresholds of response for individual biomarkers, individual genes, and common measures associated with liver cancer
  - Supports the idea that early genomic changes can be used to establish threshold estimates or “tipping points” that are predictive of later-life outcomes
  - Thresholds will be useful tools in future toxicgenomic studies

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Support from EPA Chemical Safety for Sustainability Research Program

Supporting Materials

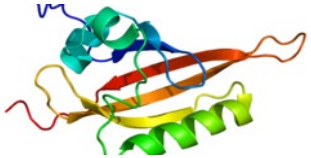
- **Adverse Outcome Pathways**
  - Link to Wiki: https://aopwiki.org/
  - General reviews of AOPs
    - Carusi et al. (2018) Sci Total Environ. 628-629:1542.
    - Ankley and Edwards (2018) Curr Opin Toxicol. 9:1.
    - Leist et al. (2017) Arch Toxicol. 91:3477.
    - Vinken et al. (2017) Arch Toxicol 91:3697.
    - Ankley et al. (2010) Environ Toxicol Chem. 29:730.
  - Using AOPs to help guide building predictive assays
    - Coady et al. (2019) Integrated Environmental Assessment and Management 15:633.
    - Wang et al. (2019) Environ Int 126:377.

- **General papers and reviews on the construction and use of gene expression biomarkers**
  - Li et al. (2017) Proc Natl Acad Sci U S A. 114:E10881-E10889.
  - Corton et al. (2019) Toxicol Appl Pharmacol. 380:114683.
  - Corton (2019) Current Opinion in Toxicol 18:54.

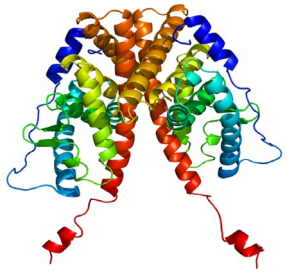
- **Construction and use of rat liver gene expression biomarkers**
  - Rooney et al. (2018) Toxicol Appl Pharmacol. 356:99.

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

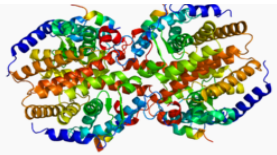
AhR



Estrogen Receptor α

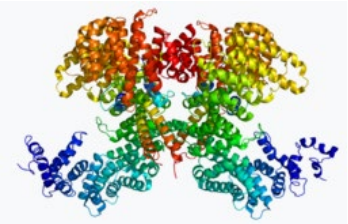


CAR

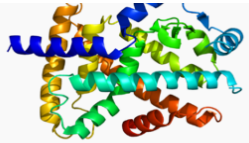


NRF2

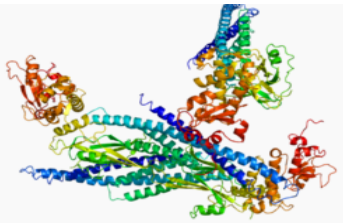
SREBP



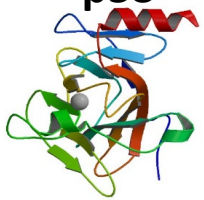
PPARα



STAT5b



p53



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- 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.
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- 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.