Towards replacing the two-year bioassay with short-term NAMs: genomic and nongenomic activation levels can identify rat liver tumorigens

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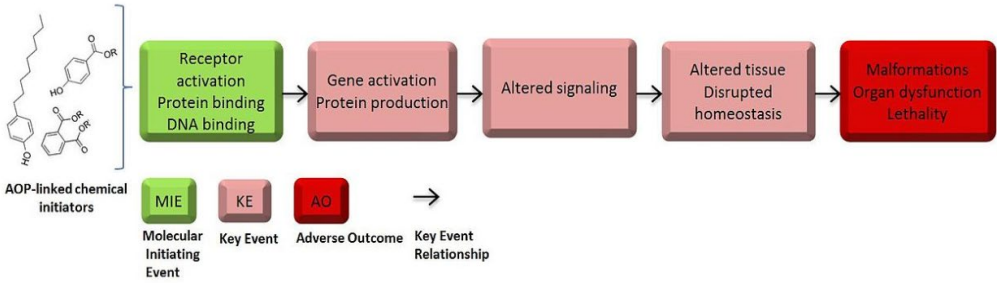
Disclaimer

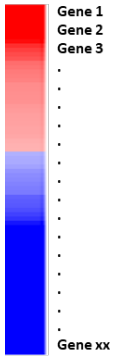
- The views expressed are those of Dr. Chris Corton and do not reflect US-EPA policy or product endorsement by the US-EPA.

Outline

- Description of methods and gene expression biomarkers used in studies
- Gene expression biomarkers in short-term animal studies can identify liver tumorigens
- Identification of biological activation levels predictive of liver cancer
  - Gene expression biomarkers
  - Individual genes
- Identification of biological activation levels predictive of liver cancer
  - 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 activation levels

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

Use of biomarkers and activation levels to inform carcinogenic risk and mode of action

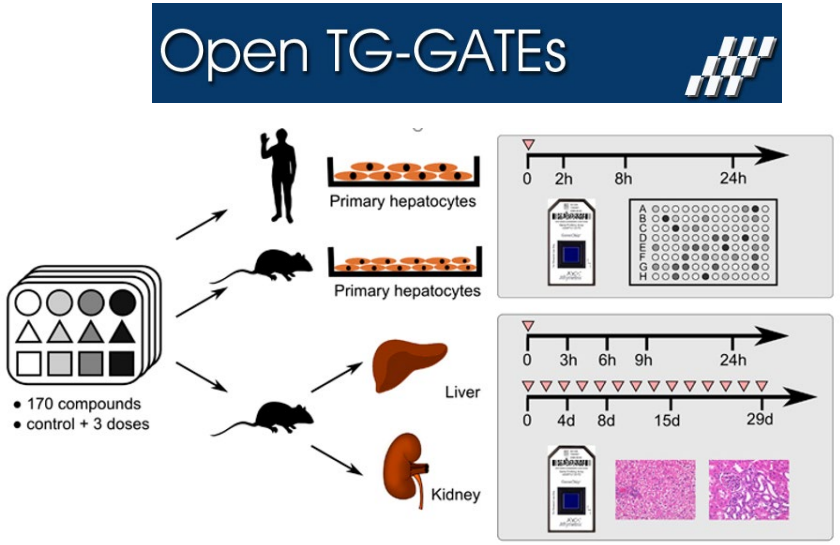
Problem: how can we better use 21st century tools in a prospective manner to avoid unnecessary 2-year bioassays?

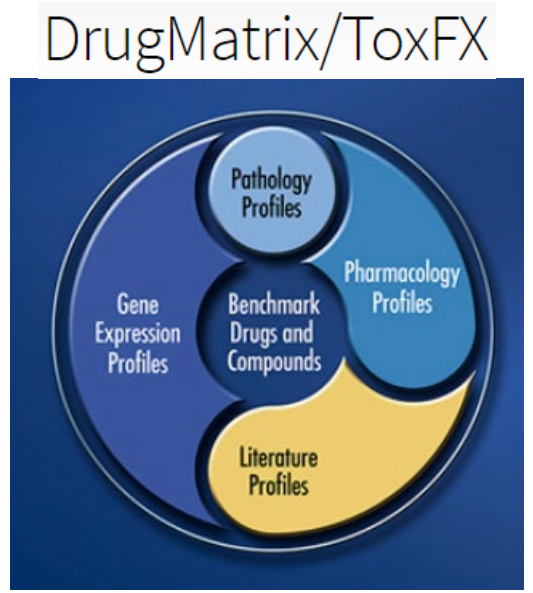
Can we predict from short-term studies:

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

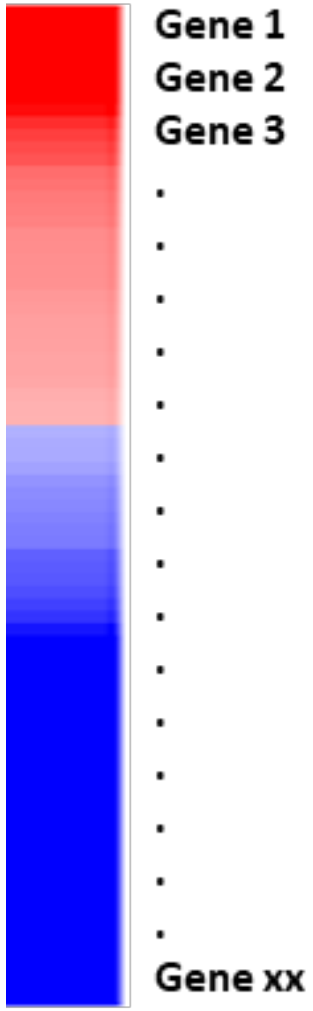
Sources of Rat Liver Tumorigenicity and Microarray Data

- TG-GATES microarray data (rat full genome)
  - ~130 chemicals, 8 time points, 3 doses
- DrugMatrix microarray data (rat full genome)
  - >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



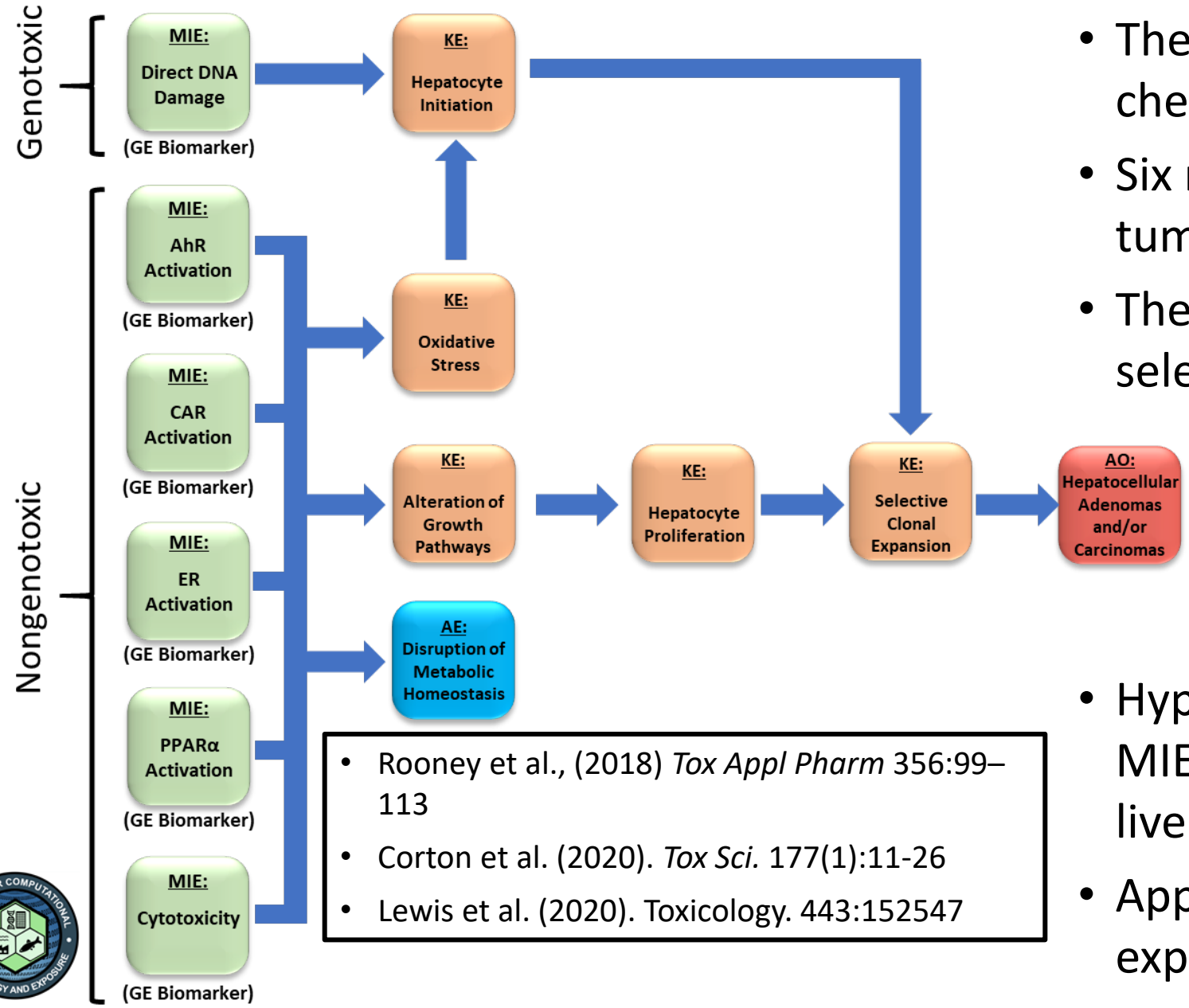


Gene Expression Biomarkers



- List of genes and associated fold-change values or ranks
- Indirectly 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
- Biomarkers that predict MIEs in mouse liver: AhR, CAR, PPARα, Nrf2, Stat5b, SREBP (multiple publications)
- Biomarkers that predict MIEs in rat liver: DNA damage, AhR, CAR, ER, PPARα, Cytotoxicity (Corton et al. (2020). *Tox Sci.* 177(1):11-26)
- Levels of biomarker activation are associated with liver tumor incidence (Hill et al. (2020) ToxSci 177(1):41-59)

Major Adverse Outcome Pathways That Lead to Rodent Liver Tumors



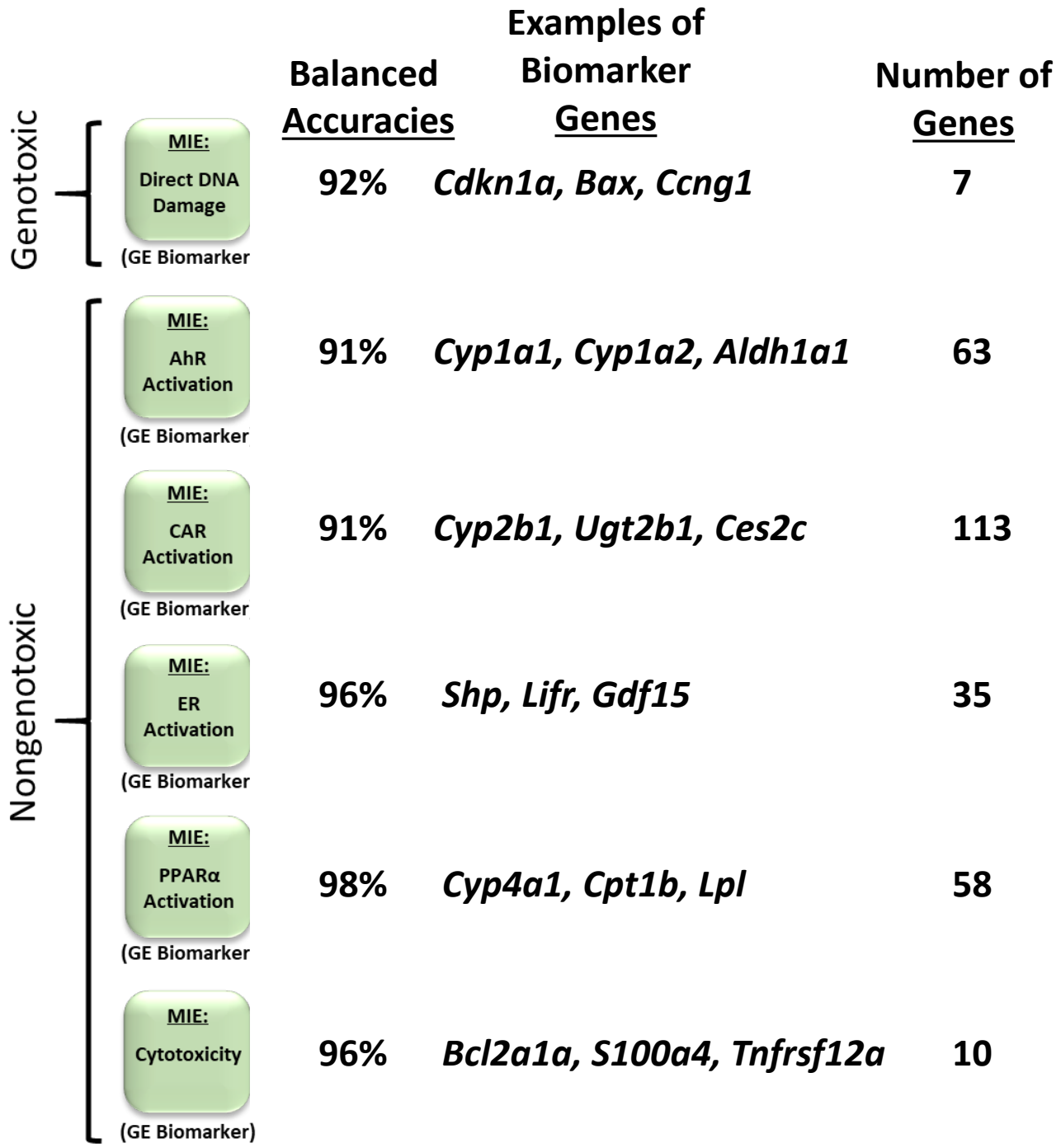
- Rooney et al., (2018) *Tox Appl Pharm* 356:99–113
- Corton et al. (2020). *Tox Sci.* 177(1):11-26
- Lewis et al. (2020). Toxicology. 443:152547

- The liver is the most frequent target of chemical tumorigens
- Six major AOPs lead to rodent liver tumors
- The AOPs converge on the key event of selective clonal expansion

- Hypothesis: measurement of the six MIEs will be sufficient to predict rodent liver tumors
- Approach: measure MIEs with gene expression biomarkers

Predictive Accuracies of Six Gene Expression Biomarkers

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



	Balanced Accuracies	Examples of Biomarker Genes	Number of Genes
Genotoxic	92%	*Cdkn1a, Bax, Ccng1*	7
Nongenotoxic	91%	*Cyp1a1, Cyp1a2, Aldh1a1*	63
Nongenotoxic	91%	*Cyp2b1, Ugt2b1, Ces2c*	113
Nongenotoxic	96%	*Shp, Lifr, Gdf15*	35
Nongenotoxic	98%	*Cyp4a1, Cpt1b, Lpl*	58
Nongenotoxic	96%	*Bcl2a1a, S100a4, Tnfrsf12a*	10

- 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

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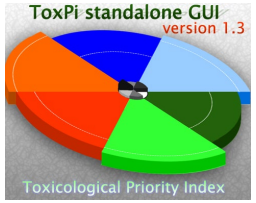
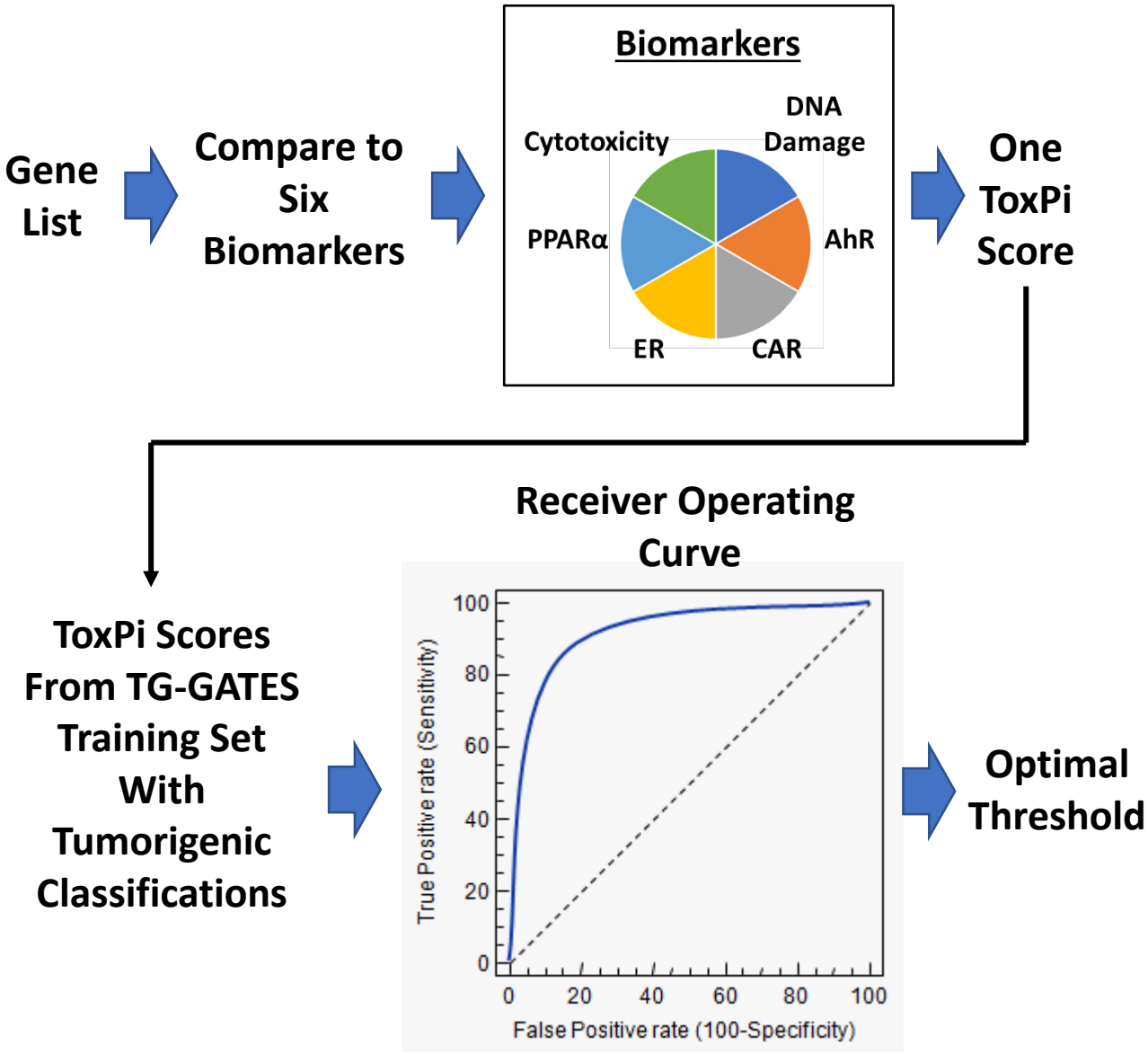
Methods for identification of tumorigenic chemicals

- 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

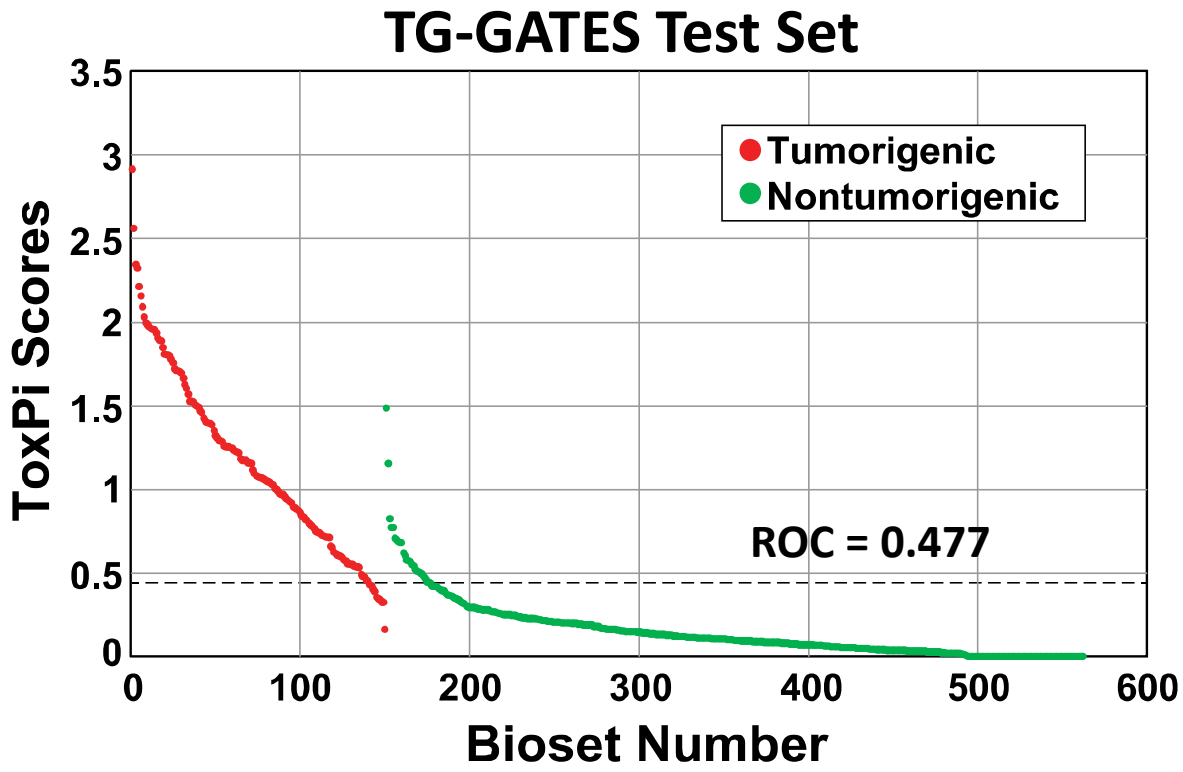


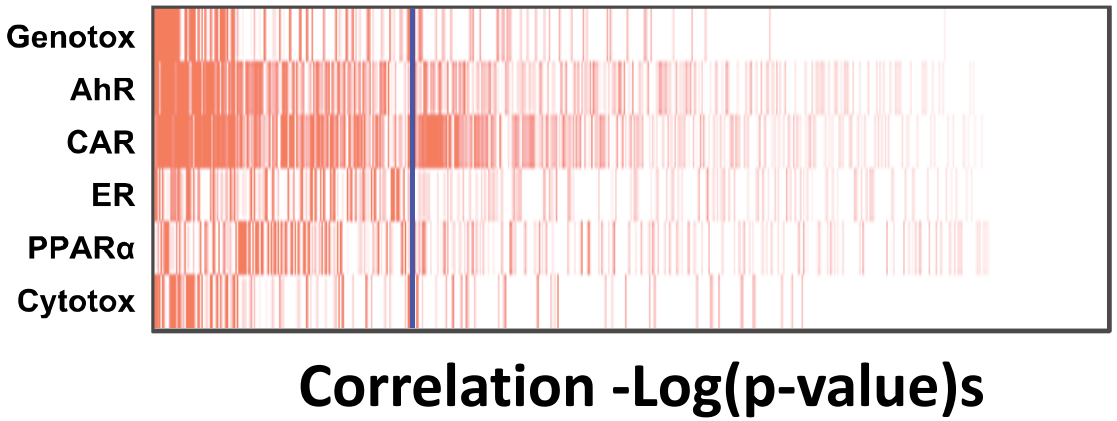
From Corton et al. (2020). *Tox Sci.* 177(1):11-26

Predictions of Six MIEs Identifies Liver Tumorigens

- Used a combination of ToxPi and Receiver Operating Curves to examine a test set of chemicals
- 90% sensitivity, 97% specificity, and a **balanced accuracy of 93%**

- Out of 38 rat liver tumorigens, only two (5%) were not predicted (acetamide, ethionine)
  - These chemicals may work through different AOPs
  - Allows a better understanding of the weaknesses of the approach





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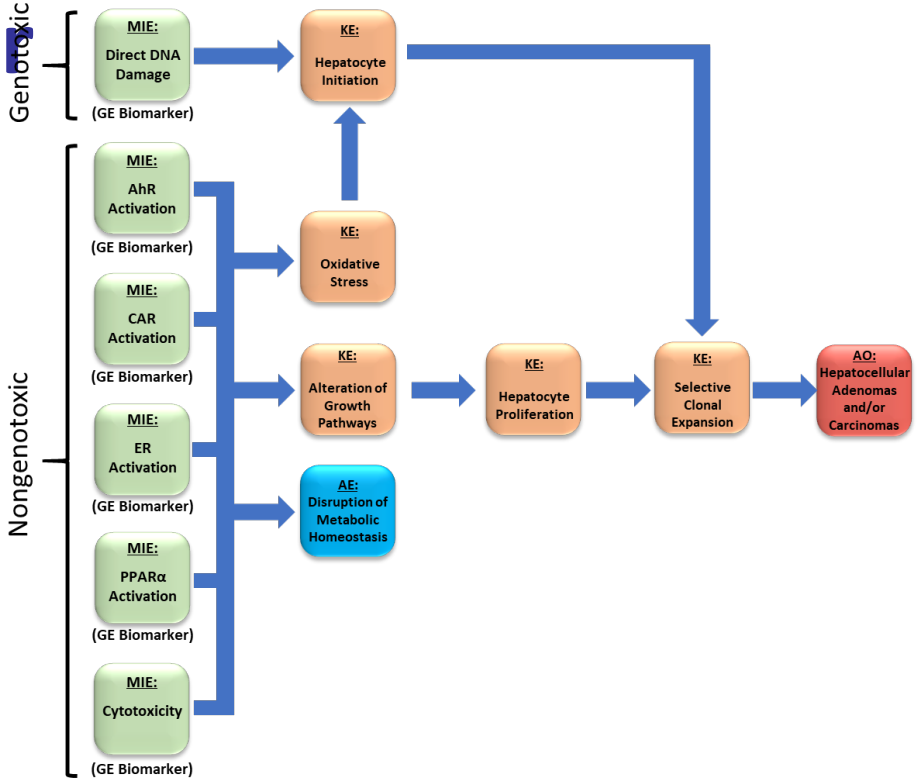
Defining biological activation levels for liver cancer

- Central premise of AOP framework: key events are necessary but not sufficient
  - Depends on the degree or amount of disruption to the particular key event

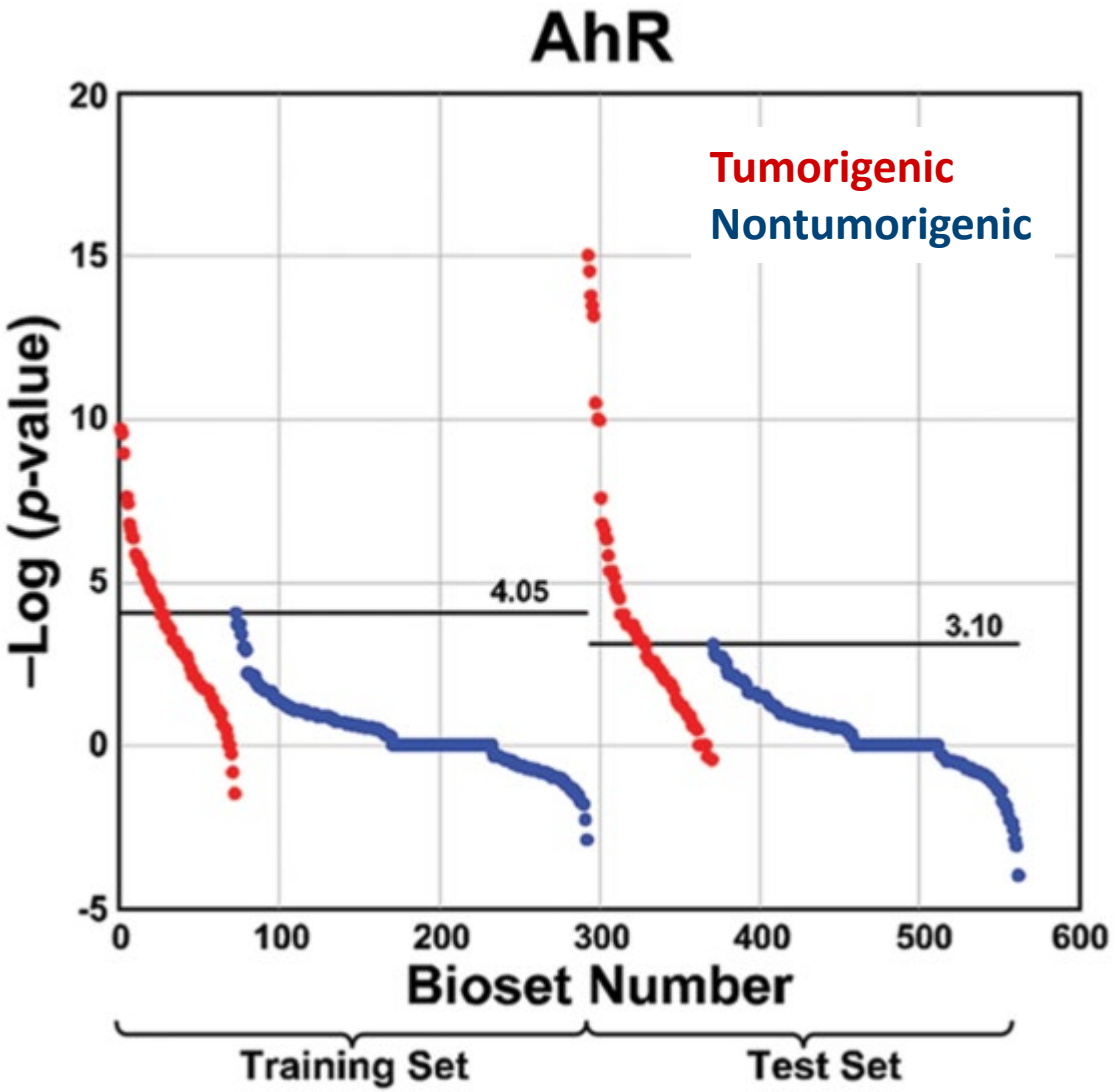
- Can we define activation levels "tipping points" for each of the MIEs?



http://www.silverdoctors.com

Identification of activation levels for gene expression biomarkers

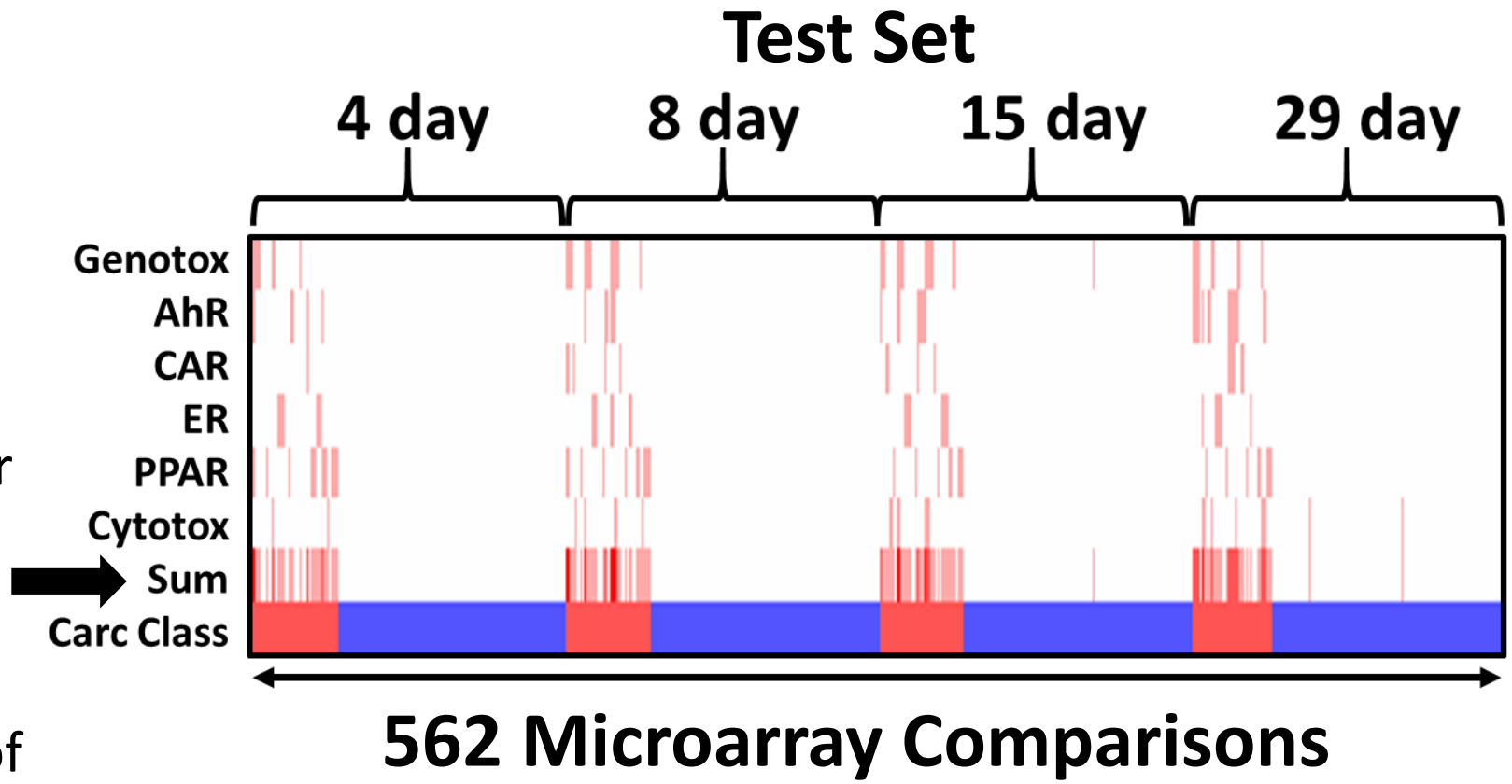
- Divided the chemical-dose conditions into tumorigenic and nontumorigenic groups and training and test sets
- Activation levels defined as the maximum value in the nontumorigenic group
- Activation levels were similar between the training and test sets
- Generated activation levels for all 6 MIEs



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

Biomarker Activation Levels Accurately Predict Liver Tumors

- Identified activation levels 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
- Most of the tumorigenic conditions exceed one or more of the 6 activation levels
- Activation levels rarely exceeded in any of the nontumorigenic conditions



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

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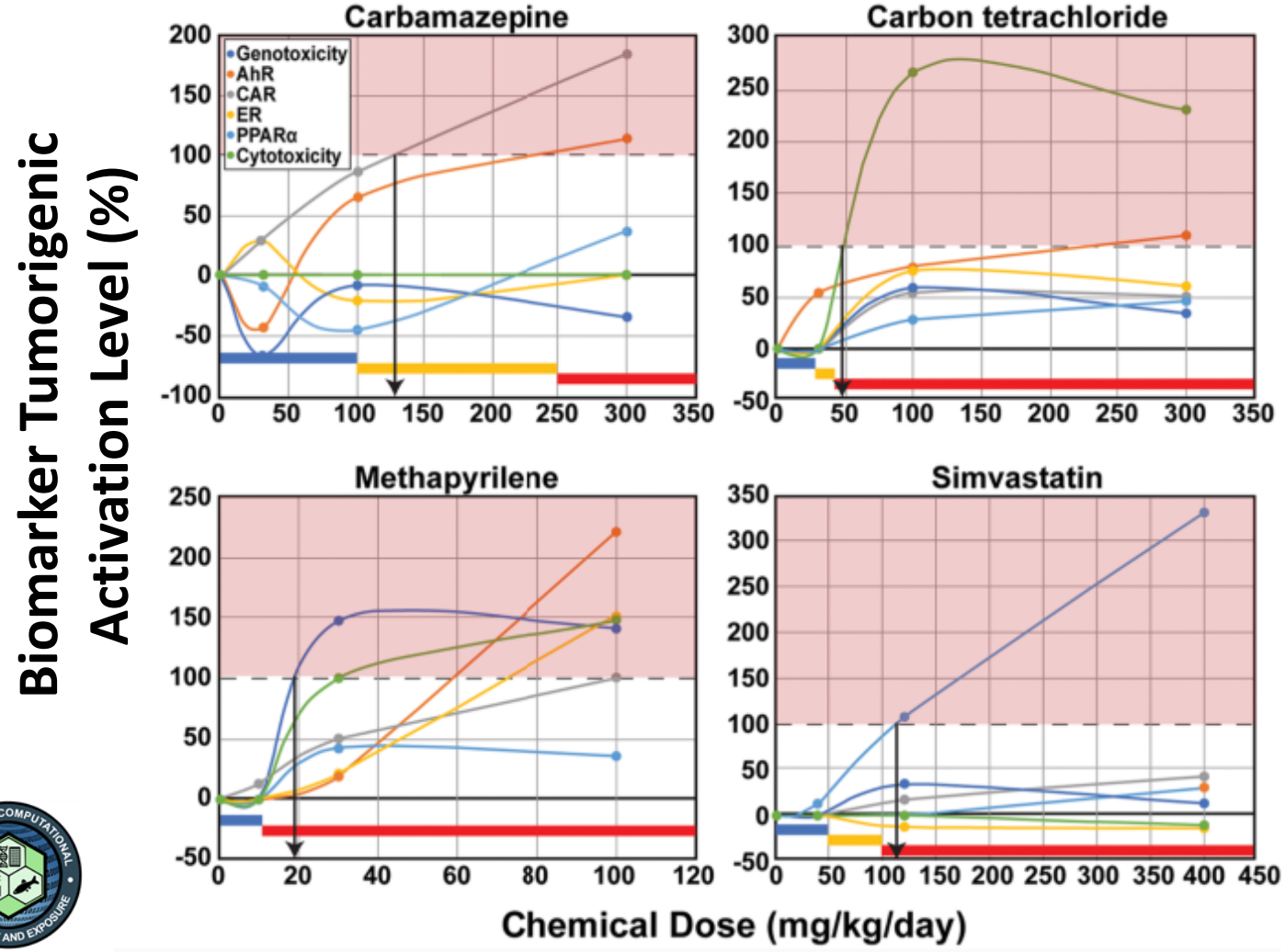
Application of Biomarkers and Activation Levels to Model Liver Tumorigens

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



Genotoxicity
AhR
CAR
ER
PPARα
Cytotoxicity

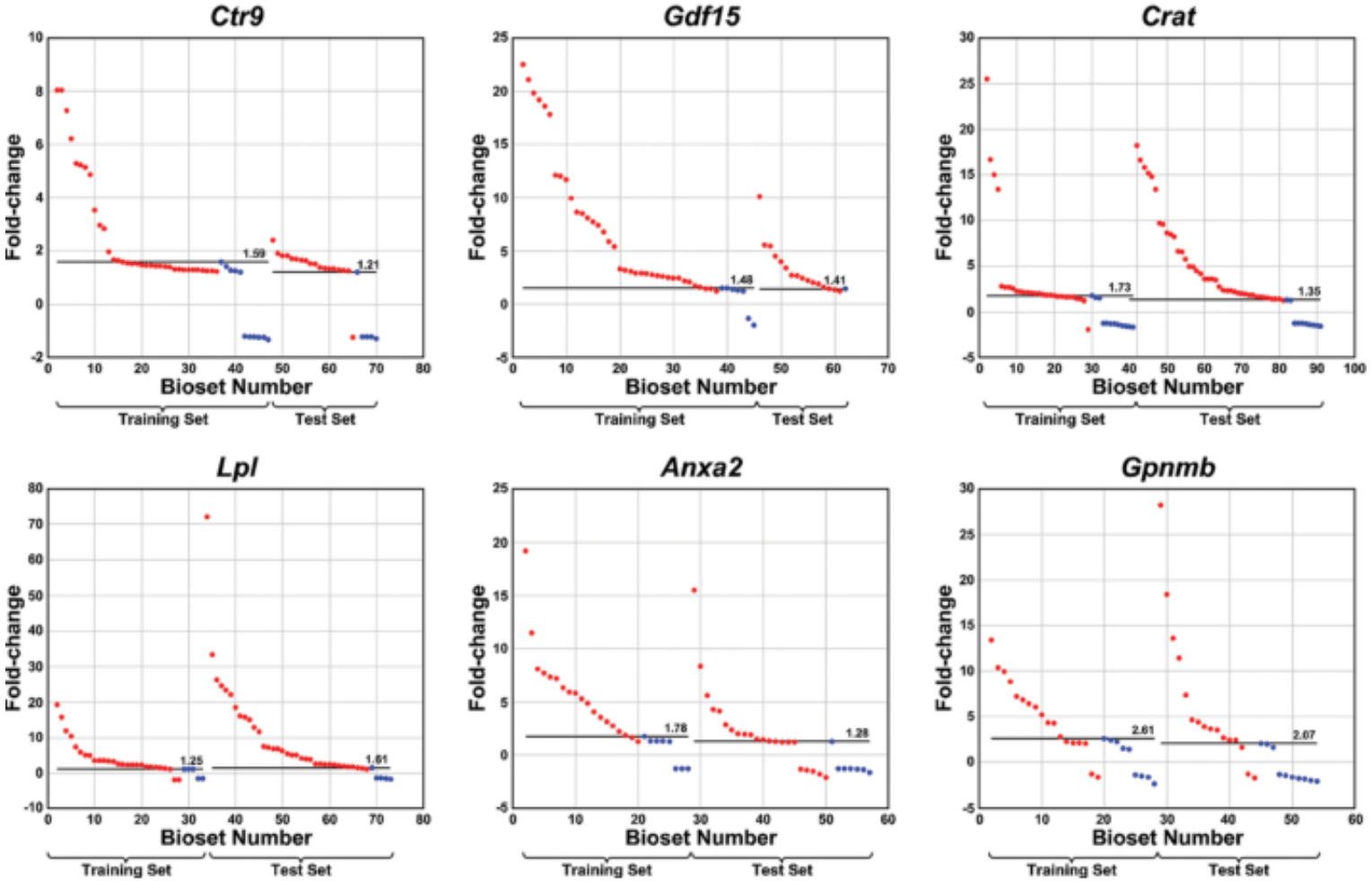
Nontumorigenic
Uncertain
Tumorigenic

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

Activation levels for individual genes are predictive of liver cancer



- Using activation levels for 12 individual genes (2/biomarker)
  - 100% sensitivity, 80% specificity, and a **balanced accuracy of 90%**

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

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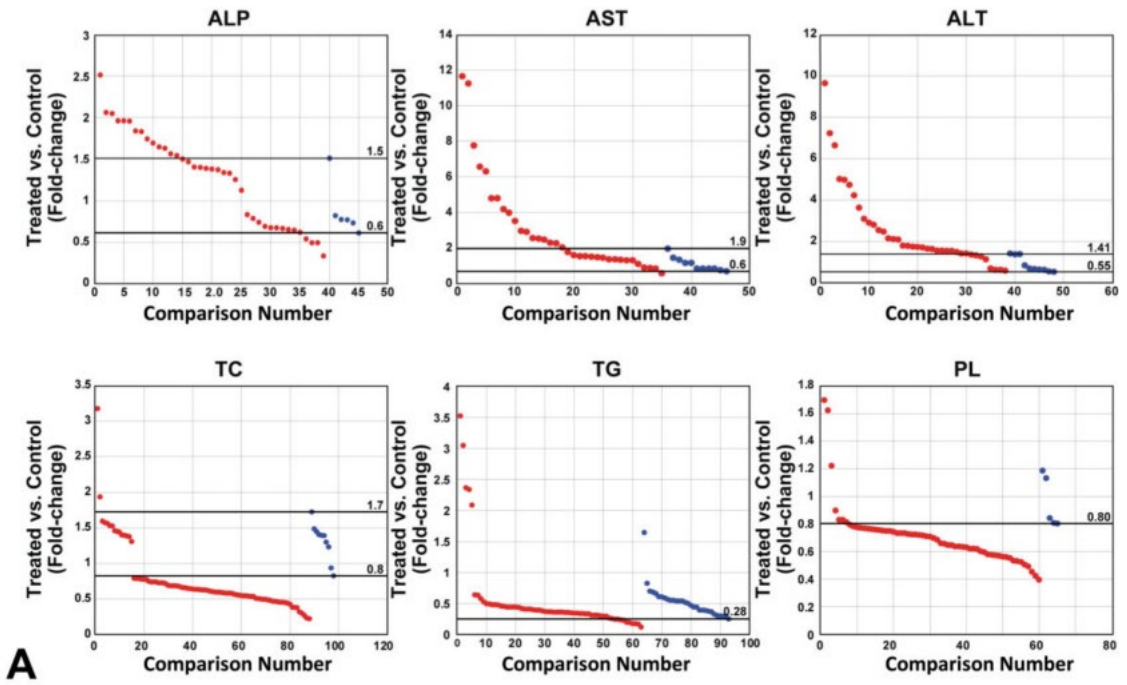
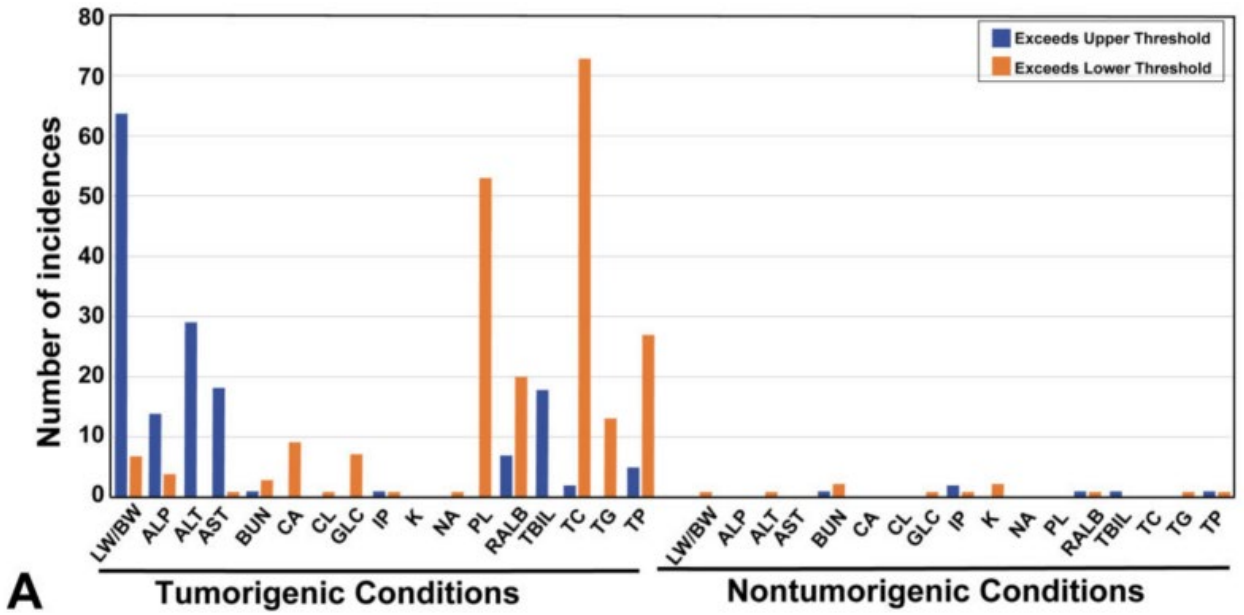
activation levels for liver weights and clinical chemistry endpoints are predictive of liver cancer

Identification of activation levels for ClinChem end points



Number of incidences the indicated upper or lower activation levels were exceeded



- Using activation levels for liver weight to body weight and clinical chemistry endpoints only
  - 88% sensitivity, 100% specificity, and a **balanced accuracy of 94%**

From Corton et al. (2020). Toxicol Path 48(7):857-874

Summary

- An AOP-guided computational approach can be used to identify liver tumorigens in prospective studies
  - Two sets of tools to apply to toxicogenomic studies
    - Gene expression biomarkers
    - Activation levels associated with tumor induction

Emerging Systems Toxicology for the Assessment of Risk Committee (eSTAR)
Carcinogenomics Subcommittee
Co-chairs: Chris Corton, EPA
Keith Tanis, Merck

- Identified clear activation levels 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

- 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

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