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Towards Reduction and Replacement of the 2-Year Rodent Bioassay Using Genomic Approaches: Update From eSTAR and Impact on ICH S1

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EMGS Meeting 09_25_21: Development and Application of Genomic Approaches to Evaluate Human Cancer Risk

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

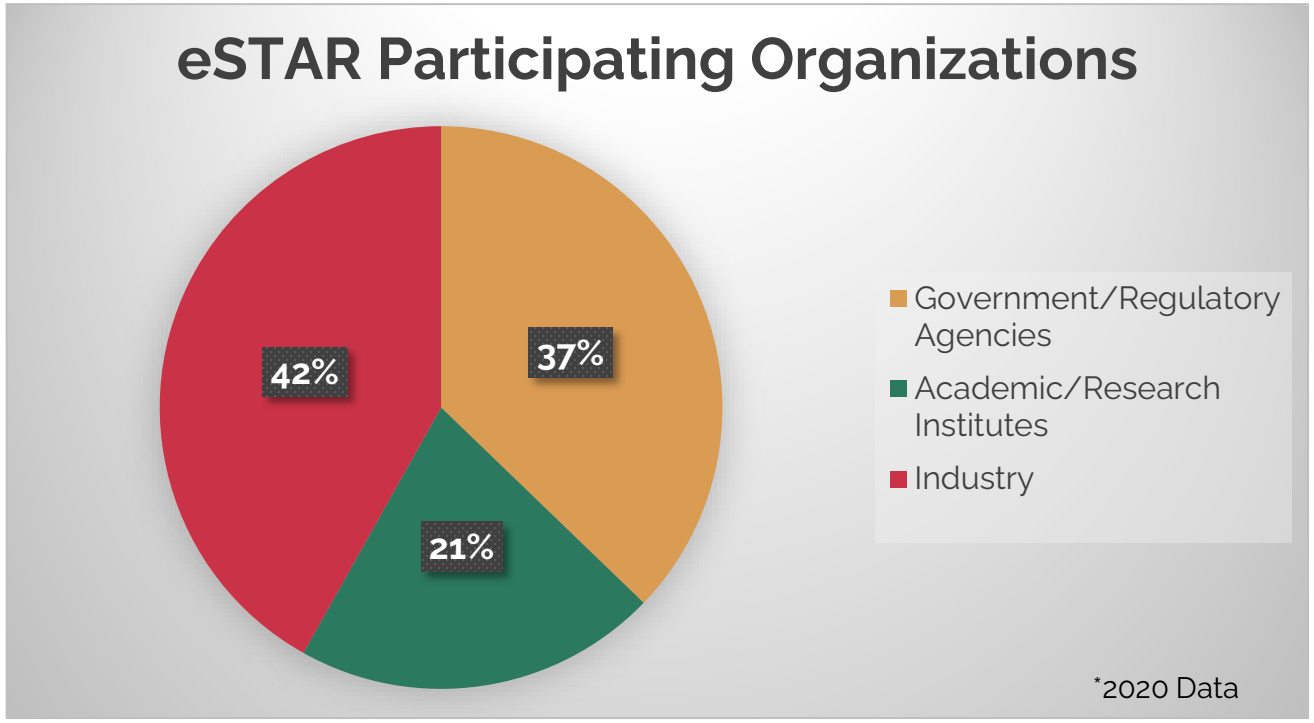
- HESI eSTAR Carcinogenomics project and key drivers
- Description of gene expression biomarkers used in studies
- Gene expression biomarkers can identify chemicals that activate the major adverse outcome pathways for liver tumor induction
- Gene expression biomarkers and their activation levels can identify liver tumorigens

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

The committee's mission is to develop and deliver innovative systems toxicology approaches for risk assessment.

Public Chair
Dr. Brian Chorley (US EPA)

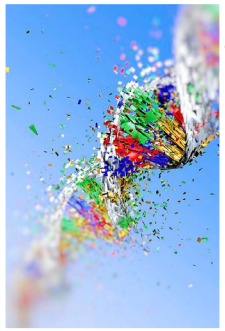
Private Chair
Dr. Kamin Johnson (Corteva Agriscience)



eSTAR Working Groups

Molecular POD

- Using transcriptomic point of departure for chemical risk assessment
- State of the science manuscript in progress

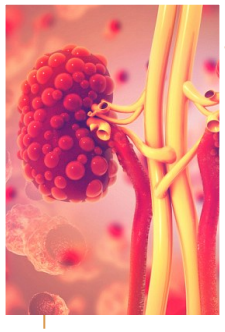


TGx-DDI

- An in vitro transcriptomic biomarker to predict probability that an agent is DDI or non DDI.
- Biomarker Qualification Plan under FDA review
- U01 grant application to fund multi lab validation study

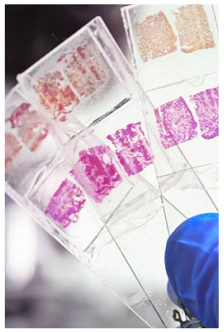
Carcinogenomics

- Goal is to develop predictive genomic tools for earlier recognition of noncarcinogenic molecules to reduce the need for two-year carcinogenicity rat studies



miRNA Biomarkers

- New experimental and/or methodology project to reduce hurdles to the use of miRNAs for translational safety assessment and in biological discovery efforts (kidney miRNAs)
- Recent manuscript accepted:



FFPE

- Developed methods to use formalin-fixed paraffin-embedded (FFPE) blocks for genomic studies
- Currently completing a manuscript on DNA de-modification analysis of clinical tumor samples
- This WG will sunset after publishing the manuscript

eSTAR Carcinogenomics Project Participants

Pharma	
Amgen:	Christine Karbowski
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Corteva:	Kamin Johnson
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GSK:	Deidre Dalmas
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U Leiden	James Stevens
UNC Chapel Hill	Julia Rager
U of Ottowa	Carole Yauk

Regulatory	
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BfArM	Roland Frötschl
EPA:	Brian Chorley Chris Corton Leah Wehmas Roman Mezencev
FDA:	Todd Bourcier Tim McGovern Shraddha Thakkur Tao Chen
Health Canada	Julie Buick Andrew Williams Scott Auerbach
NIEHS:	Jennifer Fostel Pierre Bushel Kevin Gerrish Alison Harrill* Arun Pandiri

- Scientists from 28 organizations in 5 countries

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Evolution of ICH S1

- International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) mandate is to establish and maintain standardized, international guidelines for evaluating potential human health risks of pharmaceuticals
- S1: Rodent Carcinogenicity Studies for Human Pharmaceuticals
  - 2-Year rodent bioassay, usually conducted in rat models
  - Additional lifetime or short-term assay in a second species, typically the mouse
- There is a proposal to waive the rat 2-yr bioassay given sufficient evidence determined by a number of factors including “Special studies and endpoints (**Carcinogenomics**, others)
- eSTAR Carcinogenomics WG will characterize predictive biomarkers that can provide evidence that the rat 2-year bioassay is necessary/not necessary
- The tools will impact carcinogenicity testing by both pharma and chemical industries

eSTAR Carcinogenomics Project Objectives

Objective: Drive international industry, regulatory, and academic understanding and acceptance of a **WOE** approach using new **genomic** tools and endpoints for practical application to **in vivo shorter-term rat studies** that inform on liver tumorigenic risk in the 2-year rat carcinogenicity assay

Strategy is to Measure:

Aim 1: Mechanistic Transcriptional Biomarkers

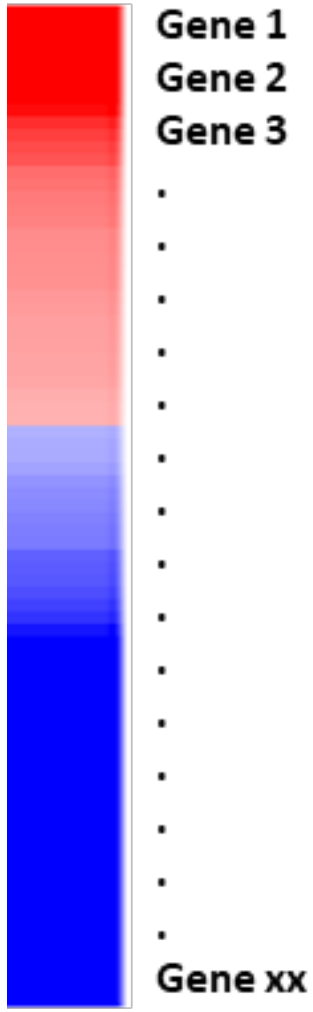
Aim 2: DNA Mutation Expansion

Aim 1: Early identification MIE/KEs of rat carcinogens using mRNA expression assays

Aim 2: Early identification of in vivo mutagenicity/expansion using error corrected sequencing

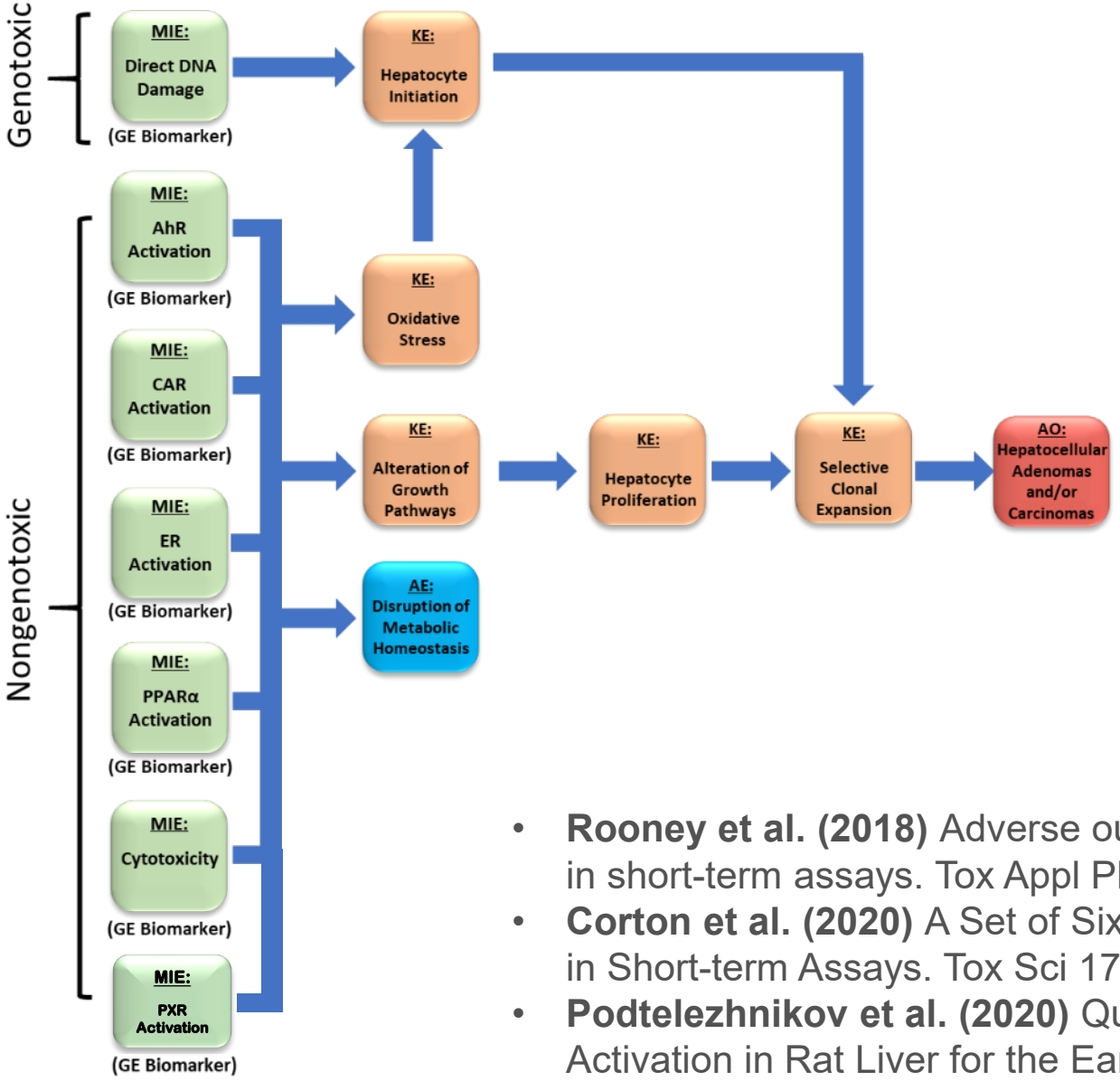
Goal is to apply these tools to pharmaceuticals and industrial chemicals

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 rat liver: DNA damage, AhR, CAR, ER, PPARα, Cytotoxicity (Corton et al. (2020). *ToxSci.* 177(1):11-26 and Podtelezhnikov et al. (2020). ToxSci. 175(1):98-112)
- The eSTAR Carcinogenomics WG is at the stage of building the biomarkers using a comprehensive set of data
  - Literature information about molecular targets of chemicals
  - Liver tumor incidence in rats after exposure to chemicals examined in microarray studies
  - Large set of microarray data (TG-GATES, DrugMatrix, iMARCAR, small academic studies)

Aim 1: Identification MIE/KEs using mRNA expression assays

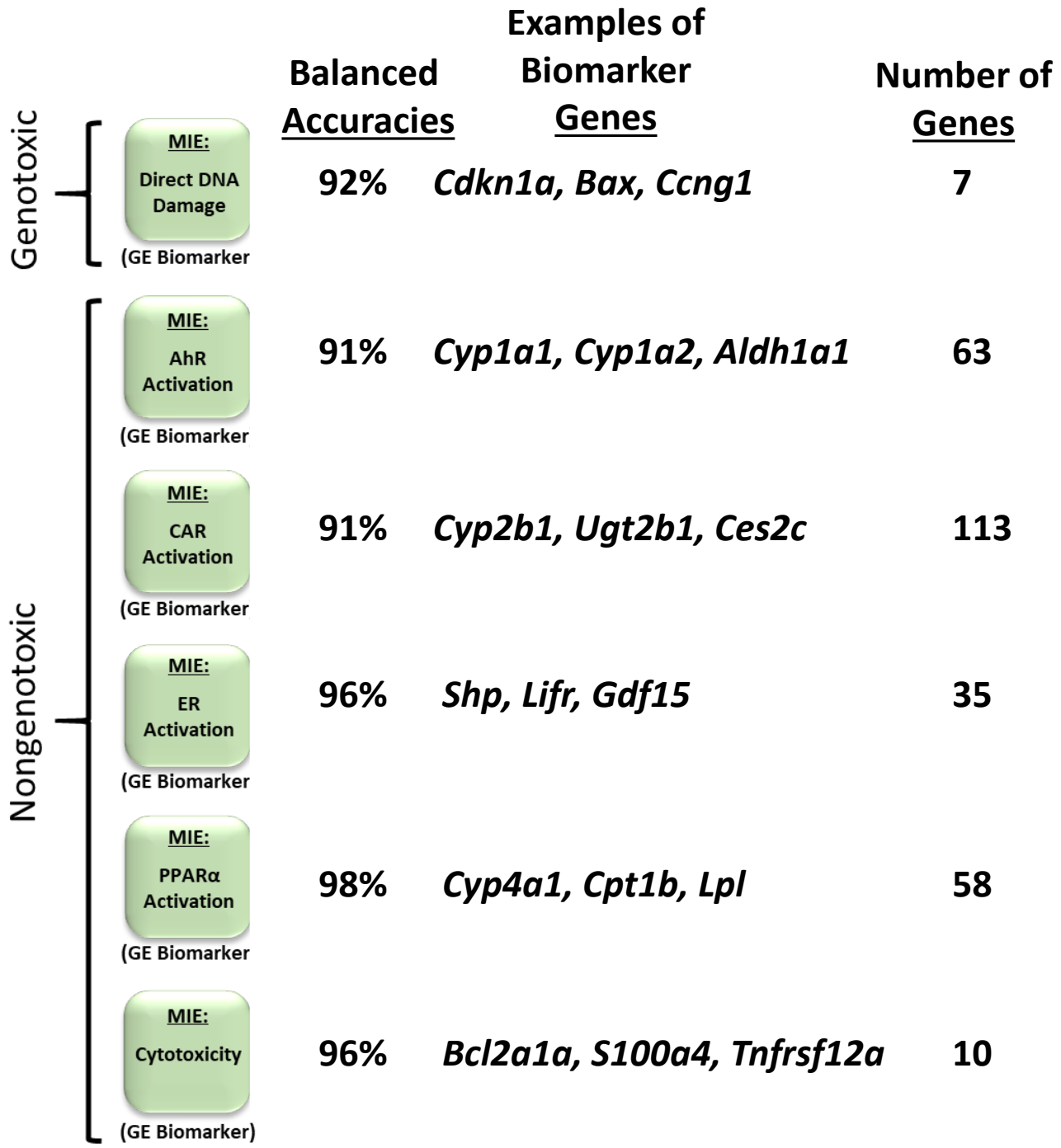


- The liver is the most common target of chemical carcinogens
- Multiple major AOPs lead to rodent cancer
- **Hypothesis 1: Measurement of MIE activation using gene expression biomarkers in short term (~1wk) rat studies will inform on 2-yr carco outcome**
- Initial reports indicate promise

- **Rooney et al. (2018)** Adverse outcome pathway-driven identification of rat liver tumorigens in short-term assays. Tox Appl Pharm 356:99-113
- **Corton et al. (2020)** A Set of Six Gene Expression Biomarkers Identify Rat Liver Tumorigens in Short-term Assays. Tox Sci 177(1):11-26
- **Podtelezhnikov et al. (2020)** Quantitative Transcriptional Biomarkers of Xenobiotic Receptor Activation in Rat Liver for the Early Assessment of Drug Safety Liabilities. Tox Sci 175(1):98-112

Predictive Accuracies of Six Gene Expression Biomarkers

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



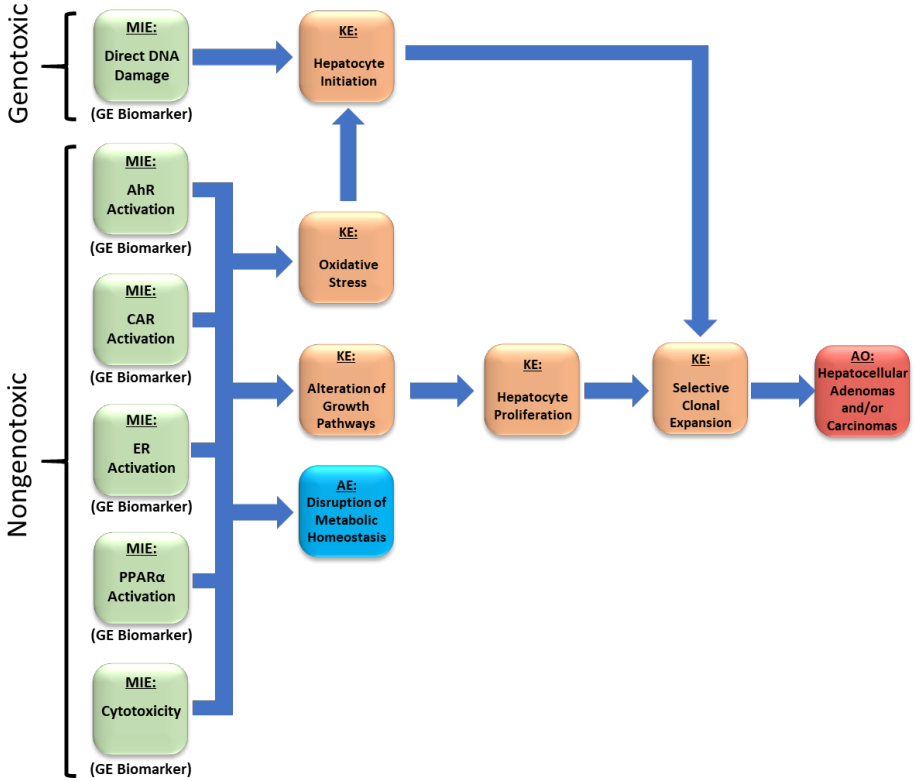
	Balanced Accuracies	Examples of Biomarker Genes	Number of Genes
Genotoxic	92%	*Cdkn1a, Bax, Ccng1*	7
Nongenotoxic	91%	*Cyp1a1, Cyp1a2, Aldh1a1*	63
Nongenotoxic	91%	*Cyp2b1, Ugt2b1, Ces2c*	113
Nongenotoxic	96%	*Shp, Lifr, Gdf15*	35
Nongenotoxic	98%	*Cyp4a1, Cpt1b, Lpl*	58
Nongenotoxic	96%	*Bcl2a1a, S100a4,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

Defining biological activation levels for liver cancer

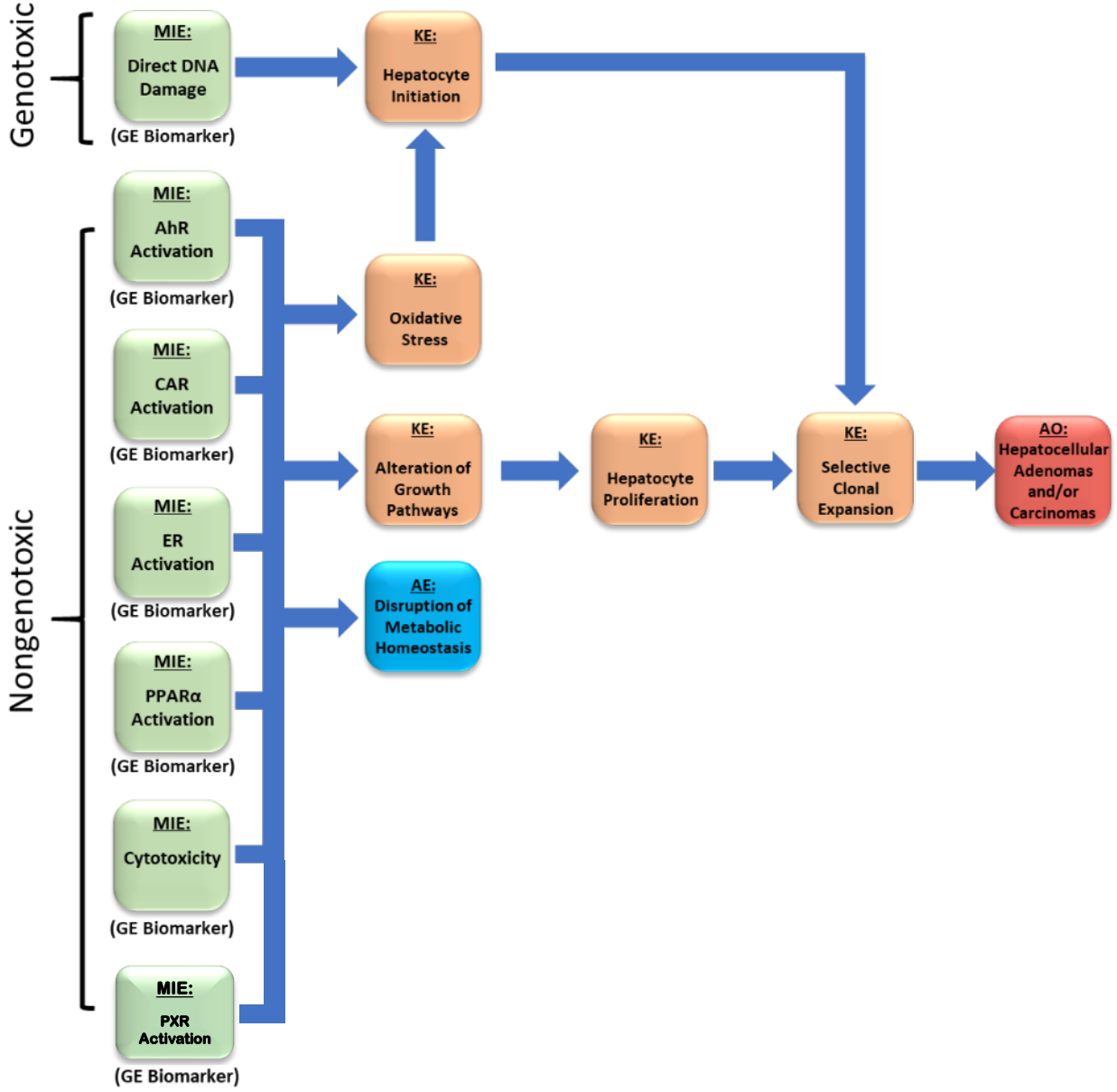
- Central premise of AOP framework: key events are necessary but not sufficient
  - Induction of an AO depends on the degree or amount of disruption to the particular key event

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



http://www.silverdoctors.com

Aim 1: Identification MIE/KEs using mRNA expression assays

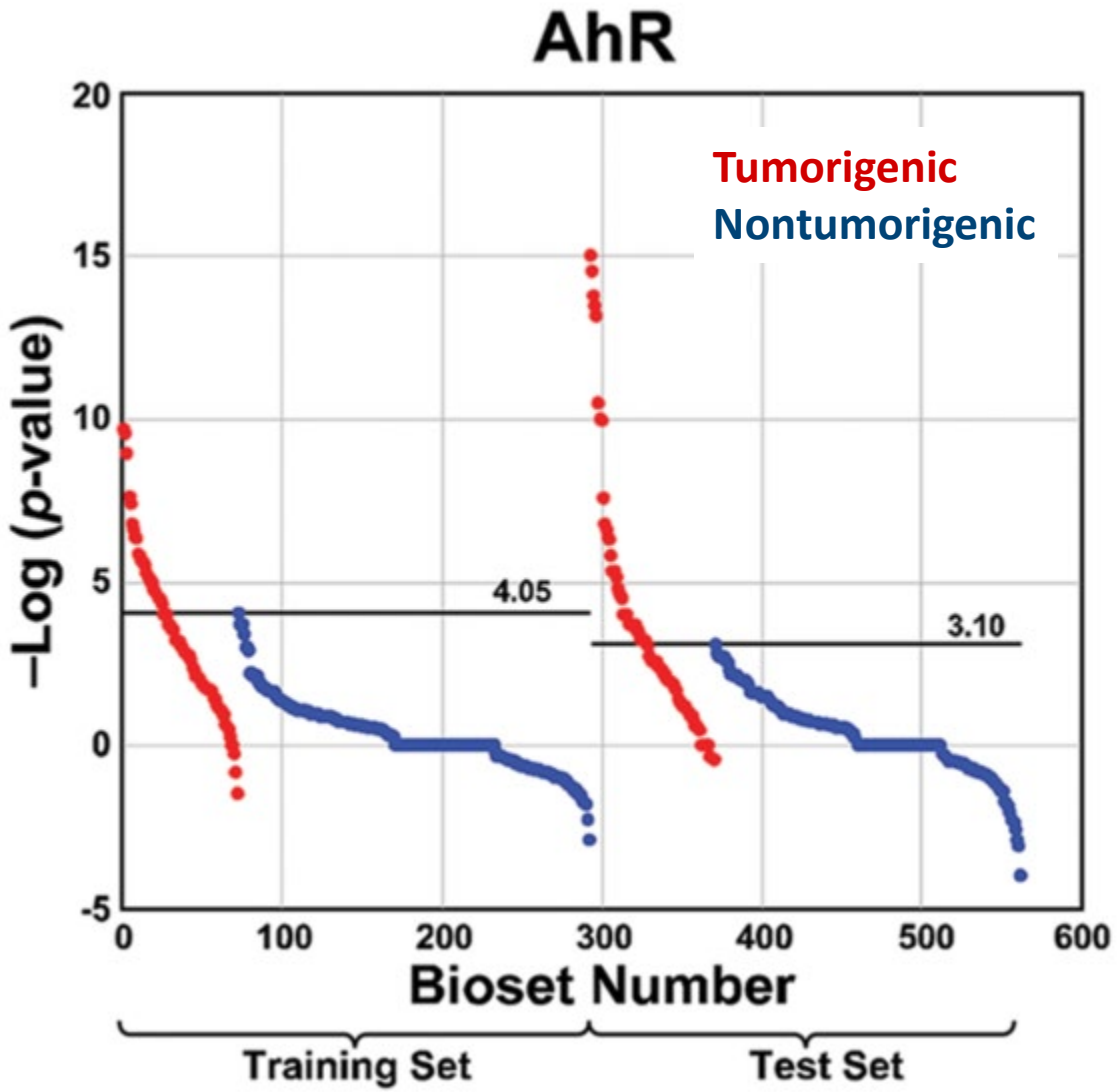


- **Hypothesis 2: MIE signature induction thresholds in short term (~1wk) rat studies will be associated with doses of carcinogenic risk**

- Qin et al. identified ranges AHR signature induction associated with increased carcinogenic risk
  - **Qin et al. (2019)** AhR Activation in Pharmaceutical Development: Applying Liver Gene Expression Biomarker Thresholds to Identify Doses Associated with Tumorigenic Risks in Rats. Tox. Sci. 171(1):46-55.
- Hill et al. identified thresholds for 6 MIE signatures that are predictive of liver tumorigens
  - **Hill et al. (2020)** Gene Expression Thresholds Derived From Short-term Exposures Identify Rat Liver Tumorigens. Tox Sci 177(1):41-59

Identification of activation levels for gene expression biomarkers

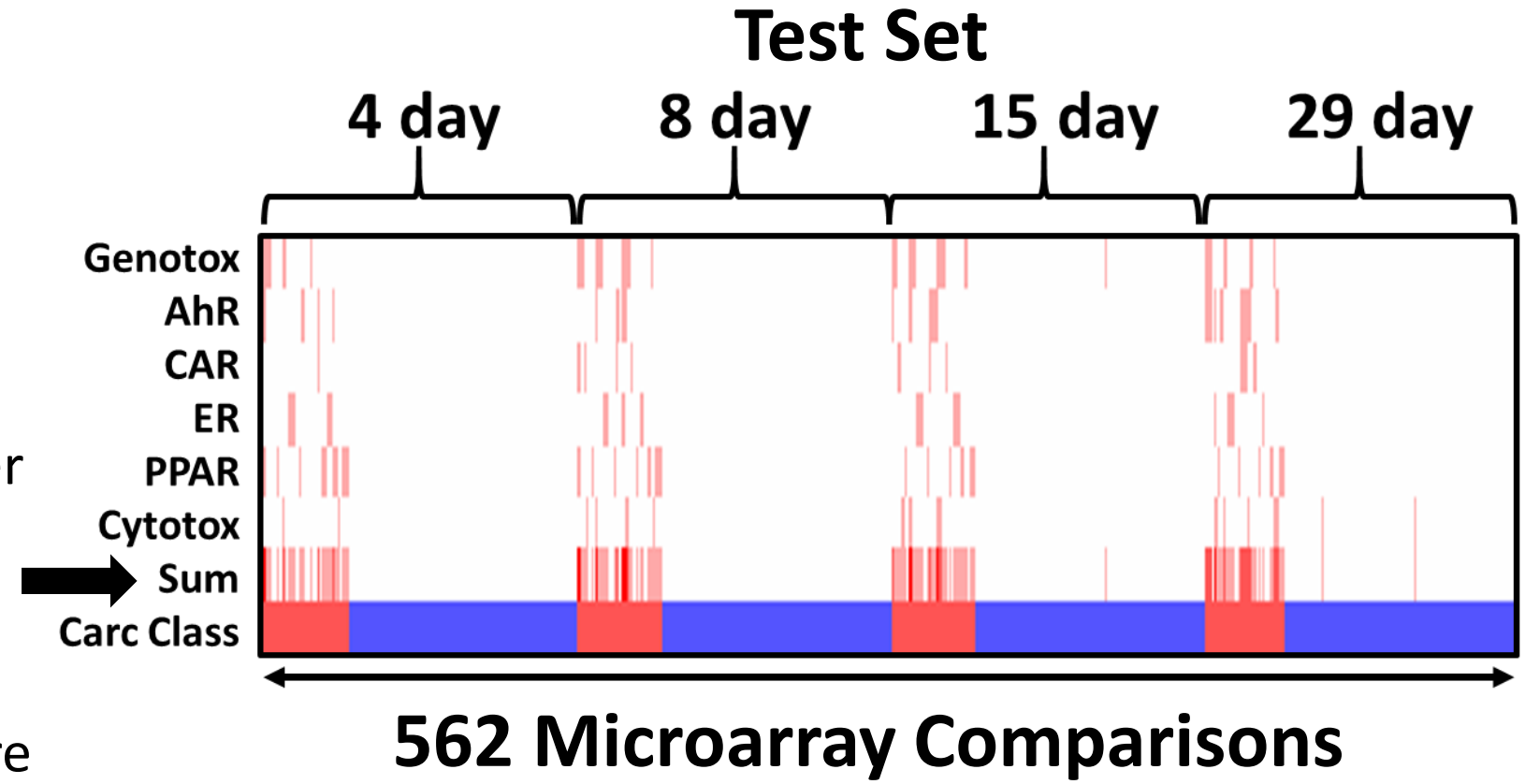
- Each gene list from a chemical-dose-time vs. control compared to each of the 6 biomarkers to generate a correlation p-value (converted to a –Log(p-value))
- Divided the chemical-dose conditions into tumorigenic and nontumorigenic groups and training and test sets
- Activation levels defined as the 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 exceeded one or more of the 6 activation levels
- Activation levels rarely exceeded in any of the nontumorigenic conditions

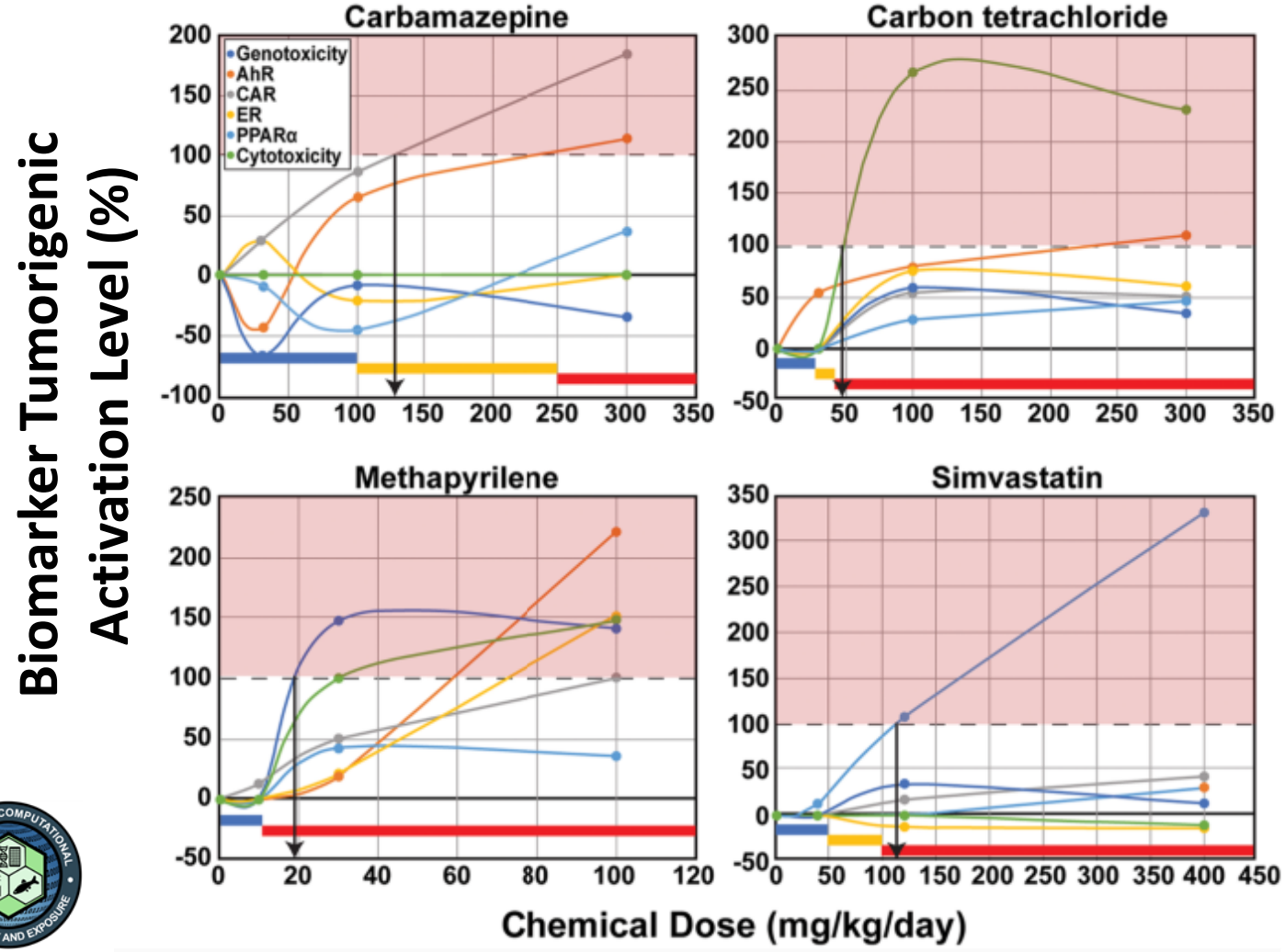


- **<u>Test set</u>: 100% sensitivity, 94% specificity, and a balanced accuracy of 97%**

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

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

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Summary

- There are opportunities to use genomic-based tools to predict tumor outcome based on short-term exposures
  - Has the potential to reduce animal testing under a number of testing requirements (e.g., S1)

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

- Identification of clear activation levels of response for biomarkers and individual genes supports the idea that early genomic changes can be used to establish activation level estimates or “tipping points” that are predictive of later-life outcomes

- The approach could be applied to predicting toxicity in other tissues

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