EPA United States Environmental Protection Agency

Systematic Analysis of High-Throughput Transcriptomics to Identify Potential Carcinogens

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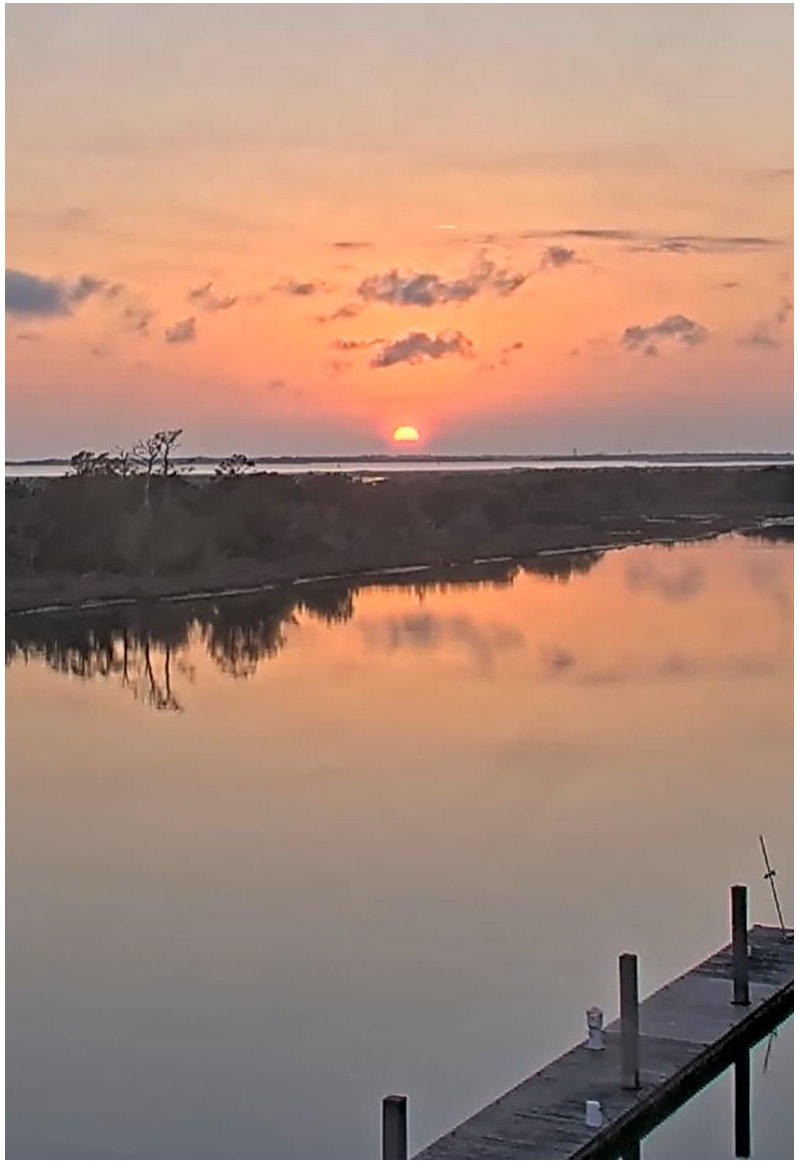
Disclaimer

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

Sunsetting the 2-year Bioassay

- The 2-year bioassay – expensive, time-consuming, many animals used, questionable relevance to humans
- Many publications arguing that it is time to use modern approaches to replace the assay
- Complex problem – how to implement a testing strategy that is health protective and can be accepted by regulatory agencies?
- Will likely require both shorter-term exposures in vivo and assessment of effects in vitro



Building an IATA to Identify Human Non-genotoxic Carcinogens (NGTxC)

Archives of Toxicology (2020) 94:2899–2923
https://doi.org/10.1007/s00204-020-02784-5

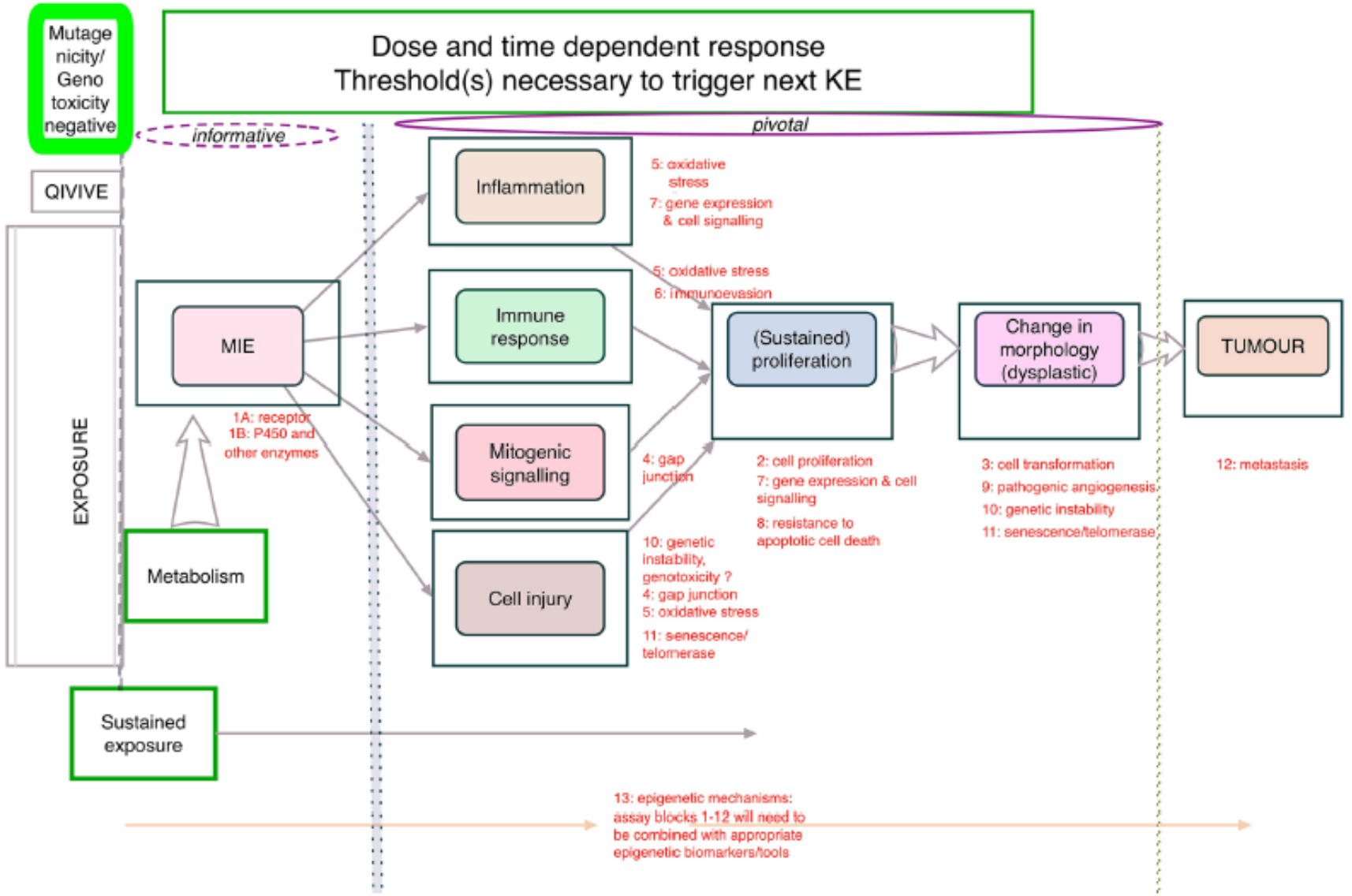
MEETING REPORTS

Chemical carcinogen safety testing: OECD expert group international consensus on the development of an integrated approach for the testing and assessment of chemical non-genotoxic carcinogens

Miriam N. Jacobs[1] · Annamaria Colacci[2] · Raffaella Corvi[3] · Monica Vaccari[2] · M. Cecilia Aguila[4] · Marco Corvaro[5] · Nathalie Delrue[6] · Daniel Desaulniers[7] · Norman Ertych[8] · Abigail Jacobs[4] · Mirjam Luijten[9] · Federica Madia[3] · Akiyoshi Nishikawa[10] · Kumiko Ogawa[10] · Kiyomi Ohmori[11] · Martin Paparella[12] · Anoop Kumar Sharma[13] · Paule Vasseur[14]

- OECD established an expert group to develop an IATA for identification of NGTxC
- Developed an overarching IATA framework based on key hallmarks of carcinogens –modules in boxes
- Identified in vitro and subchronic in vivo assays to measure the hallmarks in human cancer AOPs

A general integrated approach for the testing and assessment of non-genotoxic carcinogens



Using Transcriptomics to Augment an IATA for Non-genotoxic Carcinogens

- Proposed to use available omics database information to monitor the key events of inflammation, immune response, mitogenic signaling and cell injury, in the NGTxC IATA
- Signaling pathways contributing to carcinogenesis linked to the key hallmarks in the IATA
- Transcriptomics would be used in conjunction with cell-based assays
- Their proposal utilizes lists of genes that are linked to key hallmarks from MSigDB
- Weaknesses of the gene lists
  - Likely cell- or tissue-specific
  - Lists of genes have not been examined for ability to predict an effect
- **Hypothesis: Biomarkers with known context of use and accuracy would complement the MSigDB gene lists to predict effects**
- Two examples of the use of biomarkers
  - Identify rat liver tumorigens
  - Identify chemicals that perturb pathways relevant to human chemical carcinogenesis
- Many predictions from one gene list

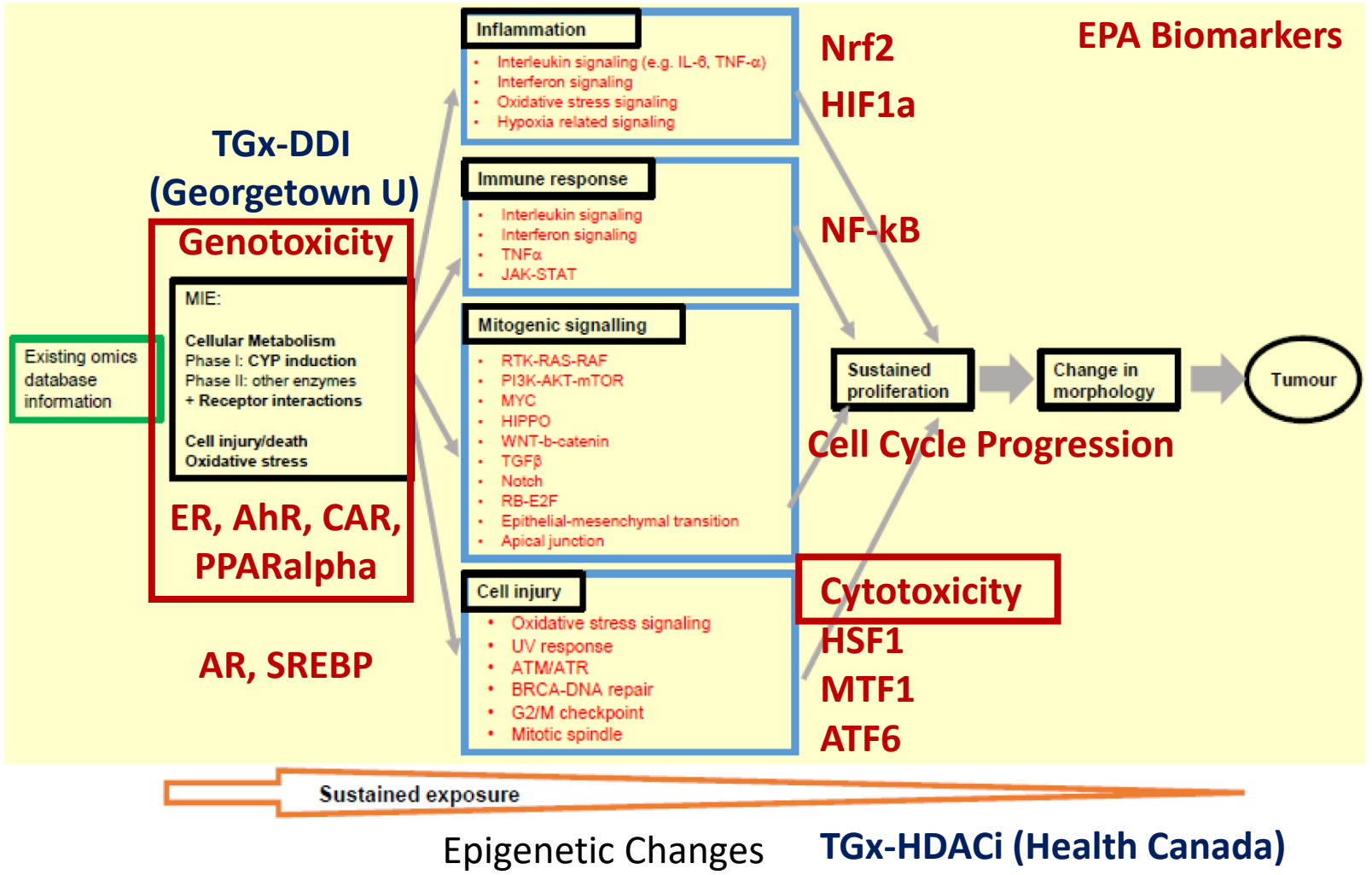
International Journal of *Molecular Sciences*

MDPI

Review

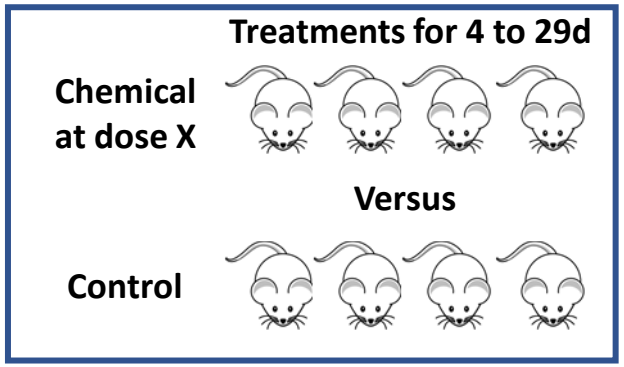
Analyses of Transcriptomics Cell Signalling for Pre-Screening Applications in the Integrated Approach for Testing and Assessment of Non-Genotoxic Carcinogens

Yusuke Oku [1,*,†], Federica Madia [2,†], Pierre Lau [3], Martin Paparella [4], Timothy McGovern [5], Mirjam Luijten [6] and Miriam N. Jacobs [7,*]



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?

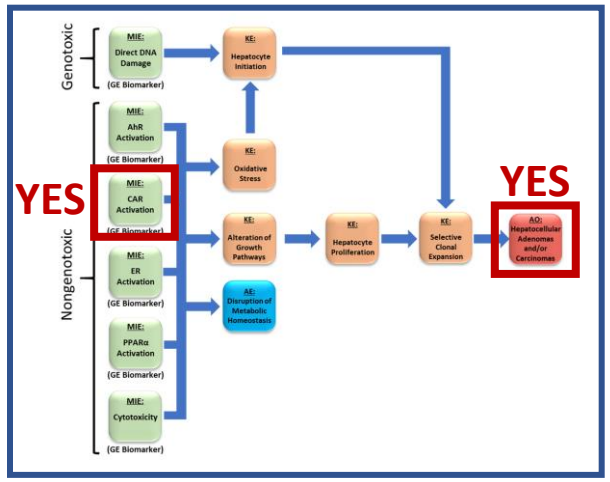


List of genes and fold-changes

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?

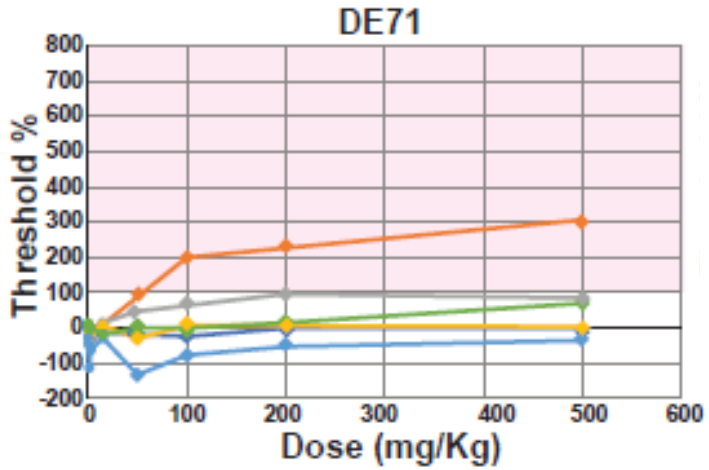
Data Used to Construct the Model

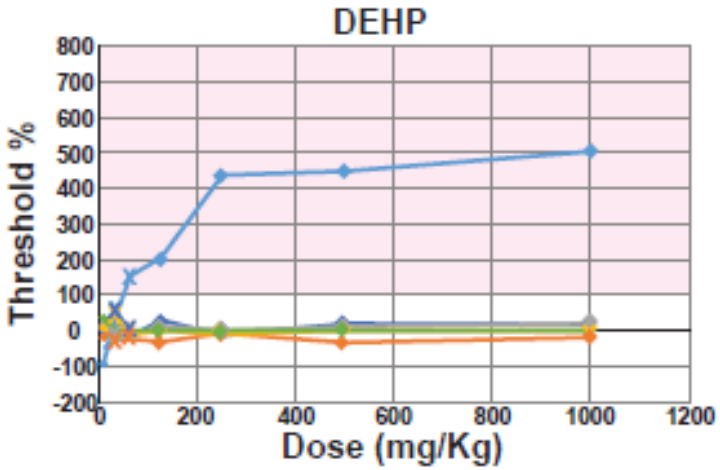
- Microarray data
  - TG-GATES
  - DrugMatrix
- 2-year cancer data
  - Lhasa carcinogenicity database

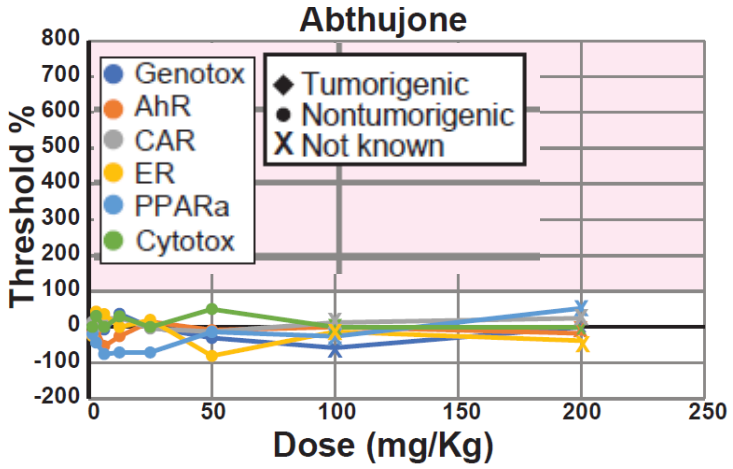
- Examined ~250 chemicals (~50 caused liver tumors)
- Accuracy was ~75-95% depending on the dataset used
- Accuracy is independent of platform used to assess gene expression

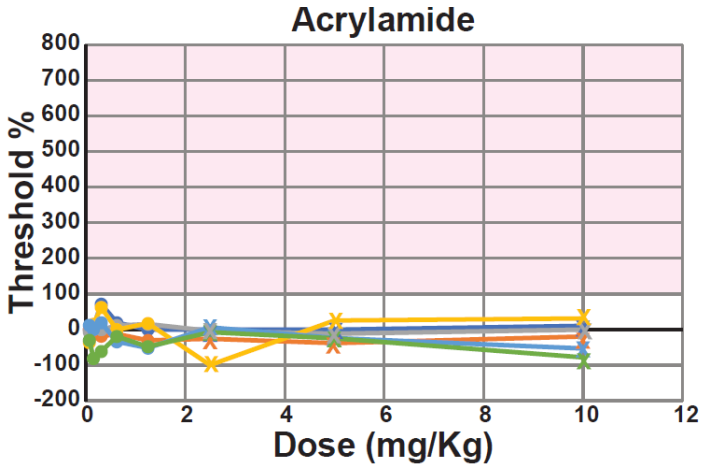
NAM identifies chemical-dose pairs that are tumorigenic in the liver using TempO-Seq

- 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





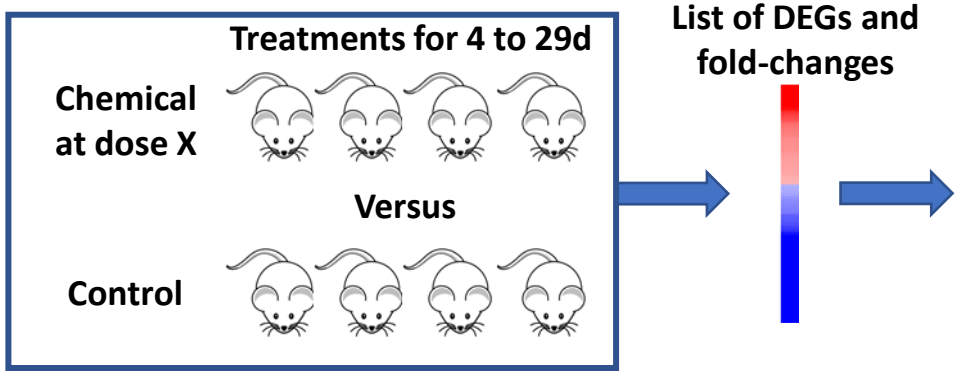




Ledbetter et al., submitted to management

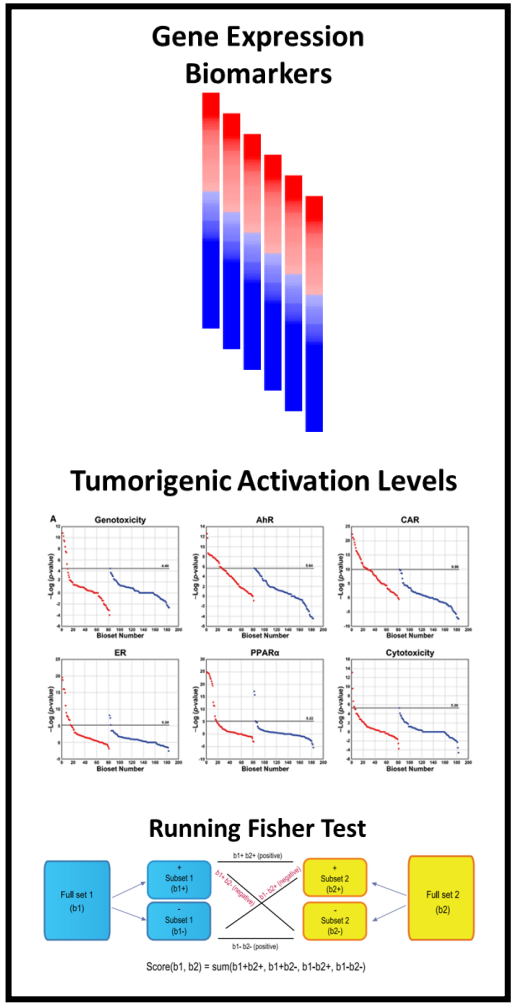
NAM: Prediction of rat liver tumor induction using toxicogenomics analysis of short-term exposures

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

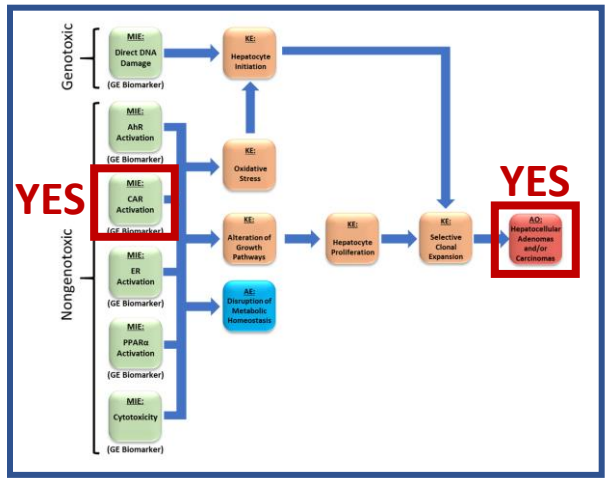


List of DEGs and fold-changes

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?**

Questions still to be addressed:

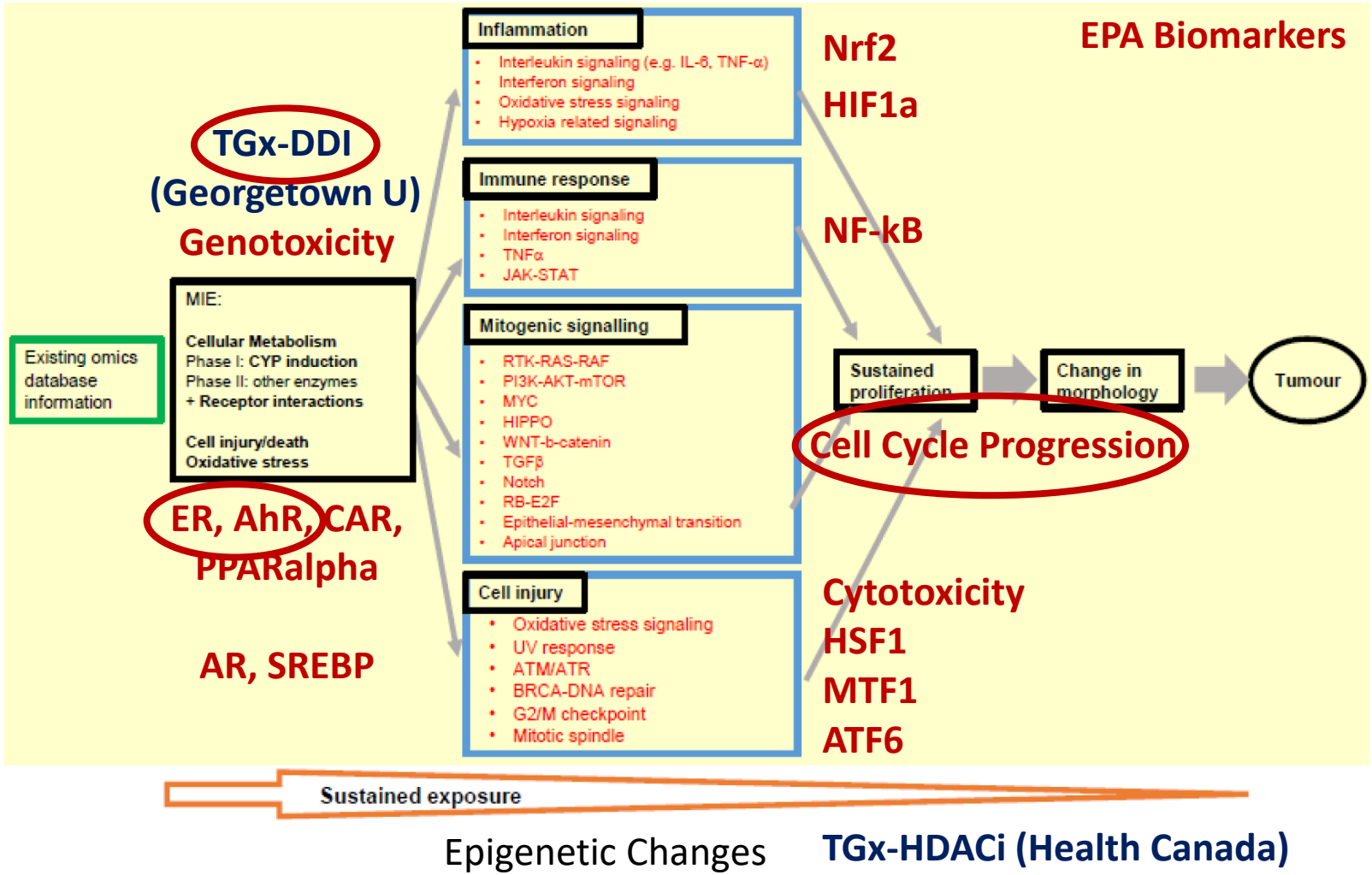
- **Can we improve accuracy by incorporating**
  - **More data?**
  - **A greater diversity of chemicals?**
  - **Wild-type and null rat comparisons?**

Future Studies:

- **Studies conducted through the HESI eSTAR Carcinogenomics Workgroup**

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

Application of biomarkers to identify effects of chemicals in human cells

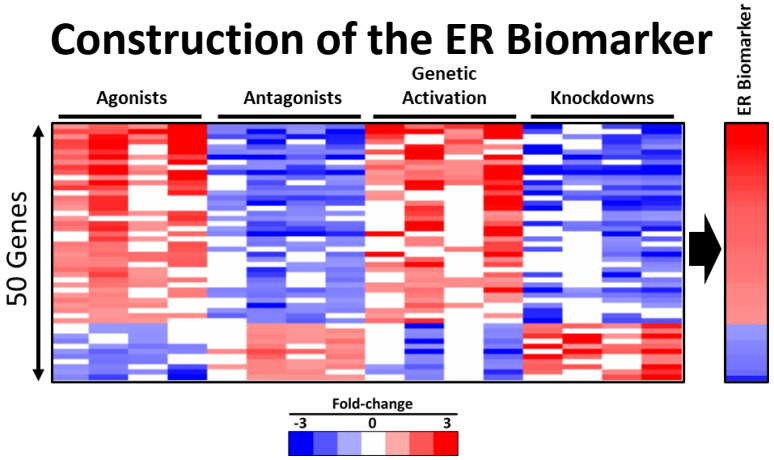


Oku et al. 2022

Creating Predictive Biomarkers

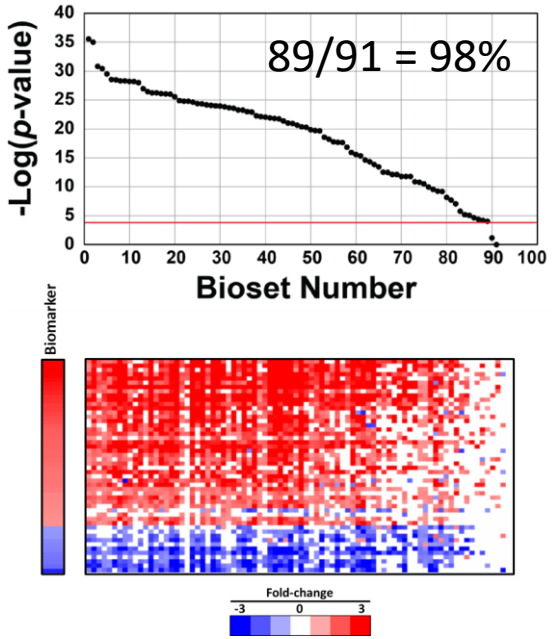
- Assemble your tools
  - Reference compounds – what is their predicted behavior?
  - Are there any examples of the gene knocked out/down or overexpressed/activated?
- Generate the profiles in which the factor is activated or suppressed in the system of interest
- Use computational approaches for identification of predictive gene sets
  - Machine learning
  - Weight of evidence

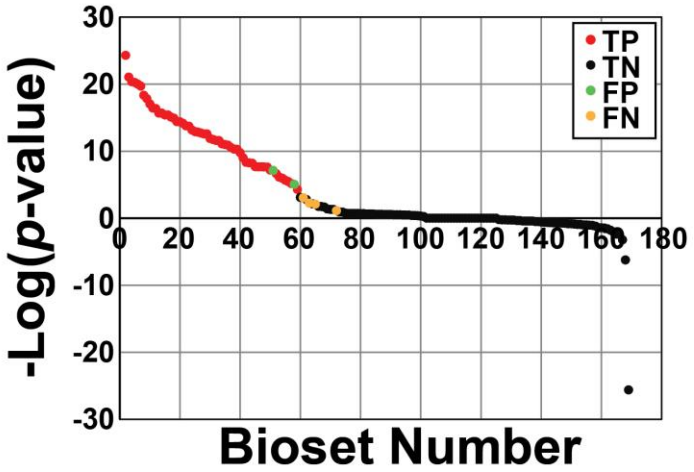
Construction of the ER Biomarker



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

The ER Biomarker identifies E2 treatments in MCF-7 cells

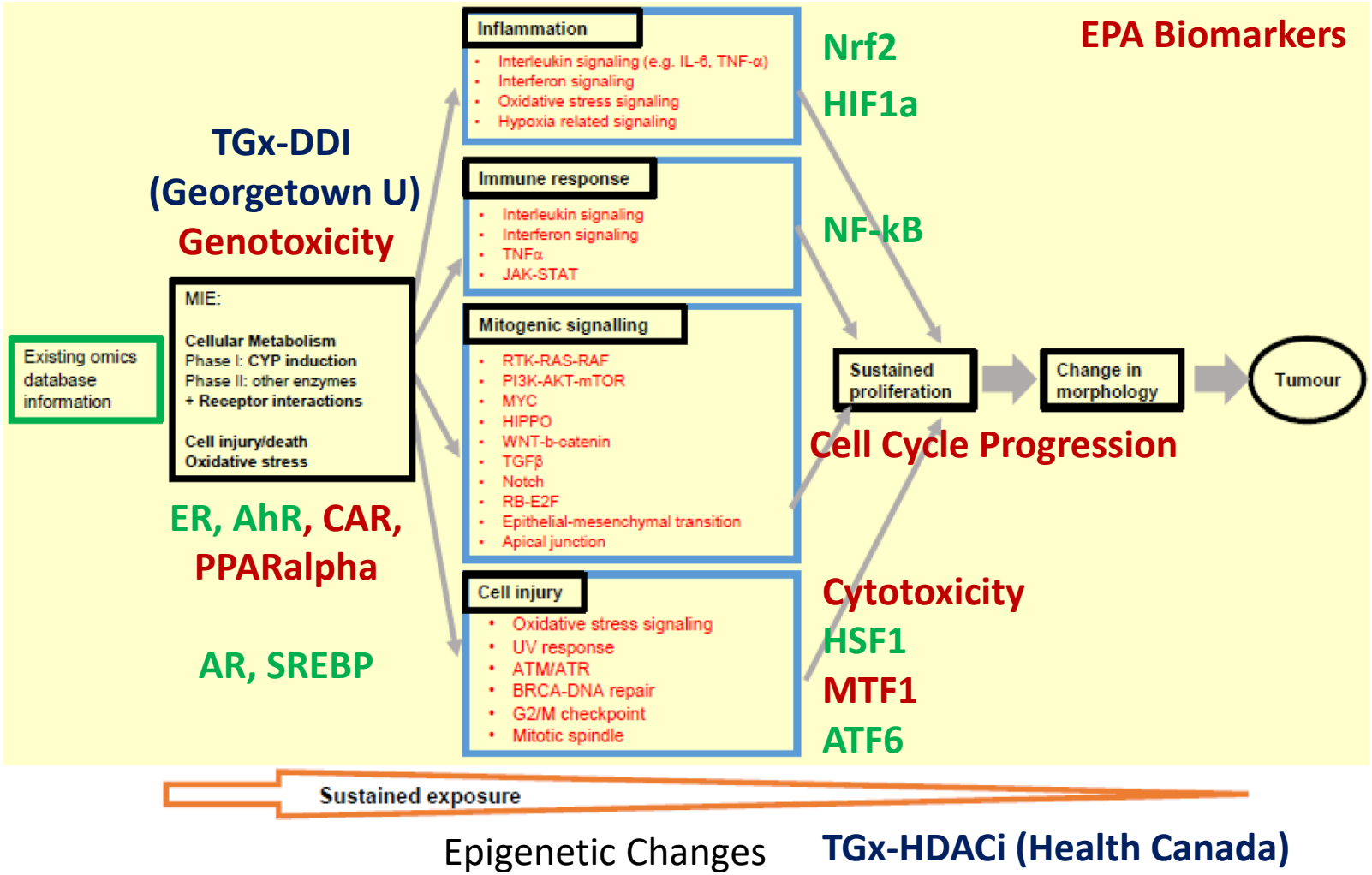




Using the NCATS Tox21 ER trans-activation assays as the reference data set:
- Balanced accuracy = 96%
- Context of use: ER positive human breast cancer cell lines

Application of biomarkers to identify effects of chemicals in human cells

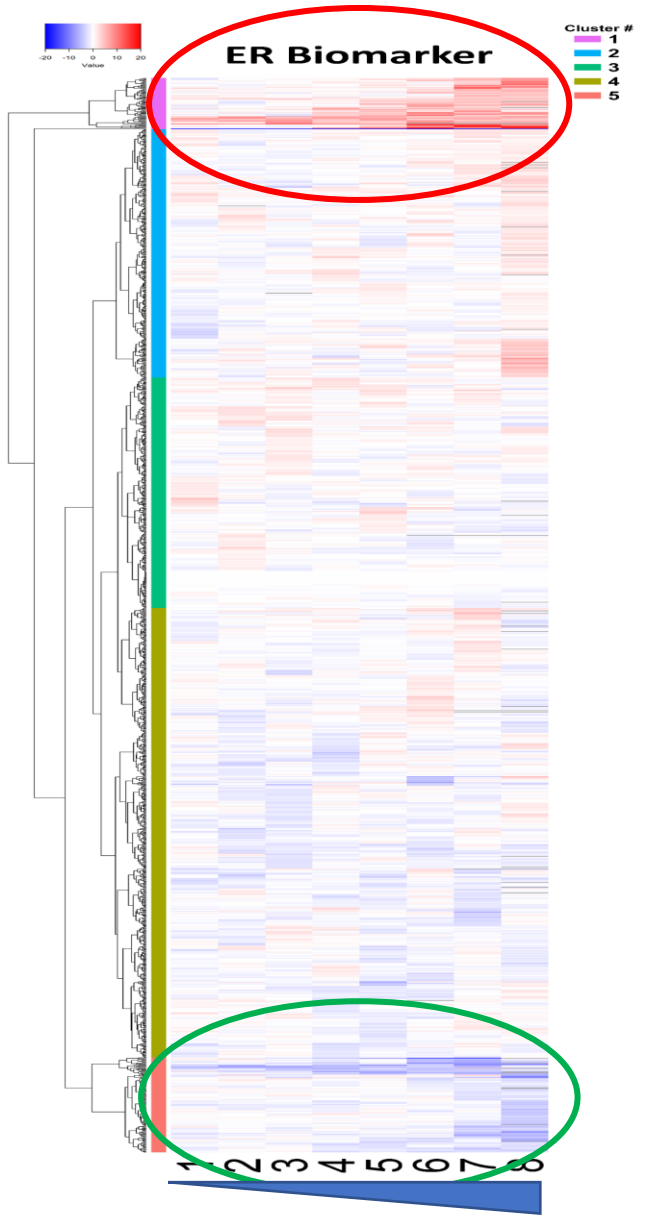
Biomarkers built using profiles from cells in which the factor was genetically modified



Oku et al. 2022

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

- Estrogen receptor activation is associated with increases in cancers of breast and ovaries
- 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
- Values expressed as –Log(p-value)s of the correlation between the profile and the ER biomarker
- 1D hierarchical clustering of chemicals across 8 concentrations



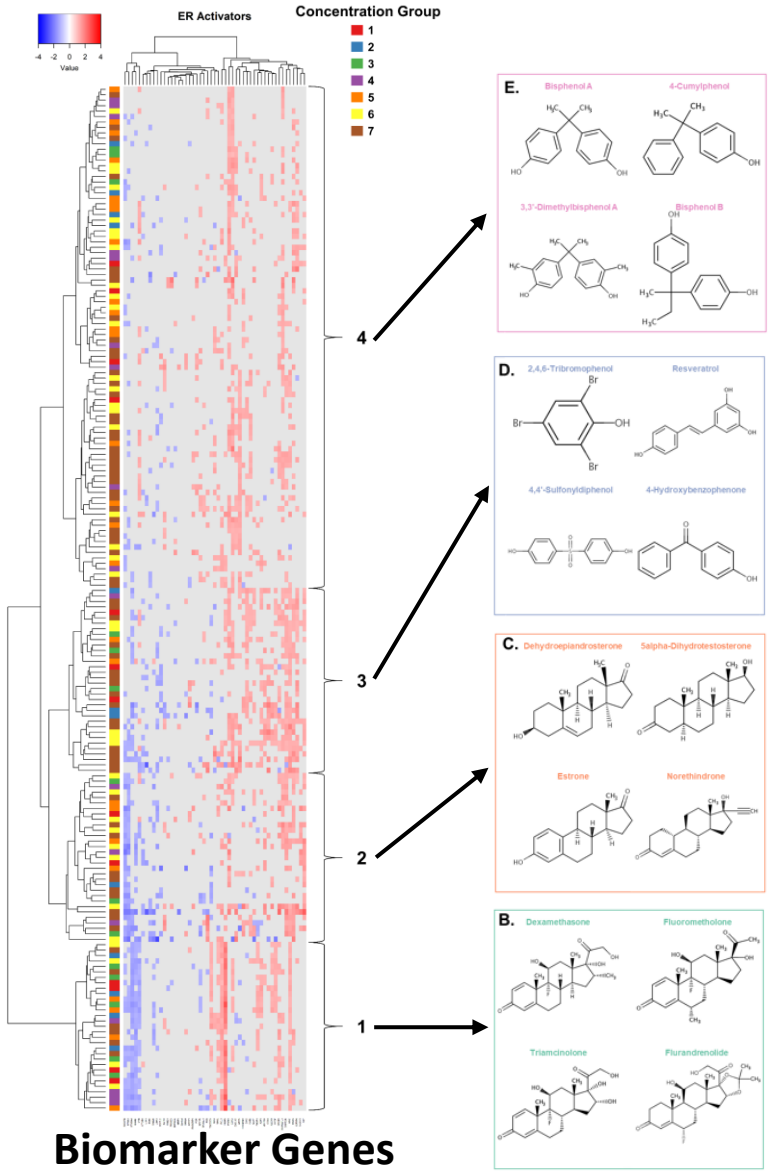
ER Activators

ER Suppressors

Robarts et al., in preparation

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

- 2D hierarchical clustering of ~120 chem-concentration pairs that activated ER vs. ER biomarker genes
- 4 major clusters of chemical-concentration pairs



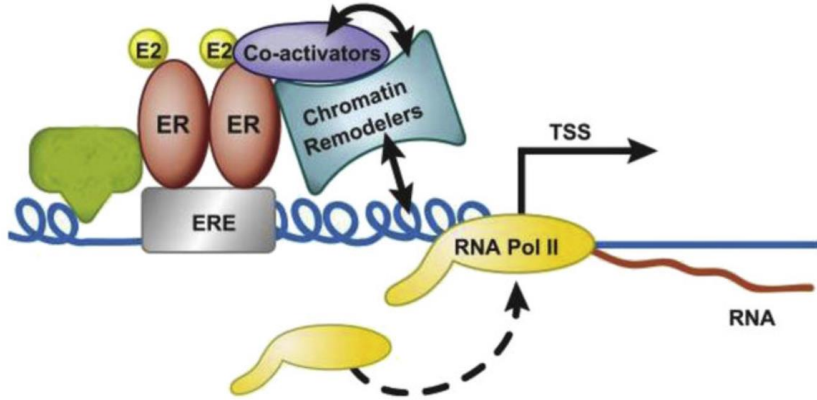
Biomarker Genes

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)
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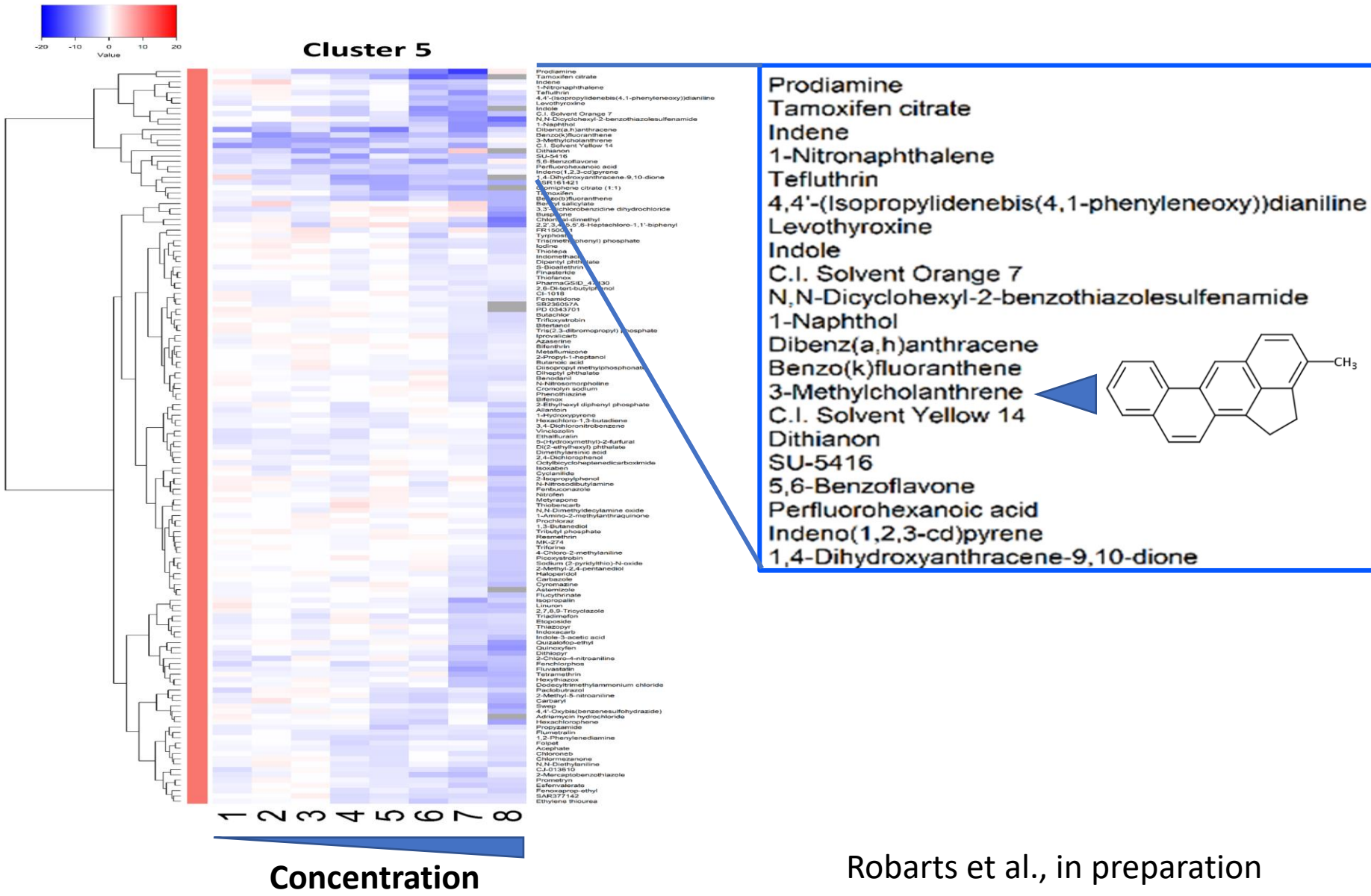
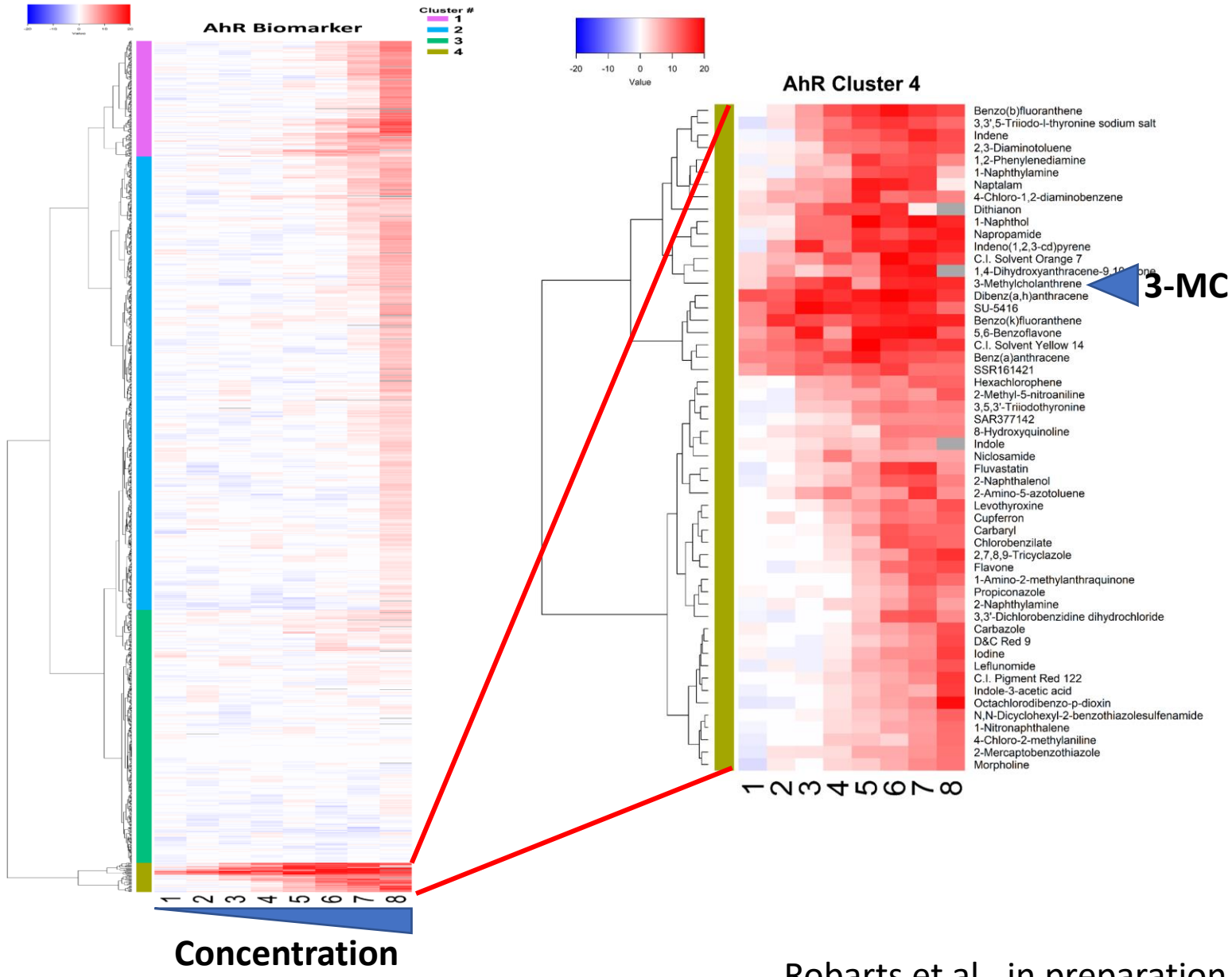


Robarts et al., in preparation

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

- Activation of AhR by TCDD is associated with a number of human tumors (e.g., breast, endometrium, testicular, liver, lung)
- 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 the ~12,800 profiles to the AhR biomarker

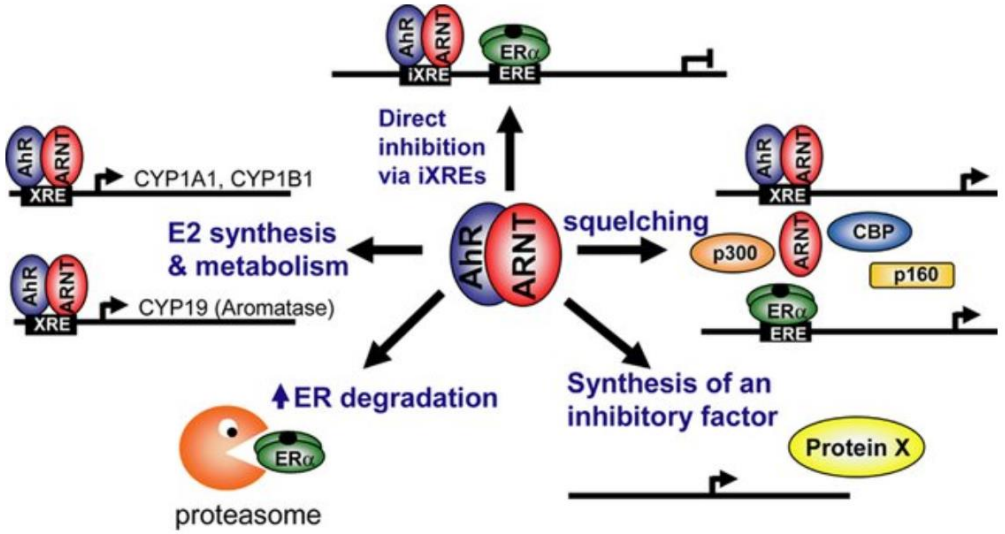


Robarts et al., in preparation

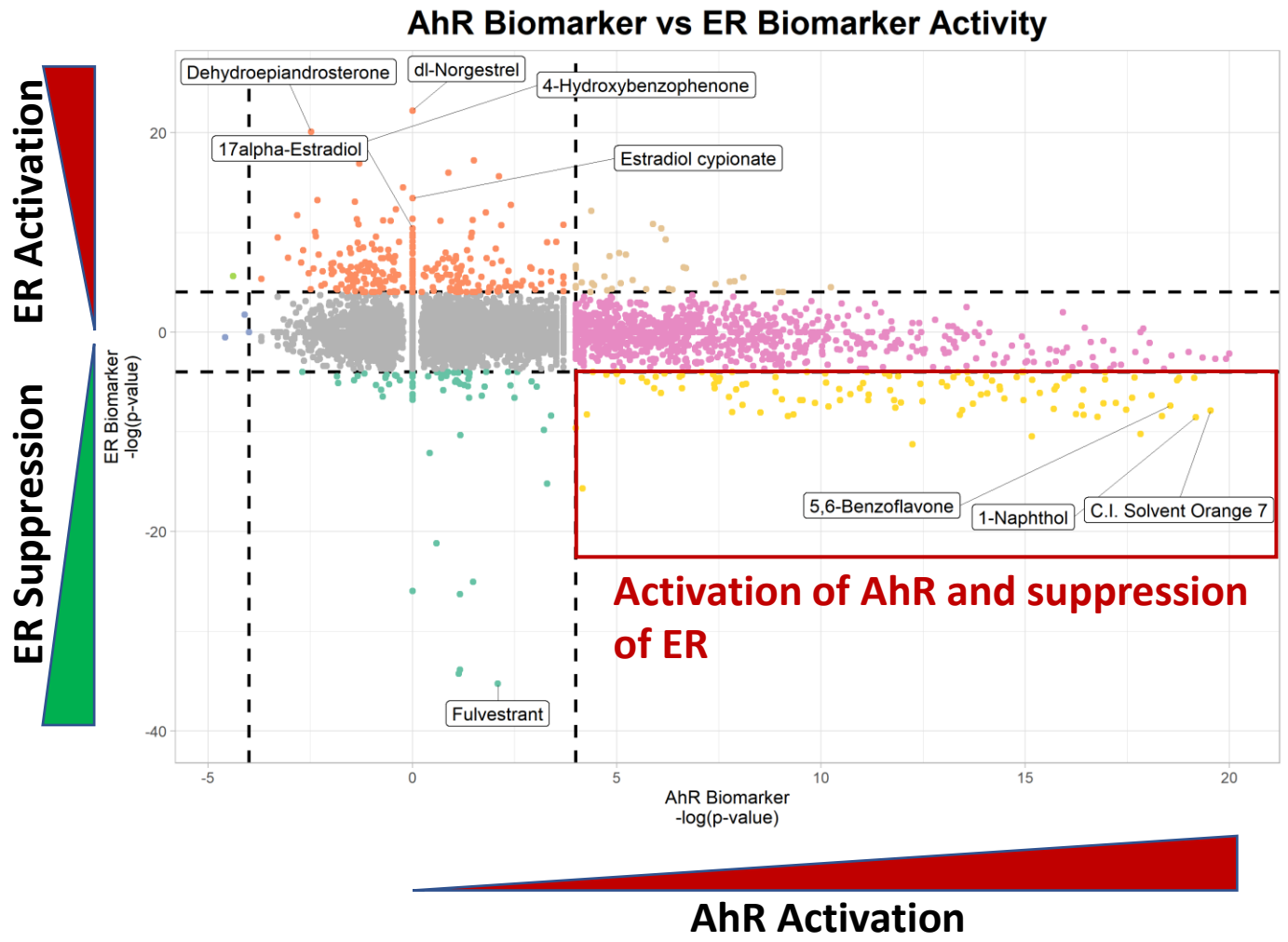
AhR activators suppress ER responses

- **Compared each of the ~12,800 profiles to the estrogen receptor (ER) and aryl hydrocarbon receptor (AhR) biomarkers**

Molecular basis for suppression of ER by AhR



From Nuclear Receptor Signaling 4(1):e016



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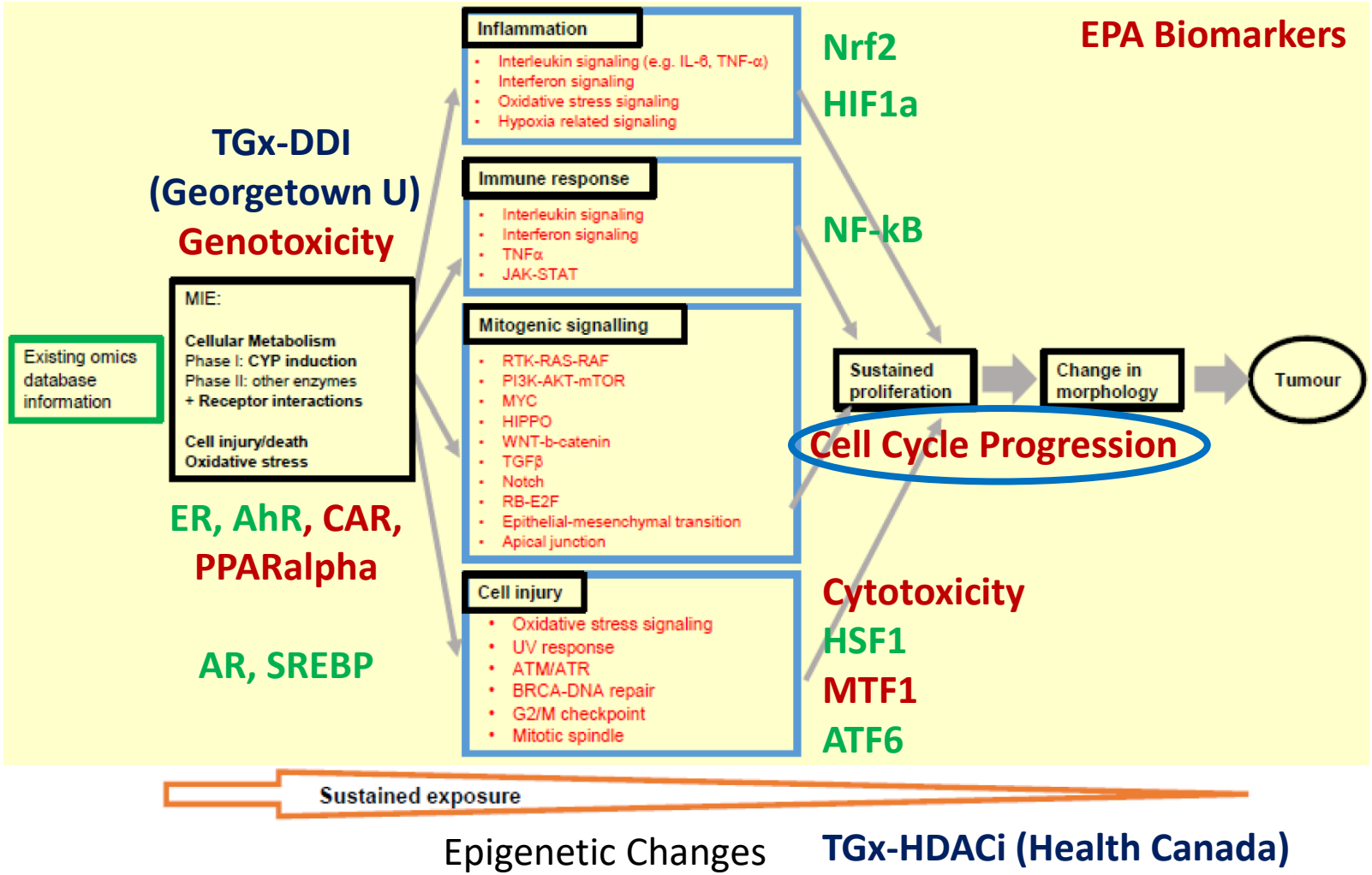
Biomarkers built using profiles from cells in which the factor was genetically modified



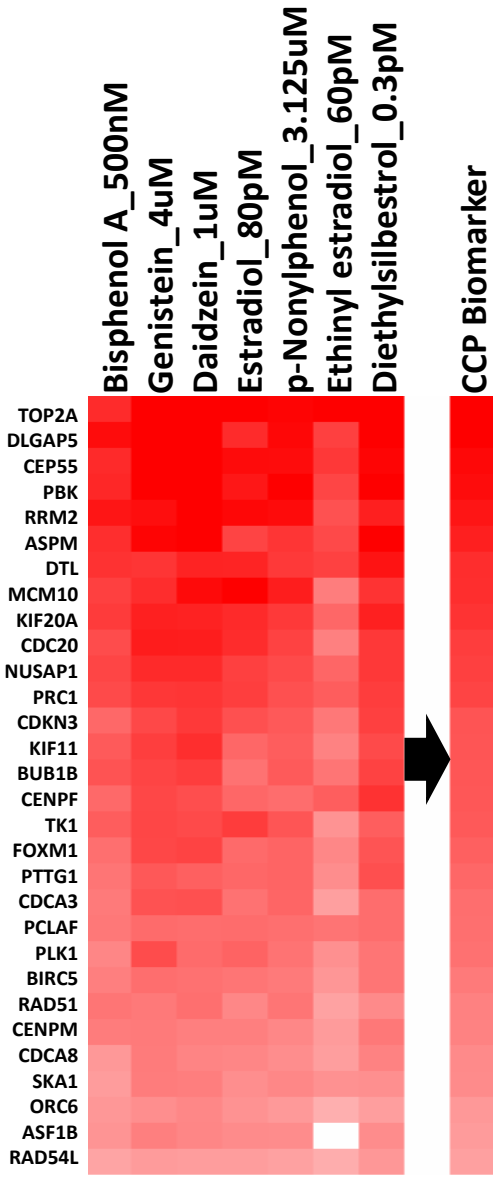
Oku et al. 2022

The Cell Cycle Progression Biomarker

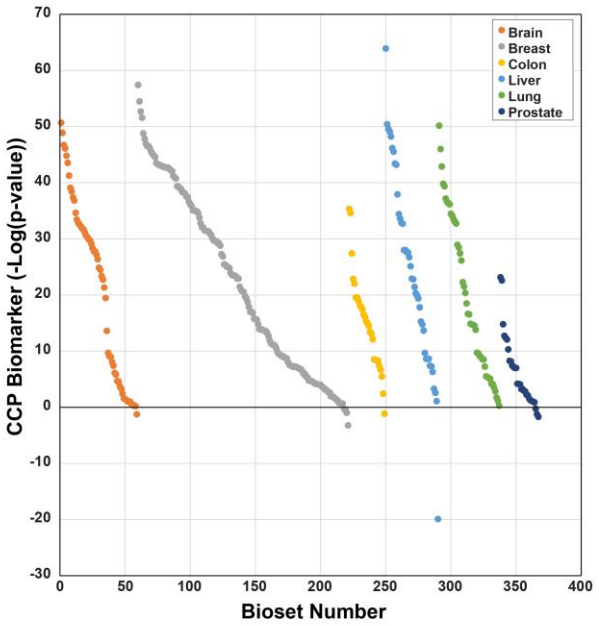
- 30 genes identified as being involved in cell cycle progression in human prostate tumors (Cuzick et al. (2011). Lancet Oncol. 12:245) – expression of genes associated with death from prostate cancer

- Examined expression of the genes after 48 hrs of treatment with 7 estrogen receptor activators in MCF-7 cells at concentrations known to induce cell proliferation

- Examined responses to chemicals and stressors in humans, rats and mice (~120K comparisons)

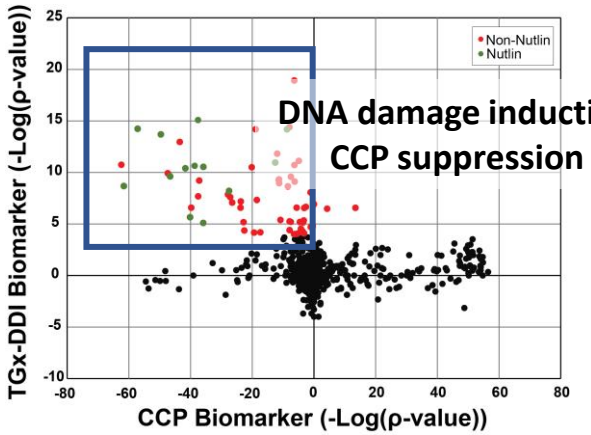


Can the biomarker identify proliferation in human tumor samples?



- Examined ~360 tumor vs surrounding tissue comparisons in 6 tissues
- Almost all give a positive response
- 84% were identified as positive for cell proliferation

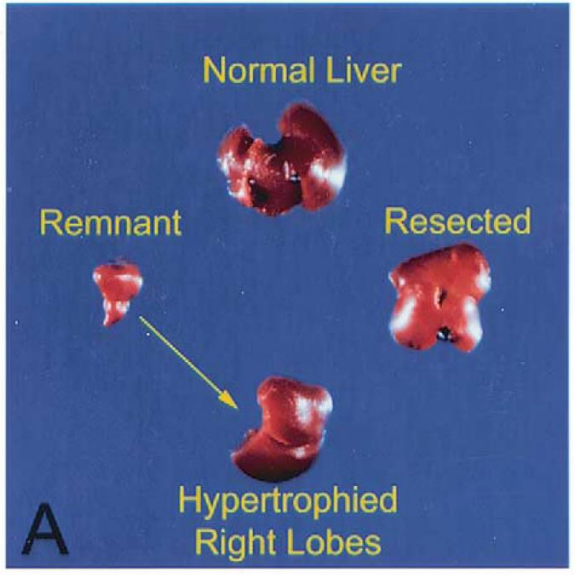
Can the biomarker identify conditions in which cell cycle is arrested in human cells?



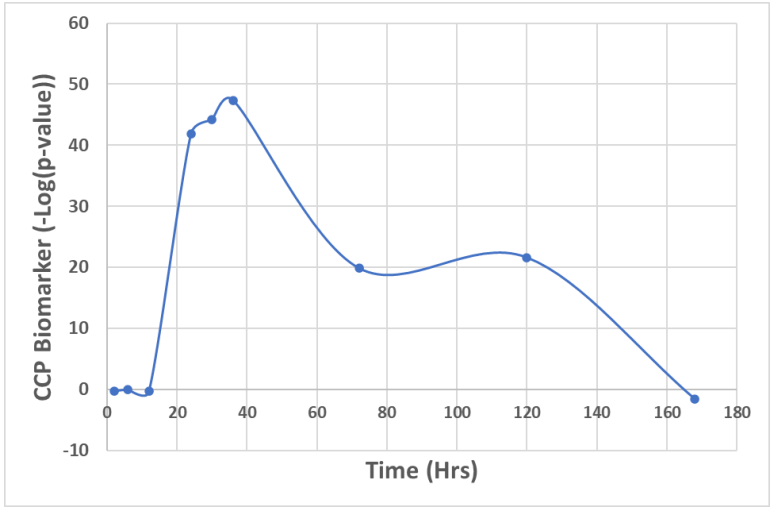
- Compared responses of the CCP biomarker to TGx-DDI biomarker
- P53 activators including Nutlin (stabilizes p53) and genotoxic chemicals suppress cell proliferation

The CCP Biomarker Identifies Proliferation in Rats

- The 2/3rds partial hepatectomy is a classic model for studying liver regeneration

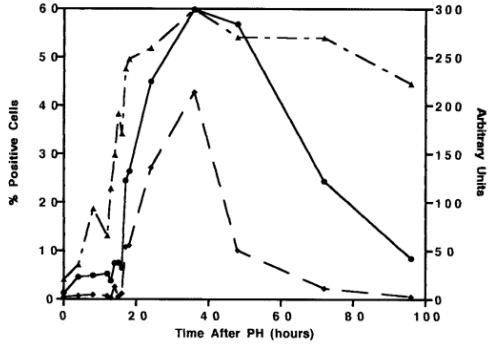


- Examined activation of CCP biomarker from 2 – 168 hrs after partial hepatectomy in male Sprague-Dawley rats
- Affymetrix data from GSE63742



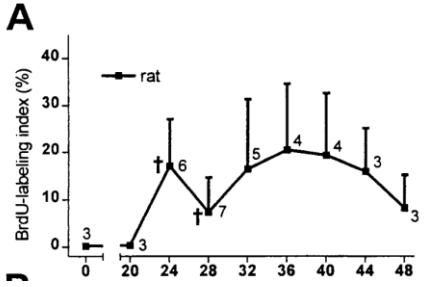
CCP Biomarker:
Peak at 36 hrs

Gerlach et al. Ki-67 Expression During Rat Liver Regeneration After Partial Hepatectomy. Hepatology 1997;26:573-578.



Male Wistar rats
BrdU: Peak at 36 hrs
Ki-67: Peak at 36 hrs

Weglarz and Sandgren Timing of hepatocyte entry into DNA synthesis after partial hepatectomy is cell autonomous. PNAS 2000 97: 12595



Male Fisher 344 rats
BrdU: Peak at 36 hrs

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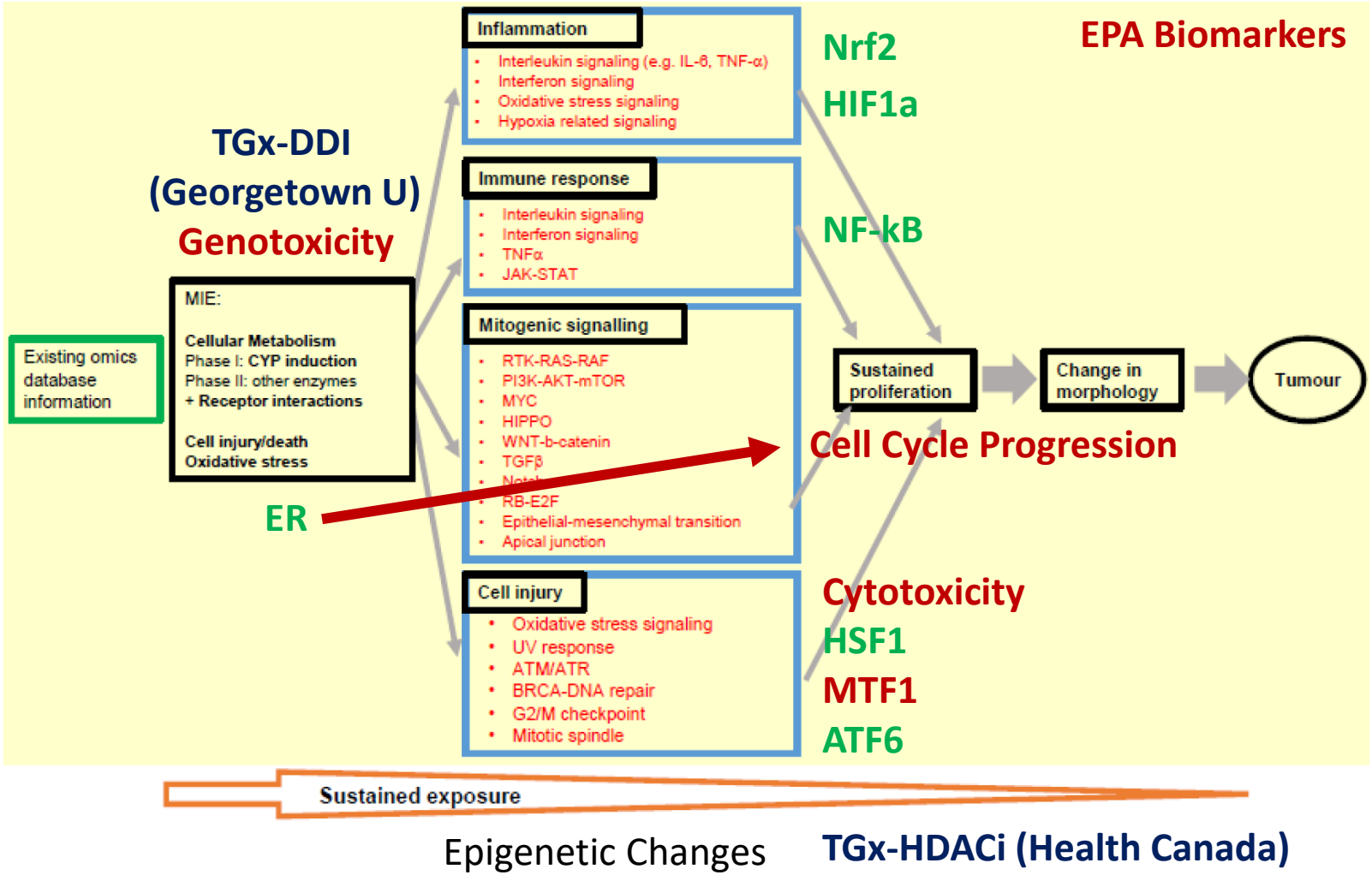
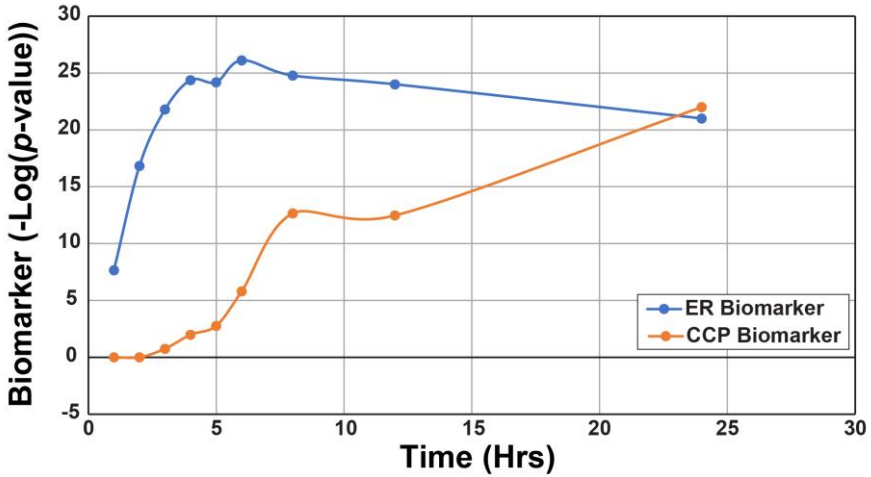


Oku et al. 2022

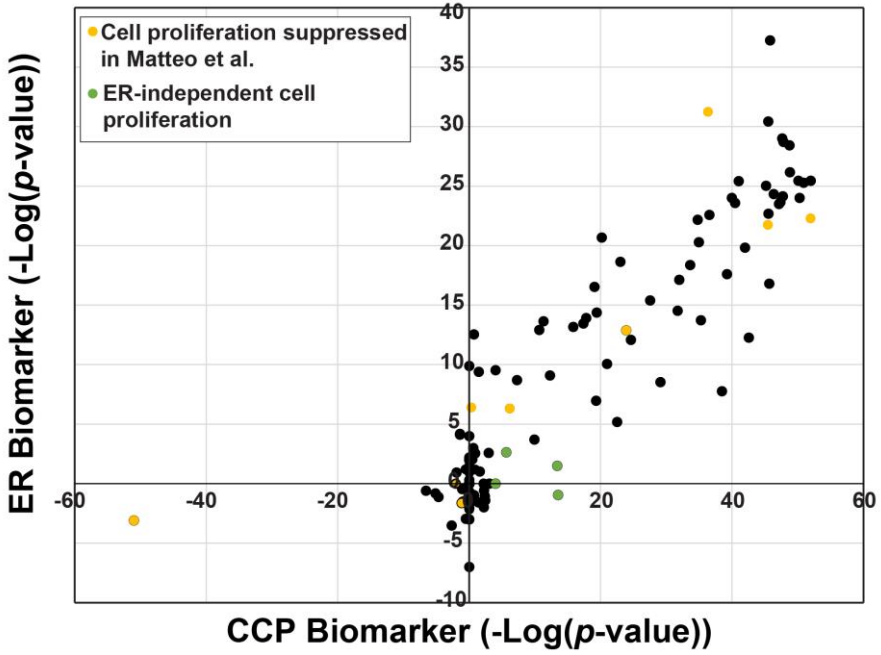
Linking Estrogen Receptor Activation with Cell Proliferation

ER activation precedes cell proliferation



- Treated MCF-7 cells with 10nM estradiol and examined gene expression out to 24 hrs
- Data from GSE78167 (Baran-Gale et al., 2016; RNA 22:1592)

Relationship between ER activation and cell proliferation across 15 BPA alternatives

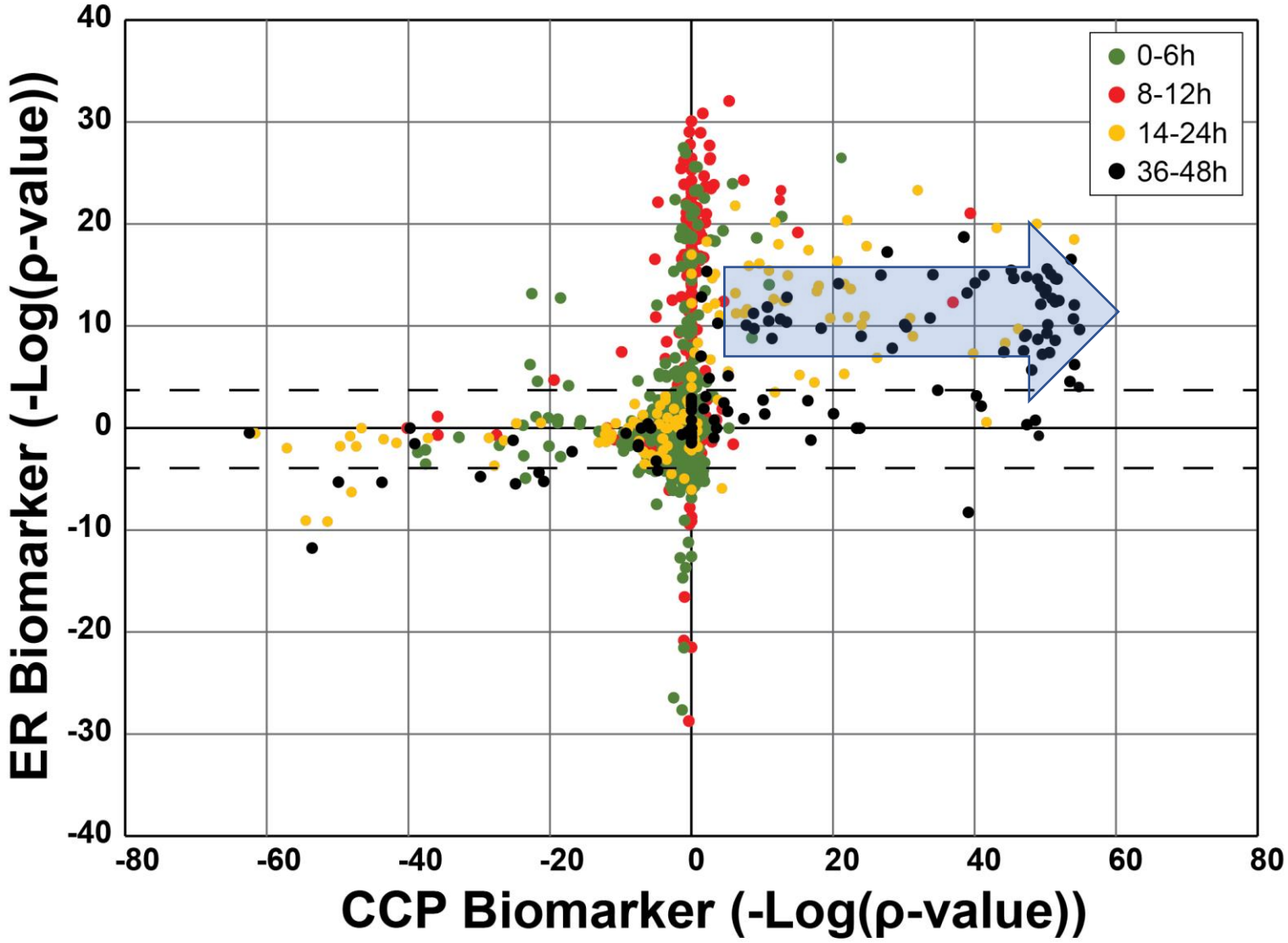


- Dataset from Matteo et al. 2023 ToxSci 191(2):266-275
- MCF-7 cells treated with BPA and 15 alternatives
- 0.0005-100 µM for each chemical and 48 hr treatment times – 143 comparisons
- The level of ER activation determines the level of cell proliferation response – is there a threshold?

Linking Estrogen Receptor Activation with Cell Proliferation

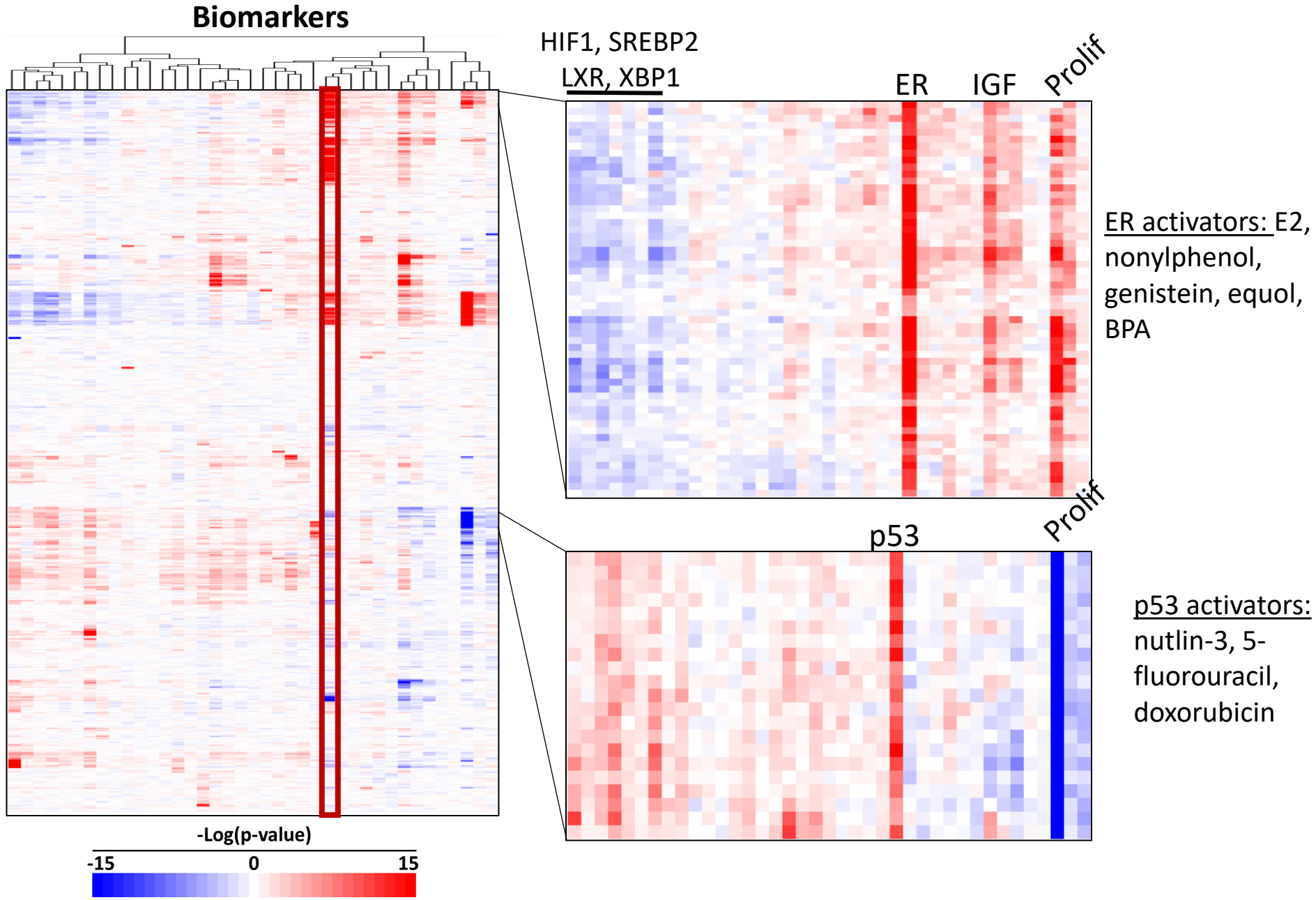
- Compared 2006 chemical treatments in MCF-7 cells (1431 chemicals) to the ER and CCP biomarkers
- Grouped by time of treatment
- In general, the longer the exposure the greater the activation of the CCP biomarker

ER activation precedes cell proliferation for a large number of ER activators



Behavior of Biomarkers in MCF-7 cells

- Examined relationships between 2165 microarray comparisons in MCF-7 cells across 39 biomarkers
- Includes chemicals, various stressors, cytokines
- Two-dimensional hierarchical complete linkage clustering
- Efforts are ongoing to integrate predictions into prioritization schemes and into the AOP network



ER activators: E2, nonylphenol, genistein, equol, BPA

p53 activators: nutlin-3, 5-fluorouracil, doxorubicin

Summary

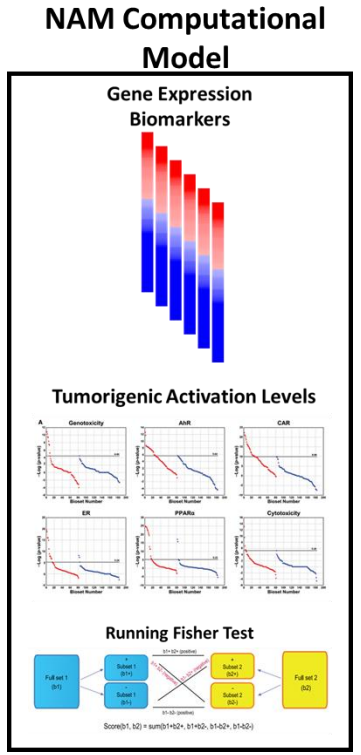
- Moving away from the 2-year bioassay will likely require both short-term exposures in vivo and assessment of effects in vitro

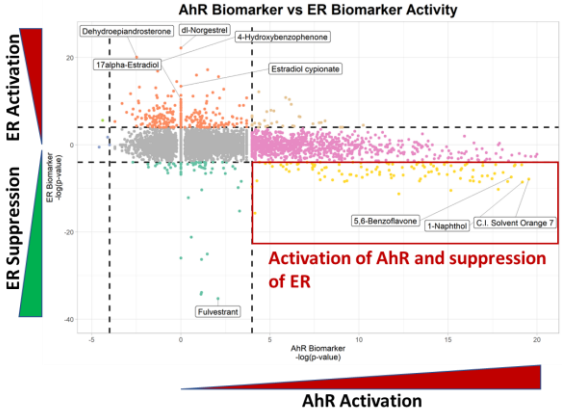
- Integration of gene expression into carcinogenicity testing will be facilitated using biomarkers with known predictive accuracies and context of use

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

- A growing number of human biomarkers are characterized for Tier 1 screening in high throughput transcript profiling
  - ER, AhR, TGx-DDI, CCP biomarkers

- Screening strategies should consider
  - Multiple cell lines (organotypic models)
  - Range of concentrations to allow response modeling
  - Range of times of exposure to capture molecular and cellular events (cell fate)





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Thanks for listening!

Questions?