Building Predictive Biomarkers from the Perspective of the Adverse Outcome Pathway

Chris Corton
US EPA/CCTE
Research Triangle Park, NC

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

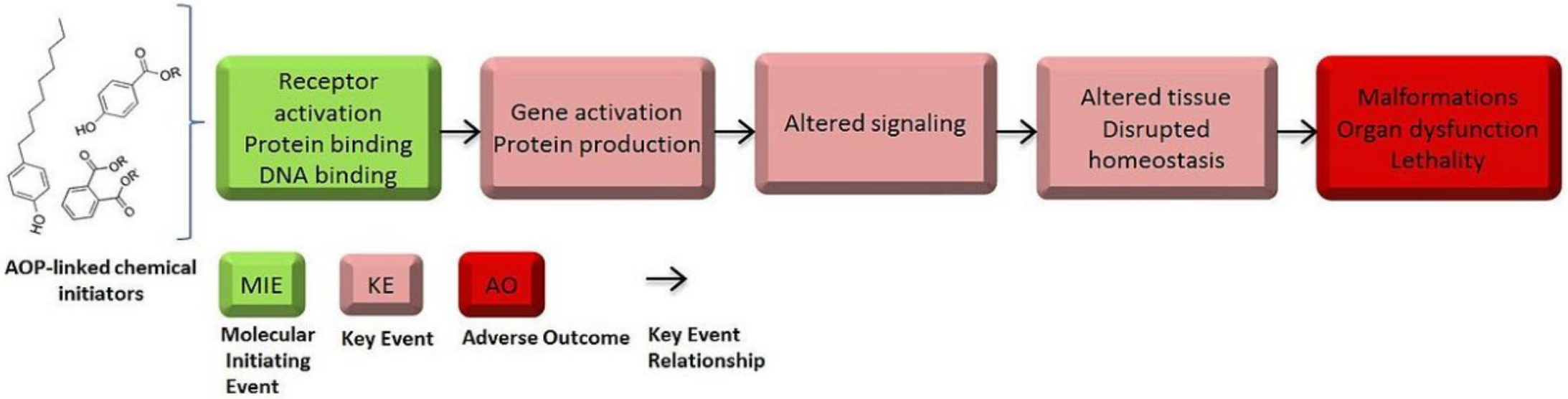
- The adverse outcome pathway
  - Use of the AOP framework as the basis for building and testing predictive assays

- Use of biomarkers to predict cancer in rodent studies

- Gene expression biomarkers
  - How to build biomarkers
  - How to test for accuracy

- Use of biomarkers to identify liver tumorigens

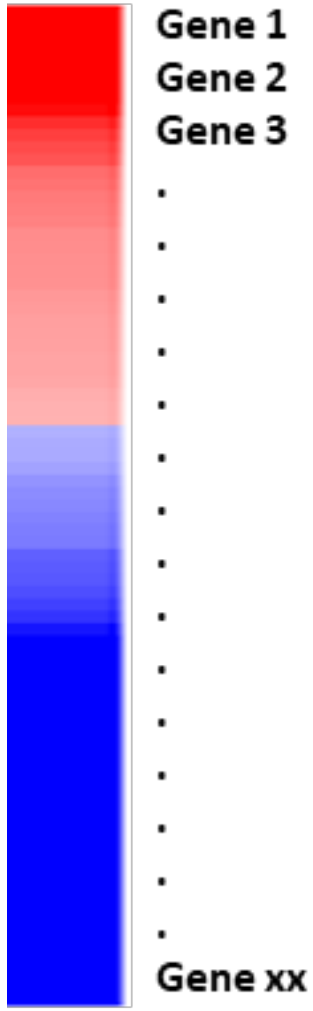
Adverse Outcome Pathways



- 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
- Provides a framework for organizing knowledge of chemical-induced effects and for identifying and prioritizing key events that can be predicted using medium- to high-throughput *in vitro* assays or biomarkers that can measure the key event in accessible tissues

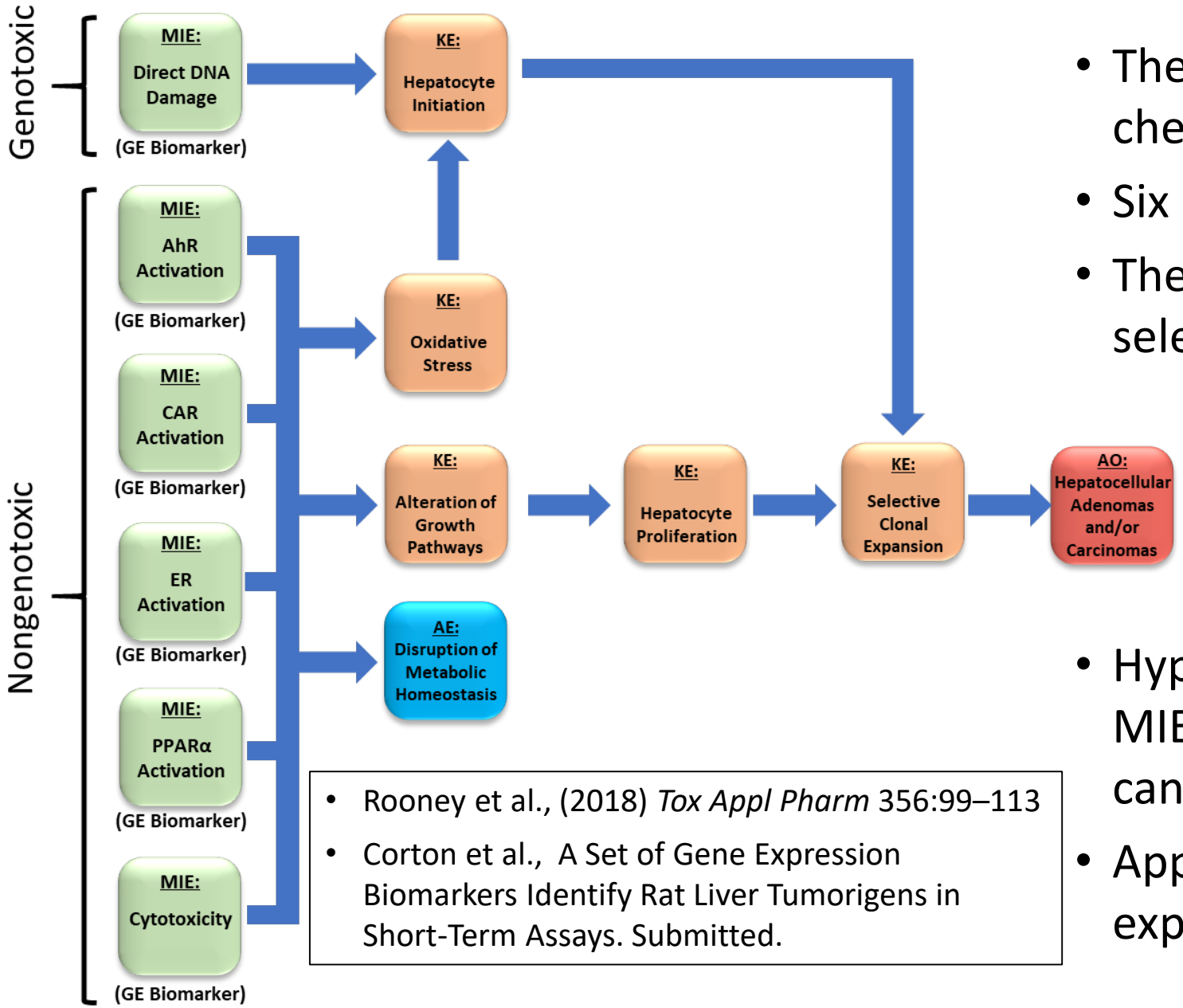
https://en.wikipedia.org/wiki/Adverse_outcome_pathway

Gene Expression Biomarkers



- 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
- Can be used to identify the mechanism of toxicity of a chemical

Major Adverse Outcome Pathways That Lead to Liver Cancer



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

- The liver is most often target of chemical carcinogens
- Six major AOPs lead to rodent cancer
- The AOPs converge on the key event of selective clonal expansion

- Hypothesis: measurement of the 6 MIEs will be sufficient to predict liver cancer
- Approach: measure MIEs with gene expression biomarkers

Use of Biomarkers to Inform Carcinogenic Risk and Mode of Action

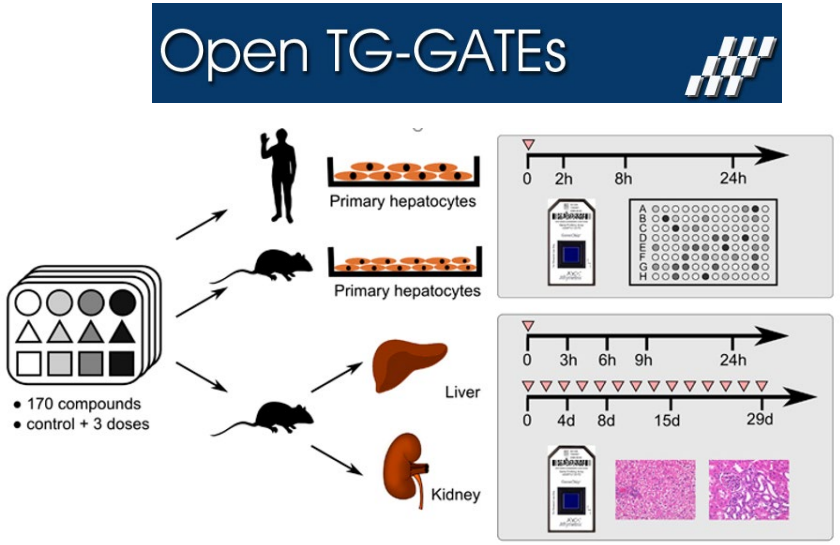
Problem: how can we better use 21st century tools in a prospective manner to avoid unnecessary 2-year bioassays?

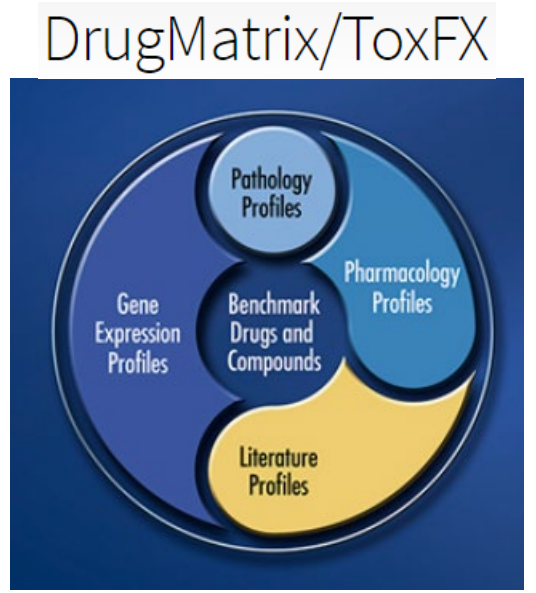
Can we predict from short-term studies:

- Chemical-dose combinations that will cause tumors?
- Mode of action by which the tumors would arise?
- And because we have prior knowledge of the AOP, whether the mechanism is human-relevant?

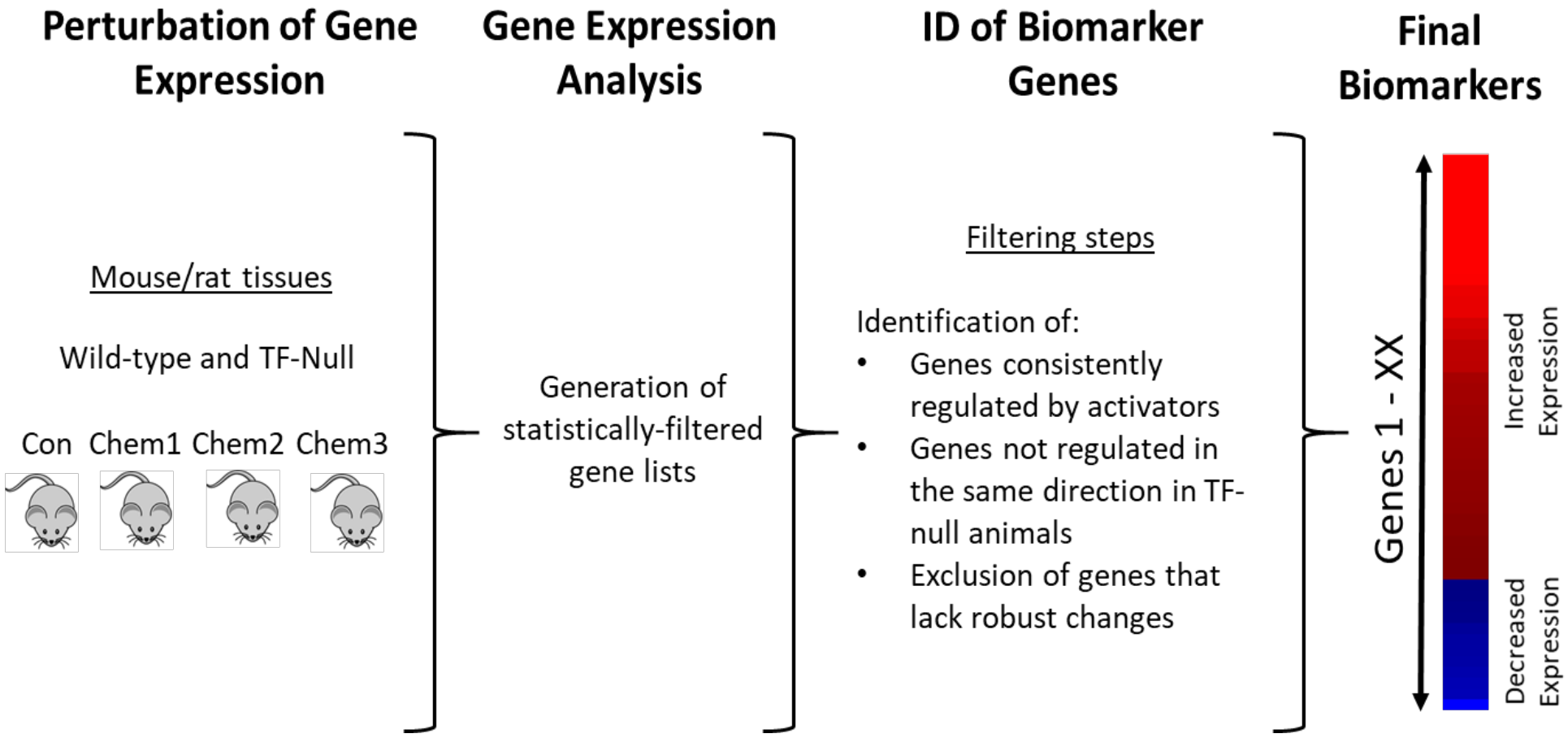
Sources of Rat Liver Tumorigenicity and Microarray Data

- **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
  - Used data to categorize the hepatotumorigenic potential of chemical-dose comparisons in TG-GATES and DrugMatrix
  - Now found at: ftp://anonftp.niehs.nih.gov/ntp-cebs/datatype/Carcinogenic_Potency_Database_CPDB/





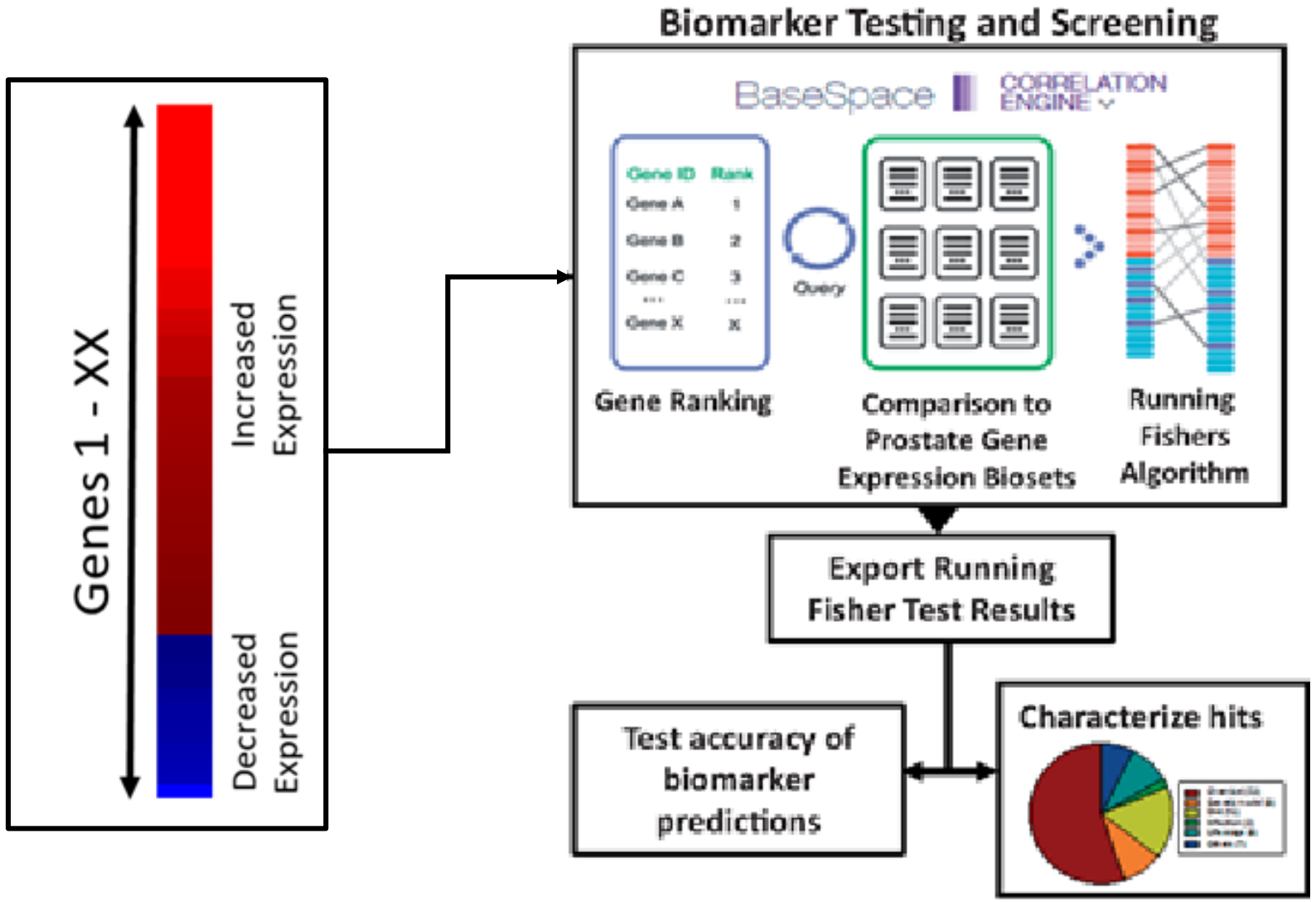
Data-Driven Weight of Evidence Construction of Biomarkers from Microarray Data Generated in Animal Tissues



- Alternative methods include machine learning

Corton (2019) *Current Opinion in Toxicol,* 18:54

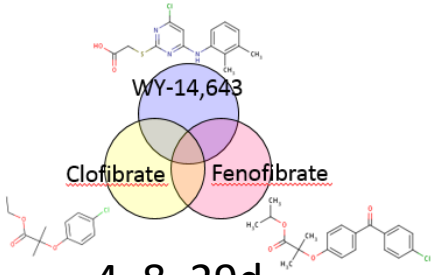
Comparing Gene Lists in BaseSpace Correlation Engine



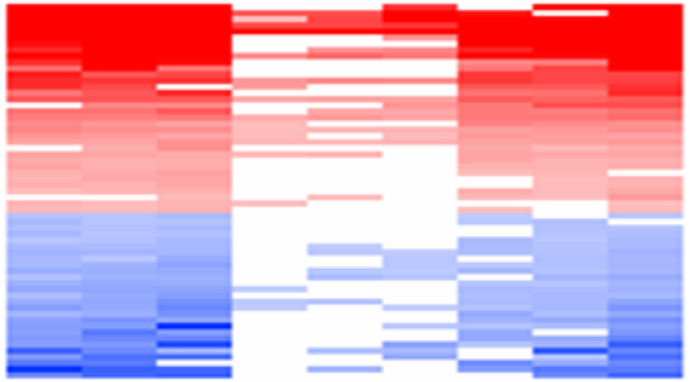
>140,000 statistically filtered gene lists from >25,000 studies

Rooney et al., *Toxicol Sci*. 166:146–162

Building a Gene Expression Biomarker: The Rat Liver PPARα Biomarker as an Example



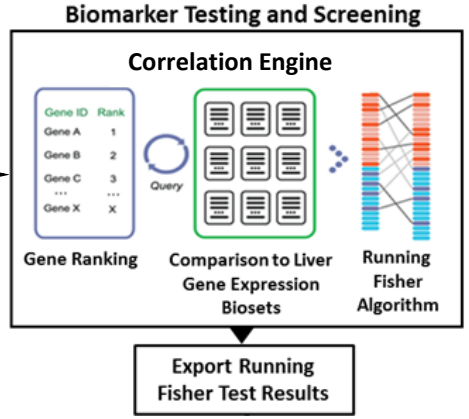
4, 8, 29d
4, 15, 29d



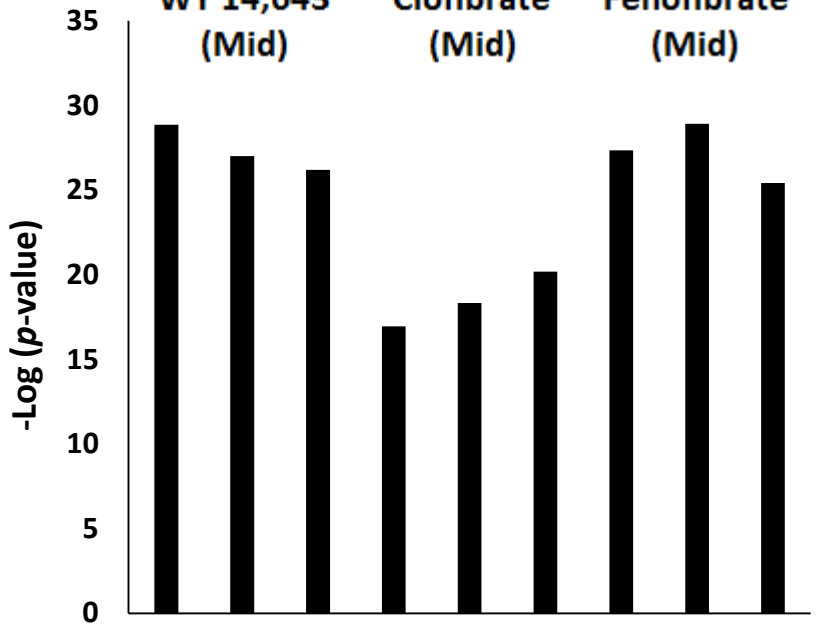
Time (days) 8 15 29 4 8 29 4 8 29

WY 14,643 (Mid) Clofibrate (Mid) Fenofibrate (Mid)

PPARα Biomarker (58 genes)



Microarray data sets from TG-GATES study



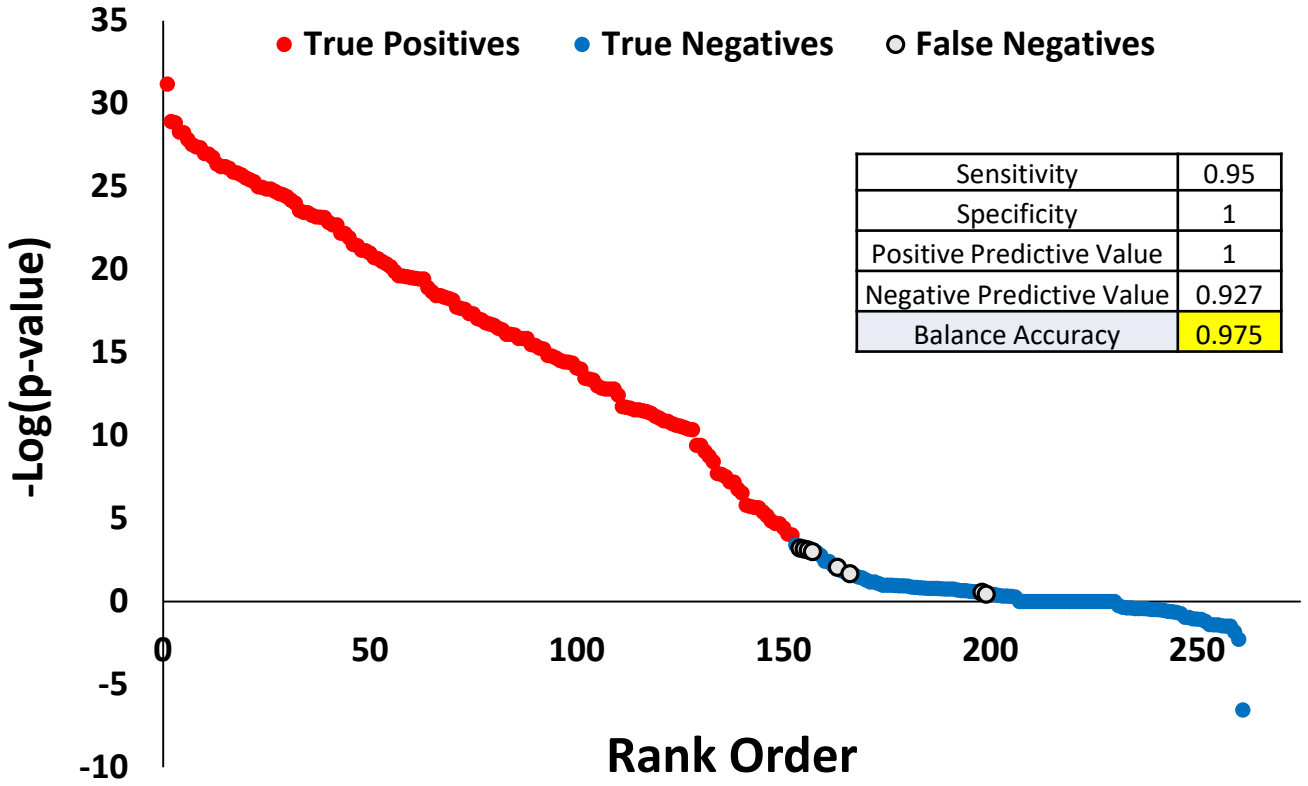
<u>Weight of evidence gene selection</u>

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

Rooney et al., (2018) *Tox App Pharmacol*

Testing the Rat Liver PPARα Biomarker for Predictive Accuracy

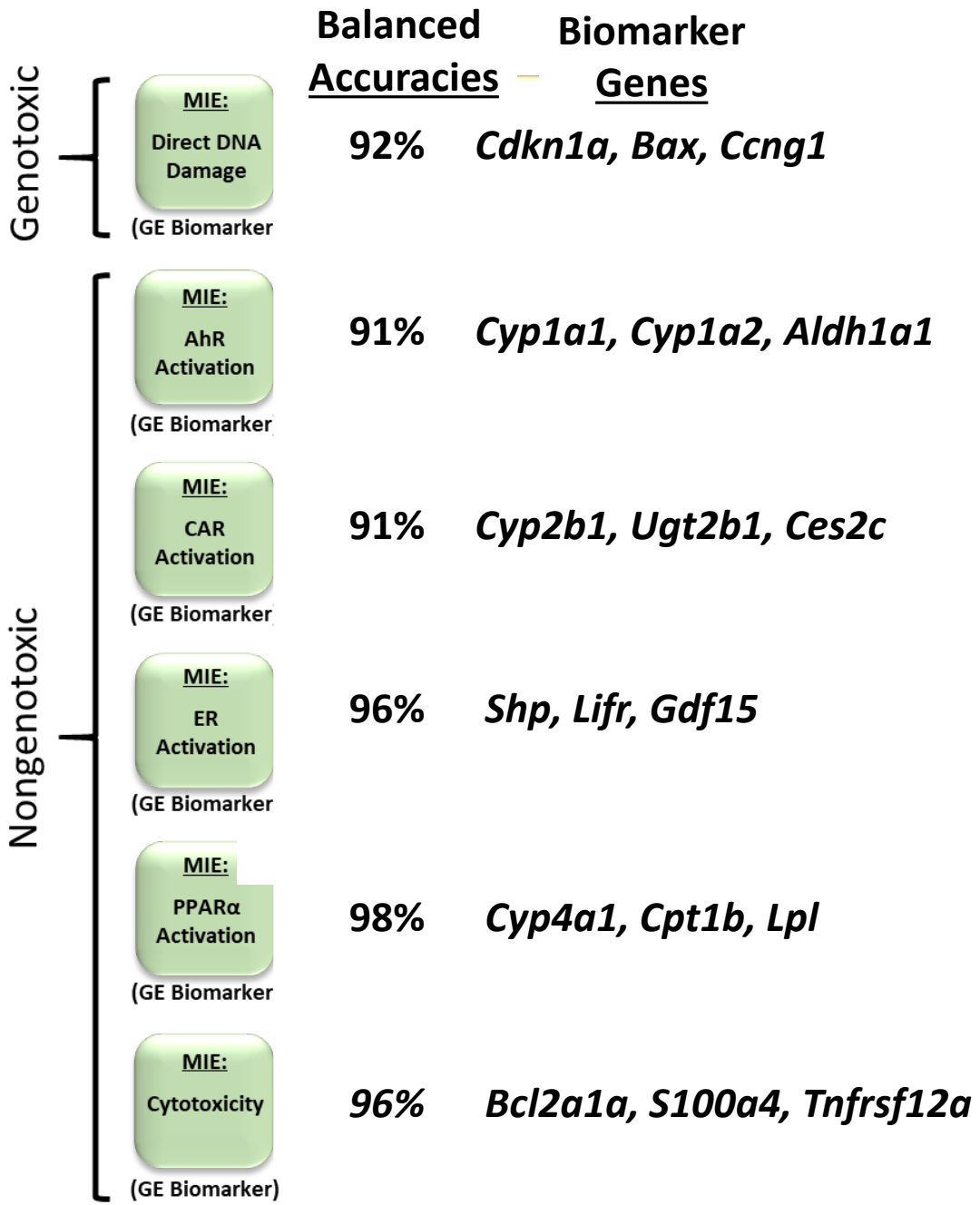
- Examined the ability of the biomarker to correctly identify comparisons known to either activate/not activate PPARα in rat liver (261 comparisons)
- A cutoff of –Log(p-value) = 4 used in prior studies
- Excluded comparisons used to create the biomarker



Rooney et al., (2018) *Tox App Pharmacol*

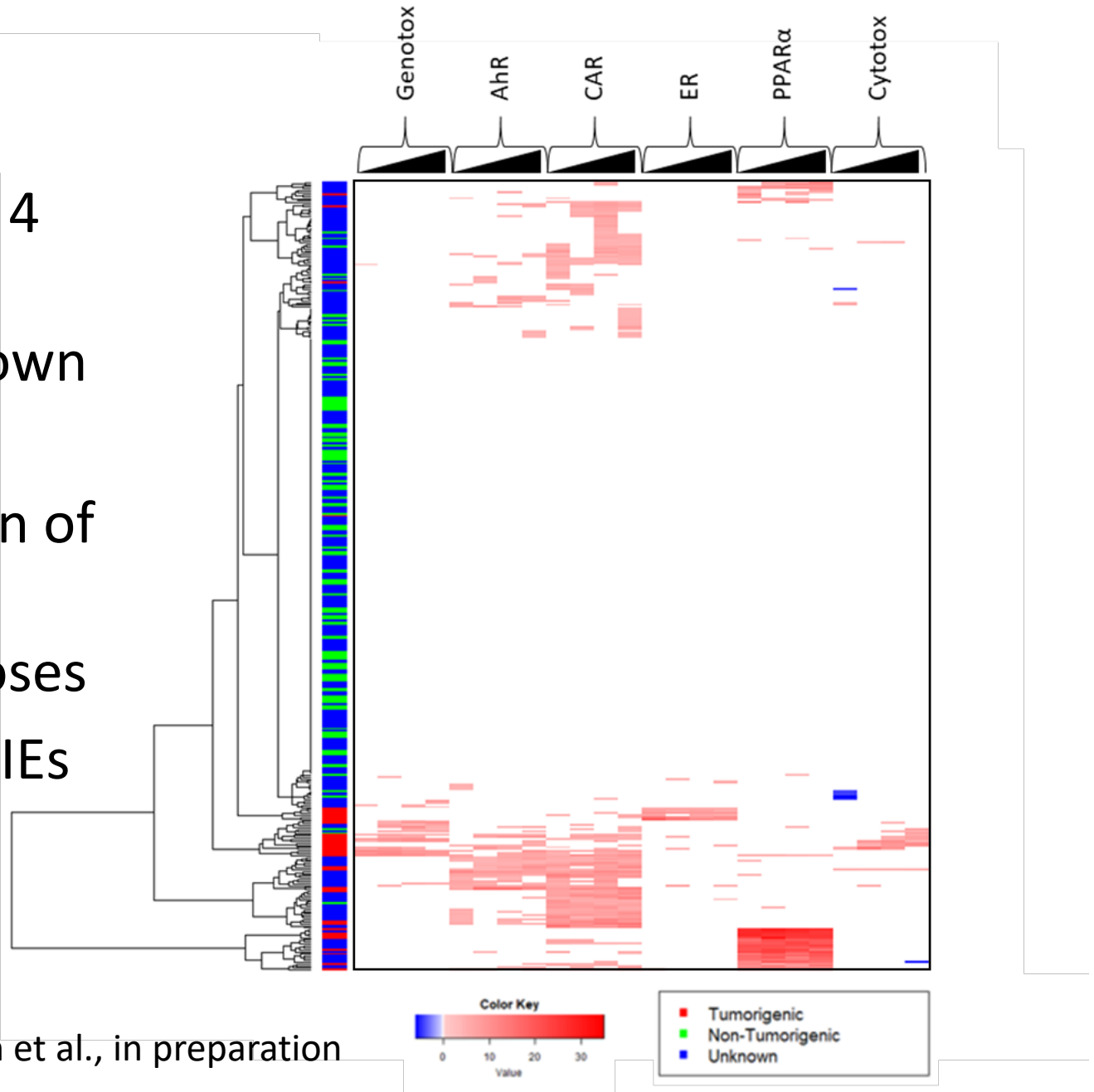
Predictive Accuracies of Six Gene Expression Biomarkers

- All biomarkers have balanced accuracies above 90%
- Genes identified are known to be regulated by the factor



Biomarker Scores across the TG-GATES Study

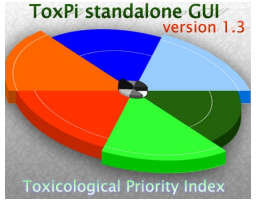
- Activation of the 6 MIEs across the 4 time points; one-dimensional clustering; -Log(p-value)s being shown
- Most of the chemical-dose-time conditions did not lead to activation of any of the MIEs and those were associated with nontumorigenic doses
- Activation of one or more of the MIEs were associated with tumorigenic doses

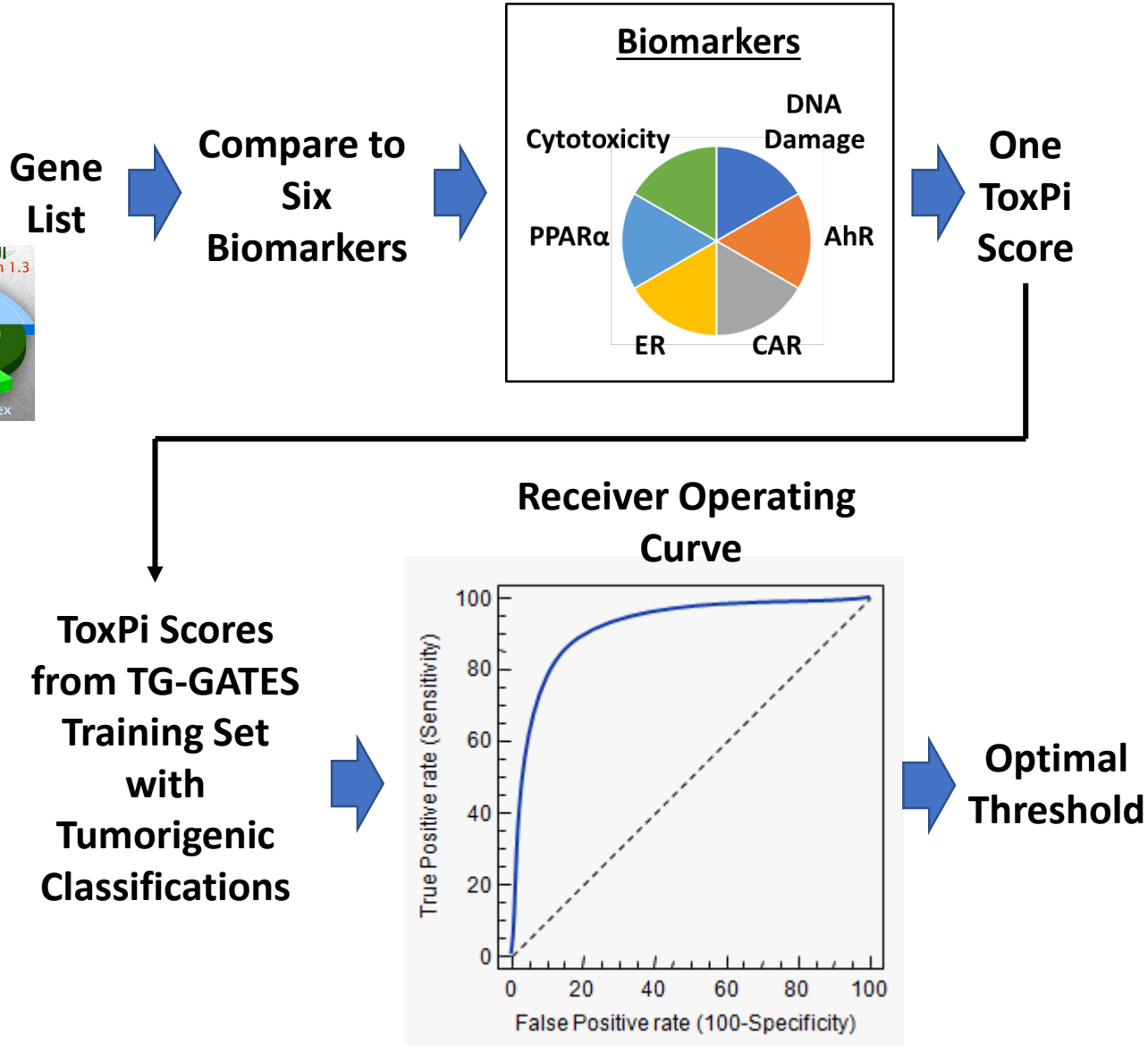


From Corton et al., in preparation

Methods for Identification of Tumorigenic Chemicals

- Compare each chemical-dose-time bioset to each of the 6 biomarkers to get one ToxPi score
  - Using the –Log(p-value)s





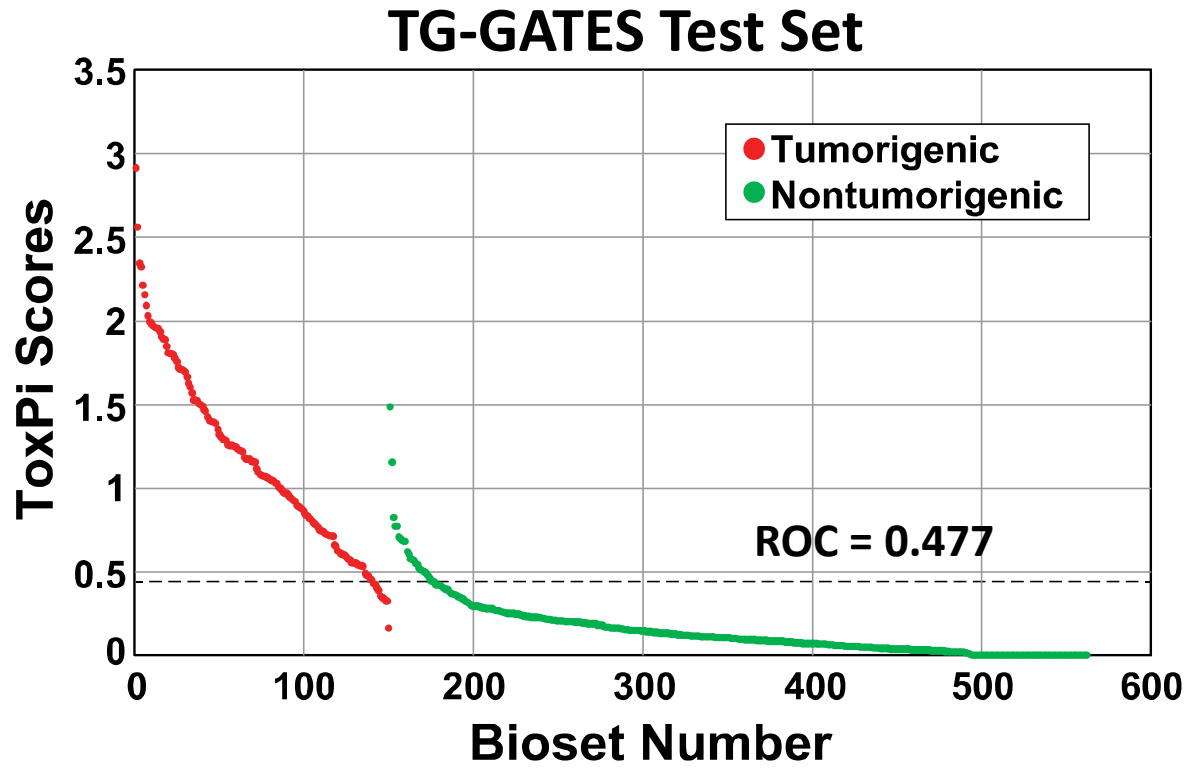
- Divided the TG-GATES study into training and test sets
- DeLong, DeLong, and Clarke-Pearson receiver operating curve (ROC) analysis to determine the optimal threshold in the training set; ROC = 0.477

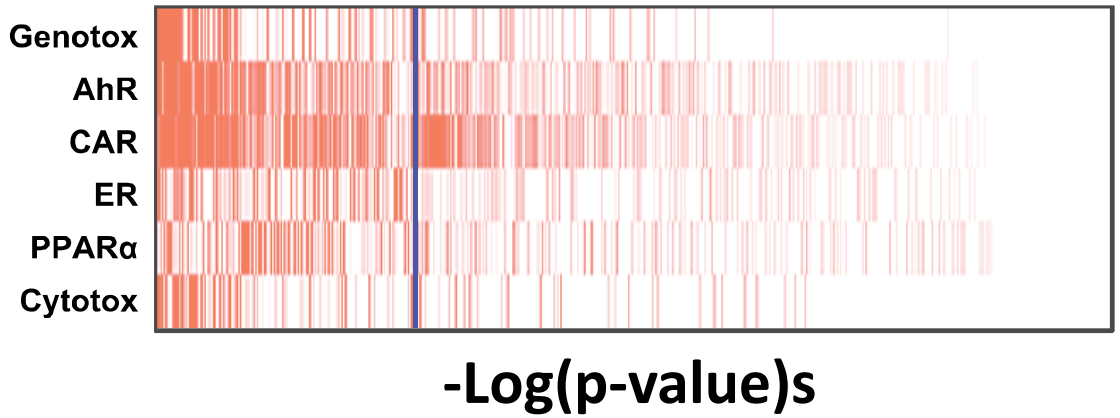
From Corton et al., in preparation

Prediction of the 6 MIEs Identifies Liver Tumorigens

- Applied the ROC = 0.477 to the test set: 90% sensitivity, 97% specificity, and a **balanced accuracy of 93%**

- Out of 44 rat liver tumorigens, only two (5%) were not predicted (acetamide, ethionine)
  - These chemicals may work through different AOPs





From Corton et al., in preparation

Summary

- Gene expression biomarkers
  - Built using transcript profiles from tissues in which the factor is known to be regulated
  - There are multiple methods that can be used to identify biomarker genes including weight of evidence and machine learning
  - To determine predictive accuracy, important to have a good reference dataset to compare to

- An AOP-guided computational approach can be used to identify tumorigens
  - Showed an example of identifying liver tumorigens in prospective studies

- Approach could be applied to predicting cancer in other tissues dependent on:
  - Knowledge of AOPs that lead to cancer
  - A robust dataset including reference chemicals

Supporting Materials

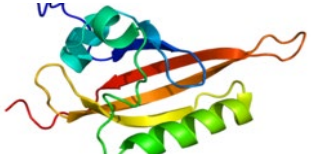
- Adverse Outcome Pathways
  - Link to Wiki: https://aopwiki.org/
  - General reviews of AOPs
    - Carusi et al., (2018) *Sci Total Environ*. 628–629:1542.
    - Ankley and Edwards (2018) *Curr Opin Toxicol*. 9:1.
    - Leist et al., (2017) *Arch Toxicol*. 91:3477.
    - Vinken et al., (2017) *Arch 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 reviewers on the construction and use of gene expression biomarkers
  - Li et al., (2017) *Proc Natl Acad Sci USA*. 114:E10881–E10889.
  - Corton et al., (2019) *Toxicol Appl Pharmacol*. 380:114683.
  - Corton (2019) *Current Opinion in Toxicol* 18:54.

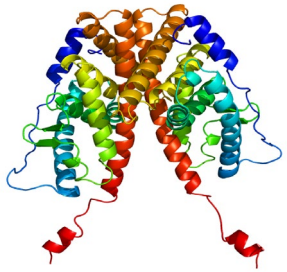
- Construction and use of rat liver gene expression biomarkers
  - Rooney et al., (2018) *Toxicol Appl Pharmacol*. 356:99.

Biomarkers That Predict Key Events in the Livers of Mice and Rats

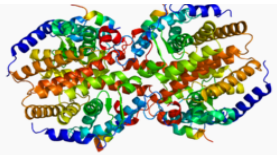
AhR



Estrogen Receptor α

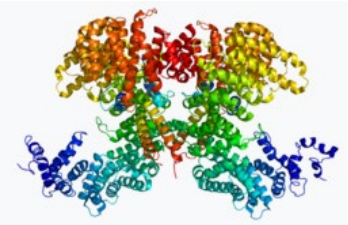


CAR

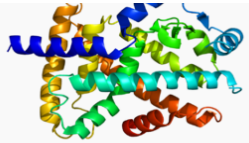


NRF2

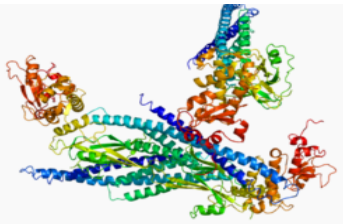
SREBP



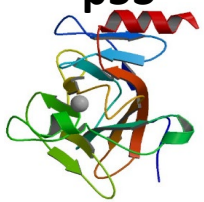
PPARα



STAT5b



p53



- 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.

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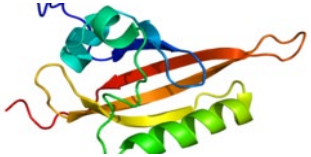
AhR



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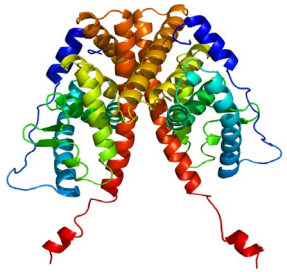
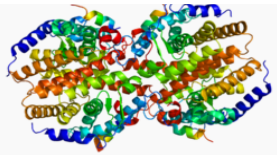
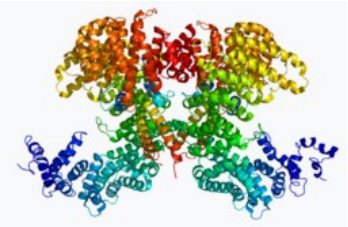


CAR

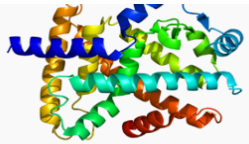


NRF2

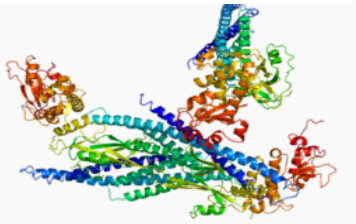
SREBP



PPARα



STAT5b



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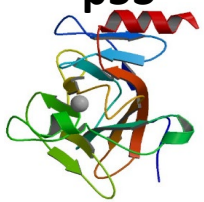

- Rooney et al., (2017). Genomic Effects of Androstenedione and Sex-Specific Liver Cancer Susceptibility in Mice. *Toxicol Sci*. 160(1):15–29.
- Rooney et al., (2018) Activation of Nrf2 in the Liver is Associated with Stress Resistance Mediated by Suppression of the Growth Hormone-Regulated STAT5b Transcription 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.