EPA United States Environmental Protection Agency

Using Transcript Profiling to Identify 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.

Outline

- Sunsetting the 2-year bioassay

- OECD efforts to build an IATA to identify human carcinogens
  - Use of annotated pathway lists from gene expression data to fill in key events

- Two examples of the use of gene expression biomarkers to identify carcinogens
  - Biomarkers for screening transcript profiles generated in rats to reduce 2-year bioassay
    - Identification of mode of action
    - Identification of chemical doses that would cause cancer
  - Biomarkers for Tier 1 screening in high-throughput transcriptomics (HTTr) profiling
    - E.g., identification of estrogen receptor modulators

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 methods to replace the assay
- Complex problem – how to implement a testing strategy that is not only health protective but can be accepted by regulatory agencies
- Will likely require both short-term exposures in vivo and assessment of effects in vitro
- Two strategies for identification of carcinogens
  - Short-term exposures in vivo to identify liver tumorigens
  - An in vitro biomarker approach

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

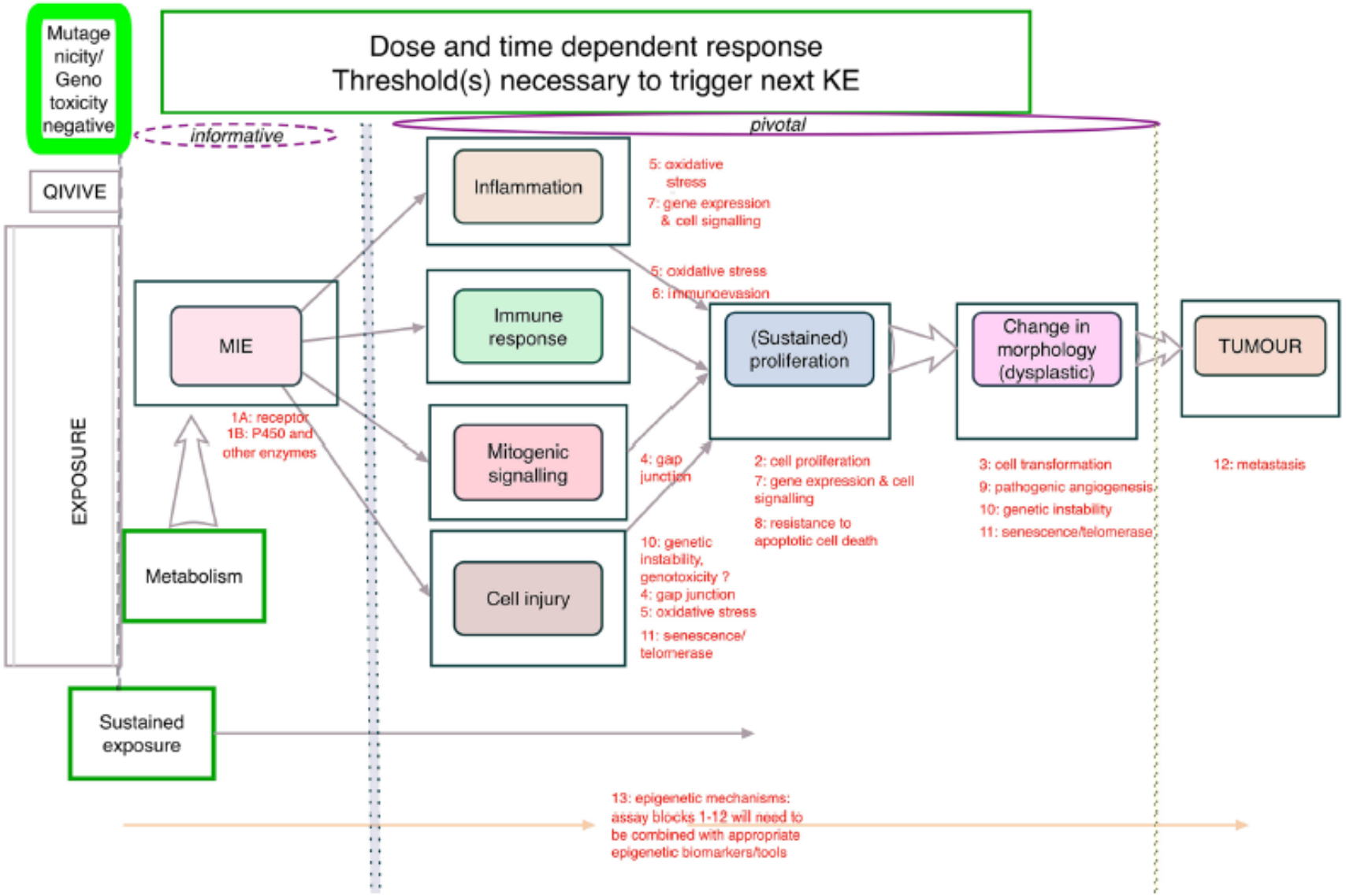
Archives of Toxicology (2020) 94:2899–2923
https://doi.org/10.1007/s00204-020-02784-5

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 characteristics of carcinogens (KCCs)
- Identified in vitro and subchronic in vivo assays to measure the key events in human cancer AOPs



Using Transcriptomics to Build an IATA for Non-genotoxic Carcinogens

International Journal of *Molecular Sciences*

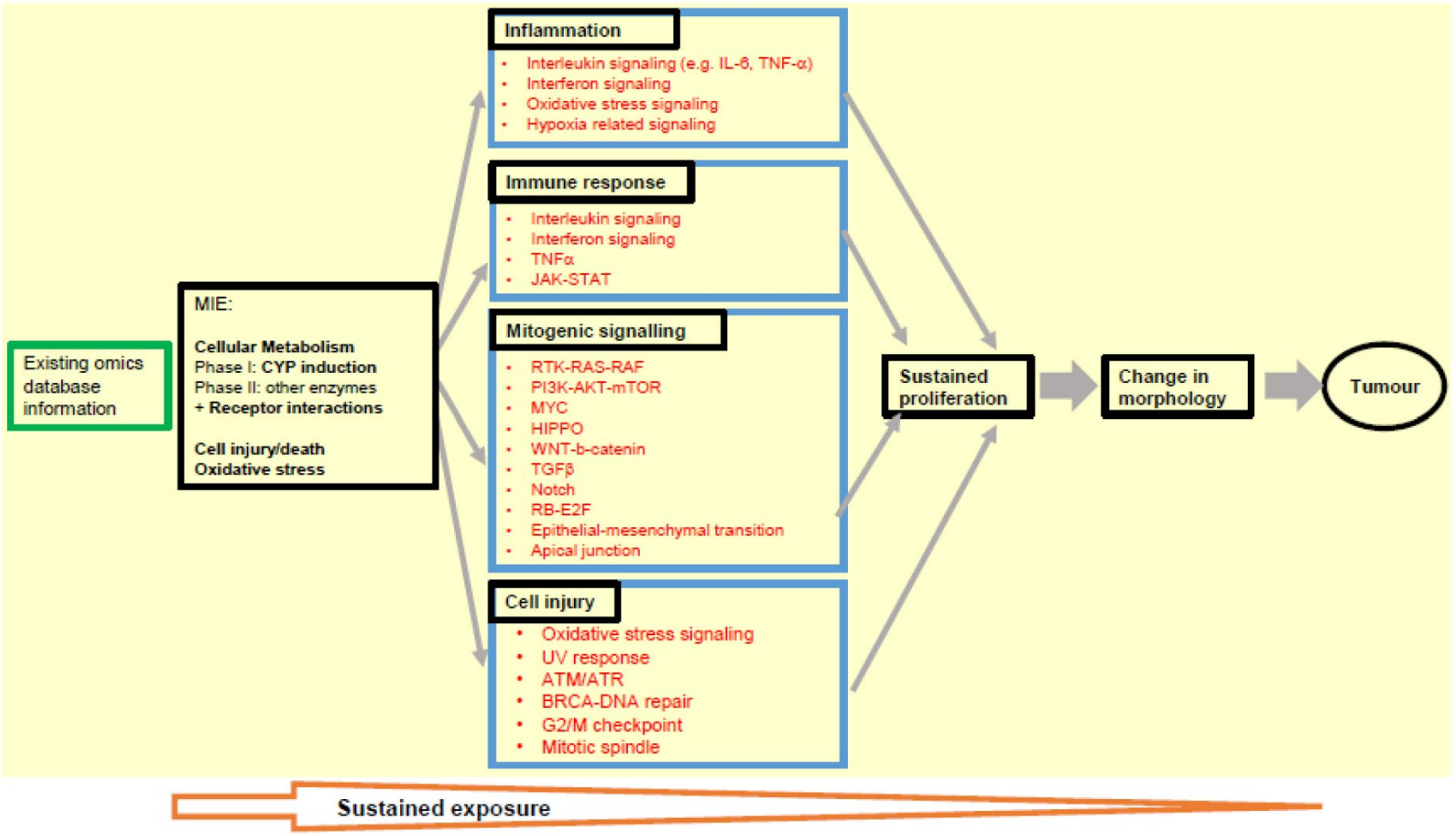
MDPI

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,*]

- Use available omics database information to monitor the key events of inflammation, immune response, mitogenic signalling and cell injury, in the NGTxC IATA
- Signaling pathways contributing to carcinogenesis (red) linked to MIEs and KEs in the IATA
- Transcriptomics would be used in conjunction with cell-based assays
- Their proposal utilizes lists of genes that are linked to key events
- Weaknesses of the approach
  - Lists are likely cell or tissue-specific
  - The lists of genes have not been examined for ability to predict an effect
- Biomarkers with known context of use and accuracy would be more useful than the off the shelf gene lists



Using Transcriptomics to Build an IATA for Non-genotoxic Carcinogens

- Use available omics database information to monitor the key events of inflammation, immune response, mitogenic signaling and cell injury in the NGTxC IATA
- Mapped signaling pathways contributing to carcinogenesis linked to MIEs and KEs in the IATA (red)
- Transcriptomics would be used in conjunction with cell-based assays
- Their proposal utilizes lists of genes that are linked to key events
- Weaknesses of the approach
  - Lists are likely cell- or tissue-specific
  - The lists of genes have not been examined for ability to predict an effect
- Hypothesis: Biomarkers with known context of use and accuracy would be more useful than the off-the-shelf gene lists

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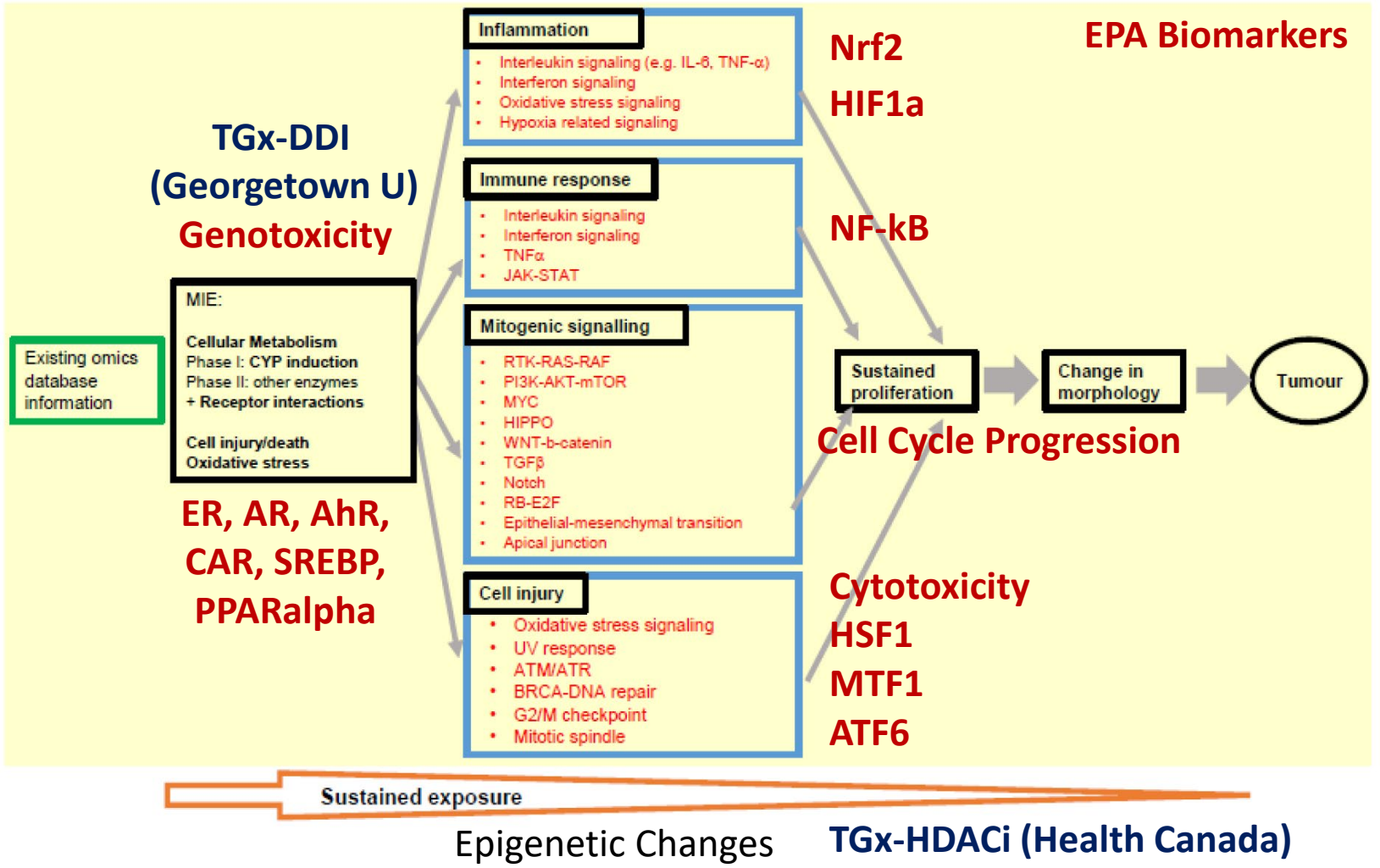
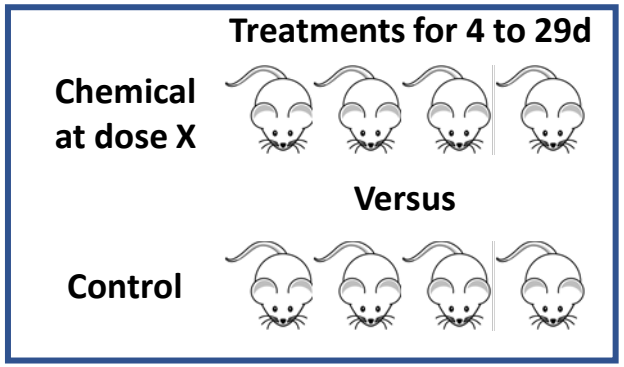


NAM: Prediction of rat liver tumor induction using toxicogenomics analysis of short-term exposures

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

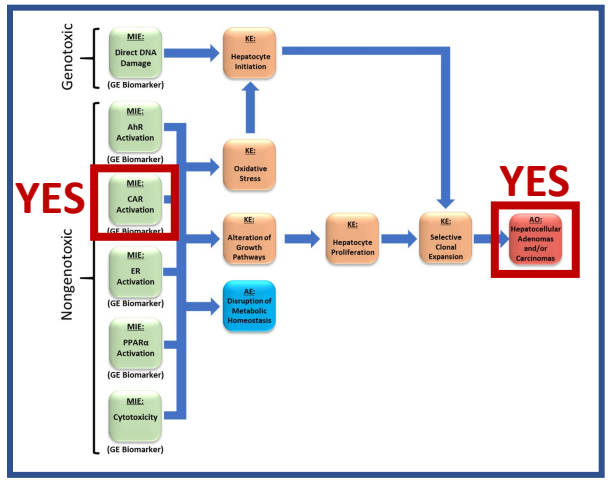


List of genes and fold-changes

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?

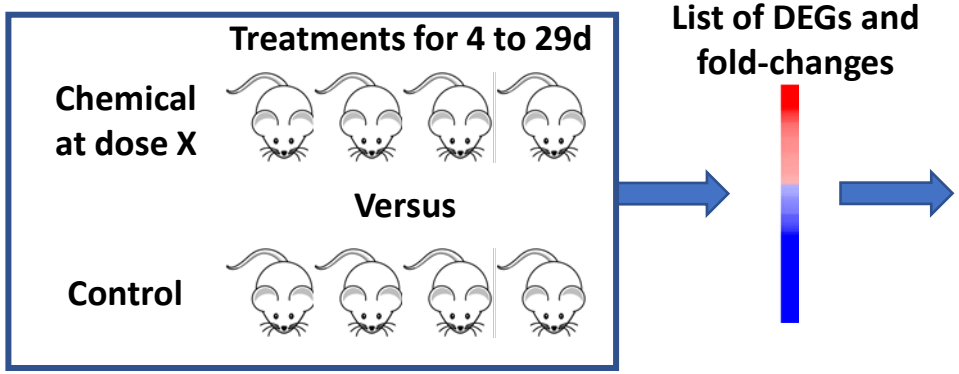
Data Used to Construct the Model

- 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: 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?



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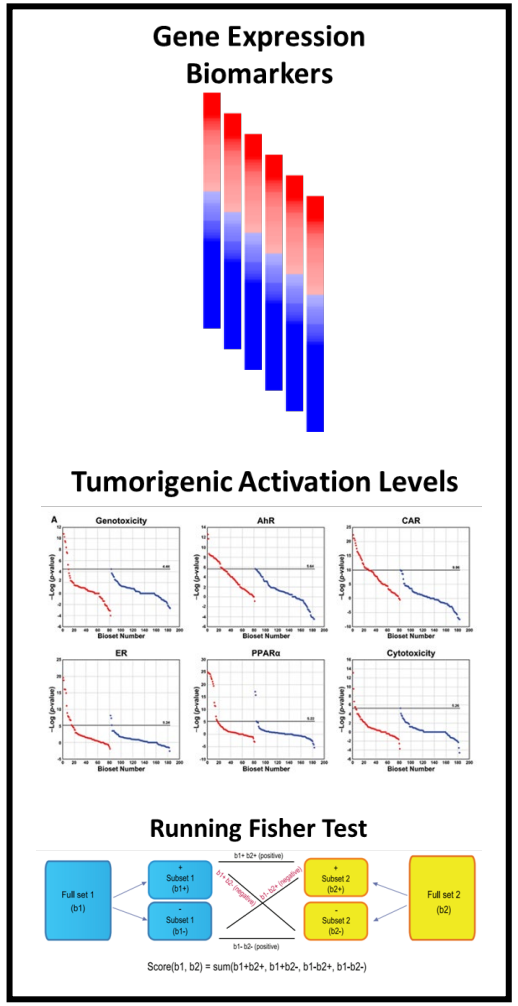
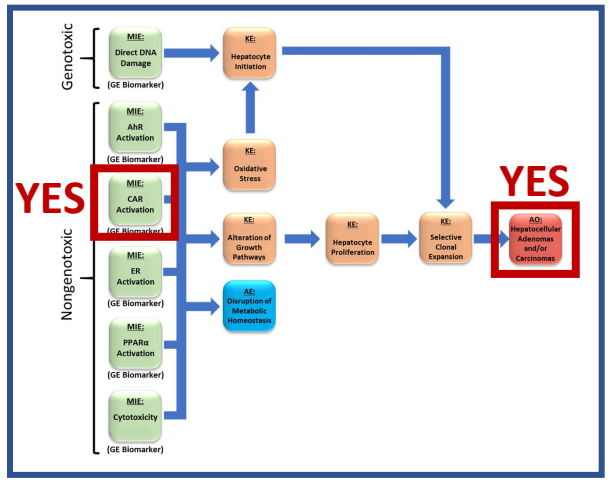


Network of Liver Cancer AOPs



- Is the dose tumorigenic?
- Which mode of action is activated?
- Is the mode 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

Biomarkers that predict key events in human cells in vitro

Used genetic profiles to develop the biomarker

Endocrine disruption

- Ryan et al. (2016). Moving Toward Integrating Gene Expression Profiling Into High-Throughput Testing: A Gene Expression Biomarker Accurately Predicts Estrogen Receptor α Modulation in a Microarray Compendium. Toxicol Sci. 151(1):88-103.
- Androgen receptor: Rooney et al. (2018). Identification of Androgen Receptor Modulators in a Prostate Cancer Cell Line Microarray Compendium. Toxicol Sci. 166:146-162.
- Robarts et al. (2023). Characterization of a 50-gene estrogen receptor biomarker. In preparation.

DNA Damage Response – TGx-DDI Biomarker

- Corton et al. (2018). Using a gene expression biomarker to identify DNA damage-inducing agents in microarray profiles. Environ Mol Mutagen. 59:772-784.
- Cho et al. (2019). Assessment of the performance of the TGx-DDI biomarker to detect DNA damage-inducing agents using quantitative RT-PCR in TK6 cells. Environ Mol Mutagen. 60:122-133.
- Corton JC, Witt KL, Yauk CL. (2019). Identification of p53 Activators in a Human Microarray Compendium. Chem Res Toxicol. 32(9):1748-1759.

Epigenetic effects – HDACi and BRDi

- Corton et al. A Gene Expression Biomarker Identifies Inhibitors of Two Classes of Epigenome Effectors in a Human Microarray Compendium. Chemico-Biological Interactions. 365:110032.

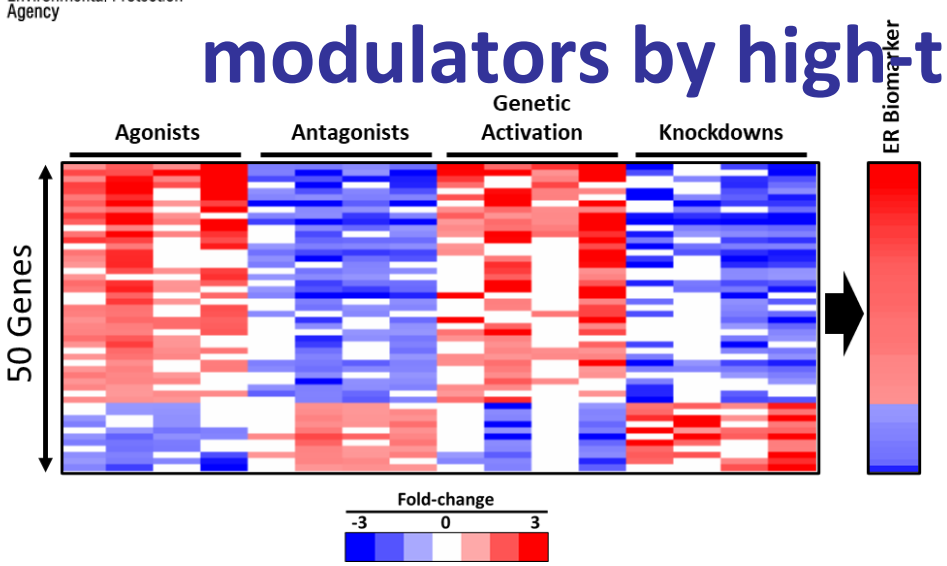
Stress factors

- Cervantes PW, Corton JC. (2021). A Gene Expression Biomarker Predicts Heat Shock Factor 1 Activation in a Gene Expression Compendium. Chem Res Toxicol. 2021 34(7):1721-1737.
- Jackson AC, Liu J, Vallanat B, Jones C, Nelms MD, Patlewicz G, Corton JC. (2020). Identification of novel activators of the metal responsive transcription factor (MTF-1) using a gene expression biomarker in a microarray compendium. Metallomics. 12(9):1400-1415.
- Korunes KL, Liu J, Huang R, Xia M, Houck KA, Corton JC. (2022). A gene expression biomarker for predictive toxicology to identify chemical modulators of NF-κB. PLoS One. 17(2):e0261854.
- Rooney JP, Chorley B, Hiemstra S, Wink S, Wang X, Bell DA, van de Water B, Corton JC. (2020). Mining a human transcriptome database for chemical modulators of NRF2. PLoS One. 15(9):e0239367.

In progress

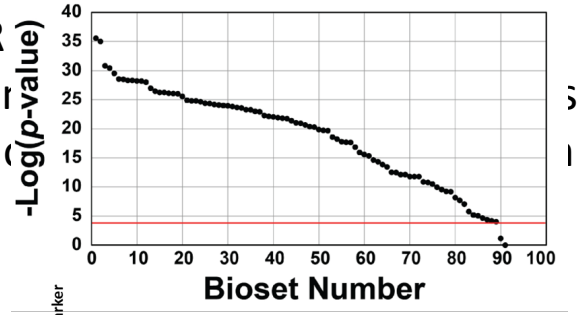
- HIF1a, Unfolded Protein Response (ATF4, ATF6, XBP1), Cell Proliferation, AhR, Epigenome Effectors

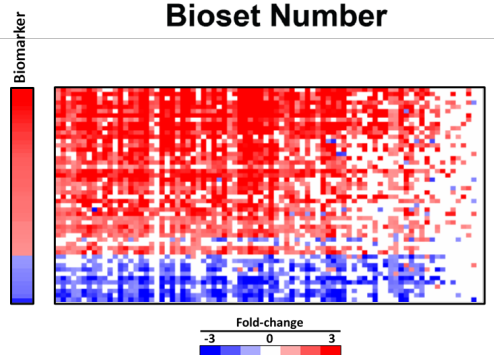
Use of an estrogen receptor biomarker to identify ER modulators by high-throughput transcriptomics (HTTr) screening

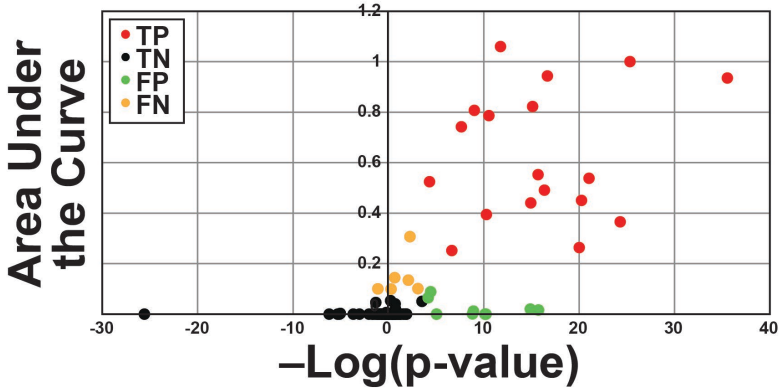


50-gene biomarker built from profiles of

- 4 ER agonists
- 4 ER
- 4 cor
- 4 kn



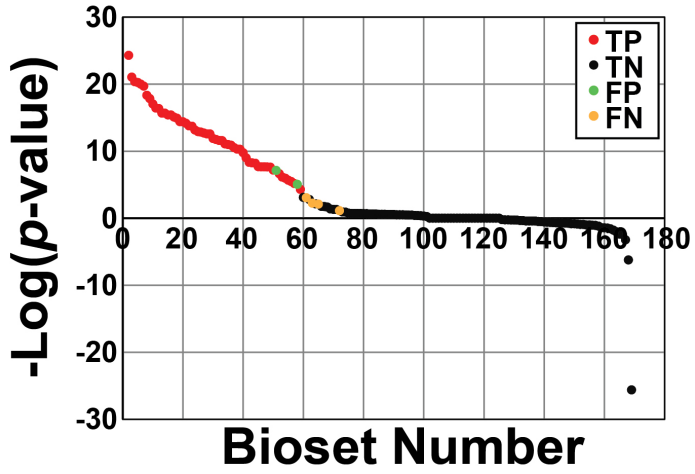




Using the ToxCast ER model as the reference data set:

- Sensitivity = 75%
- Specificity = 90%
- Balanced accuracy = 82%

- Replicates the predictions of the ToxCast ER Model based on 18 HTS assays



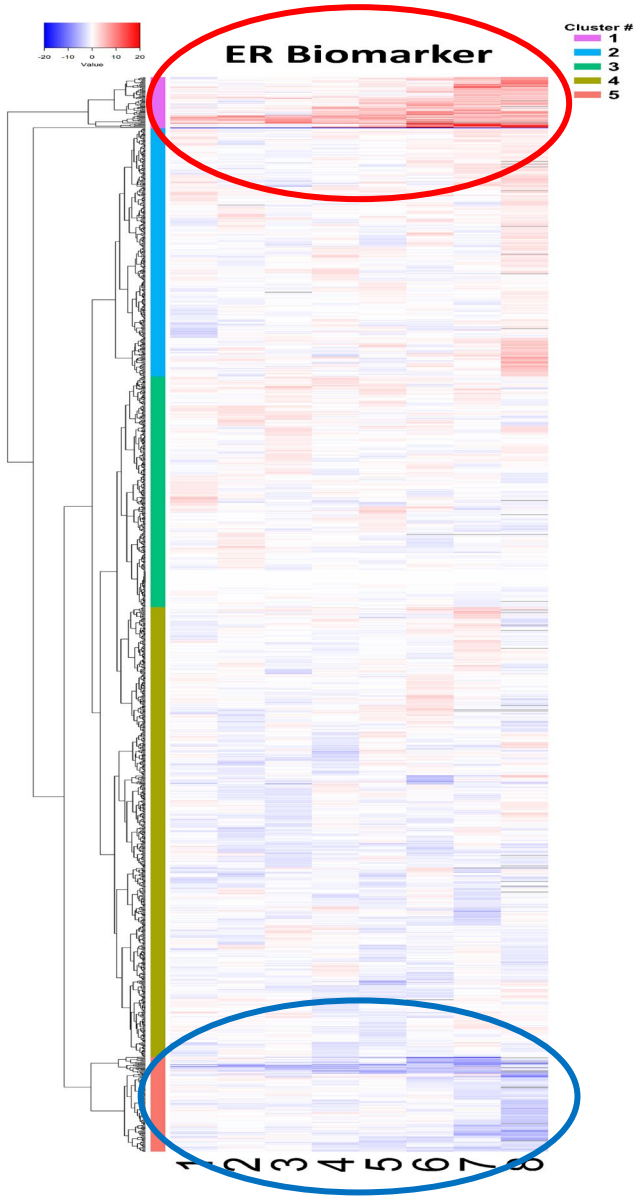
Using the NCATS Tox21 ER trans-activation assays as the reference data set:

- Sensitivity = 93%
- Specificity = 98%
- Balanced accuracy = 96%

- Excellent predictive accuracy with HTTr TempO-Seq data (Robarts et al., in prep)

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

- 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
- 2D hierarchical clustering of chemicals across 8 concentrations

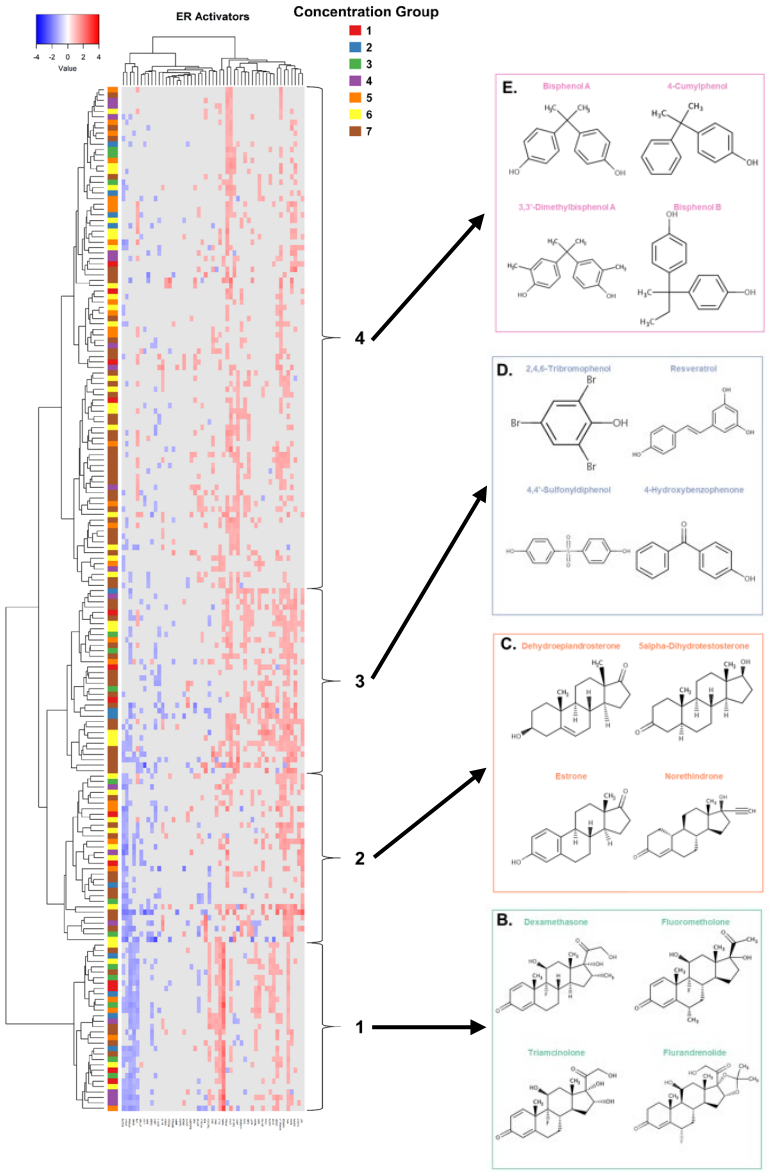


ER Activators

ER Suppressors

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

- **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**
- **2D hierarchical clustering of ~120 chem-concentration pairs that activated ER**

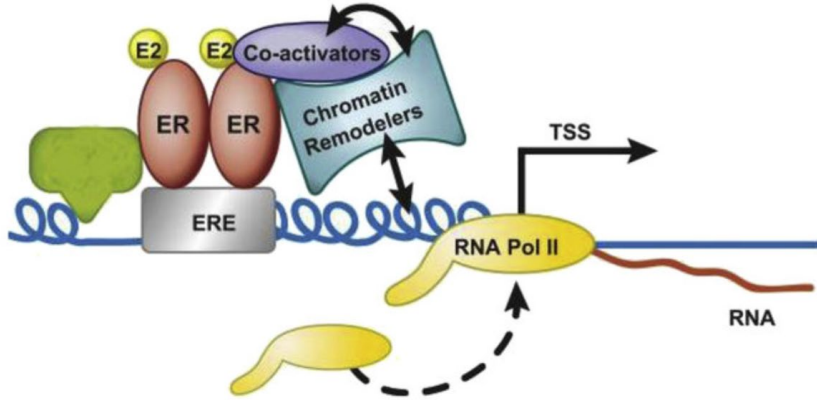


Bisphenols

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

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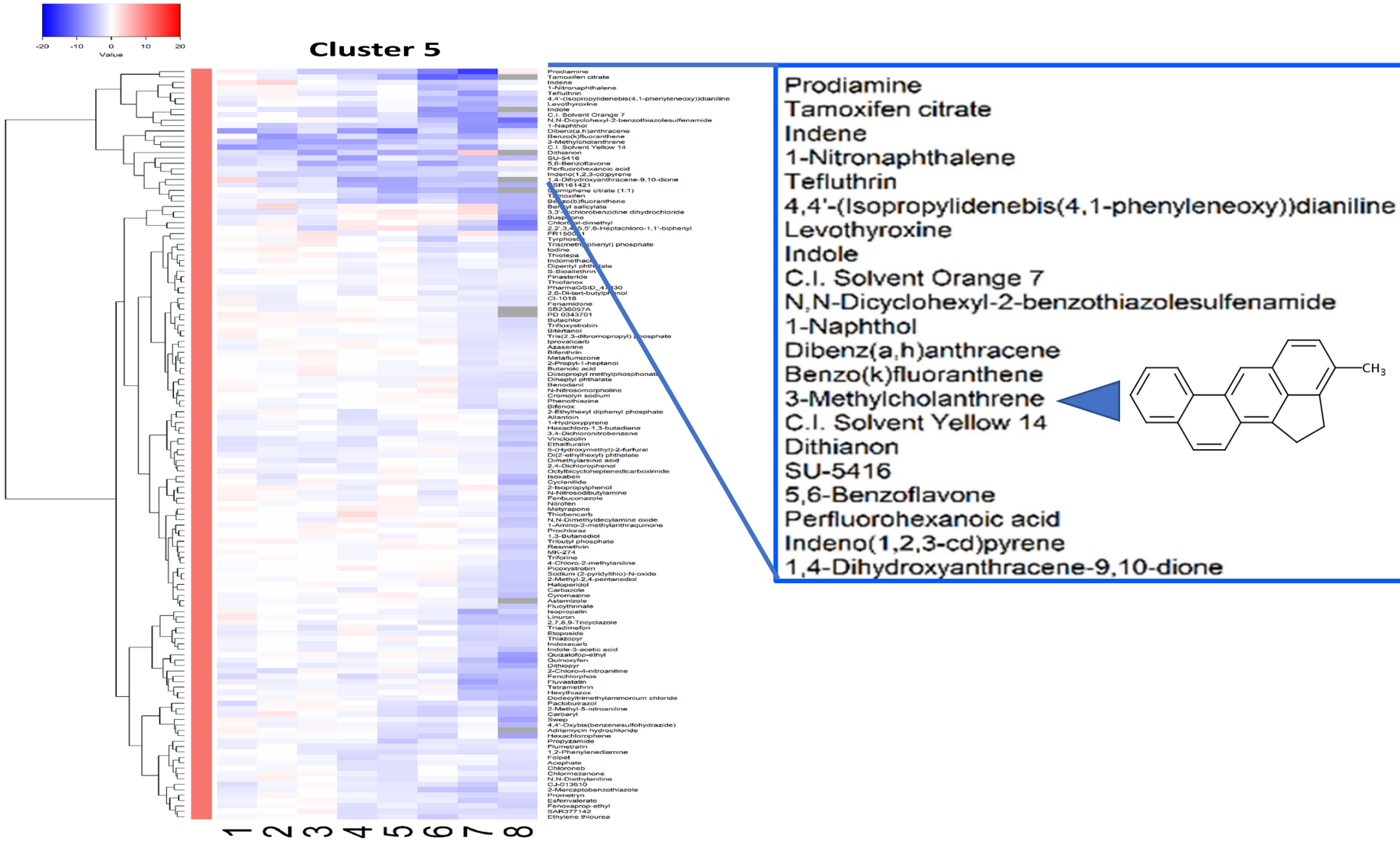
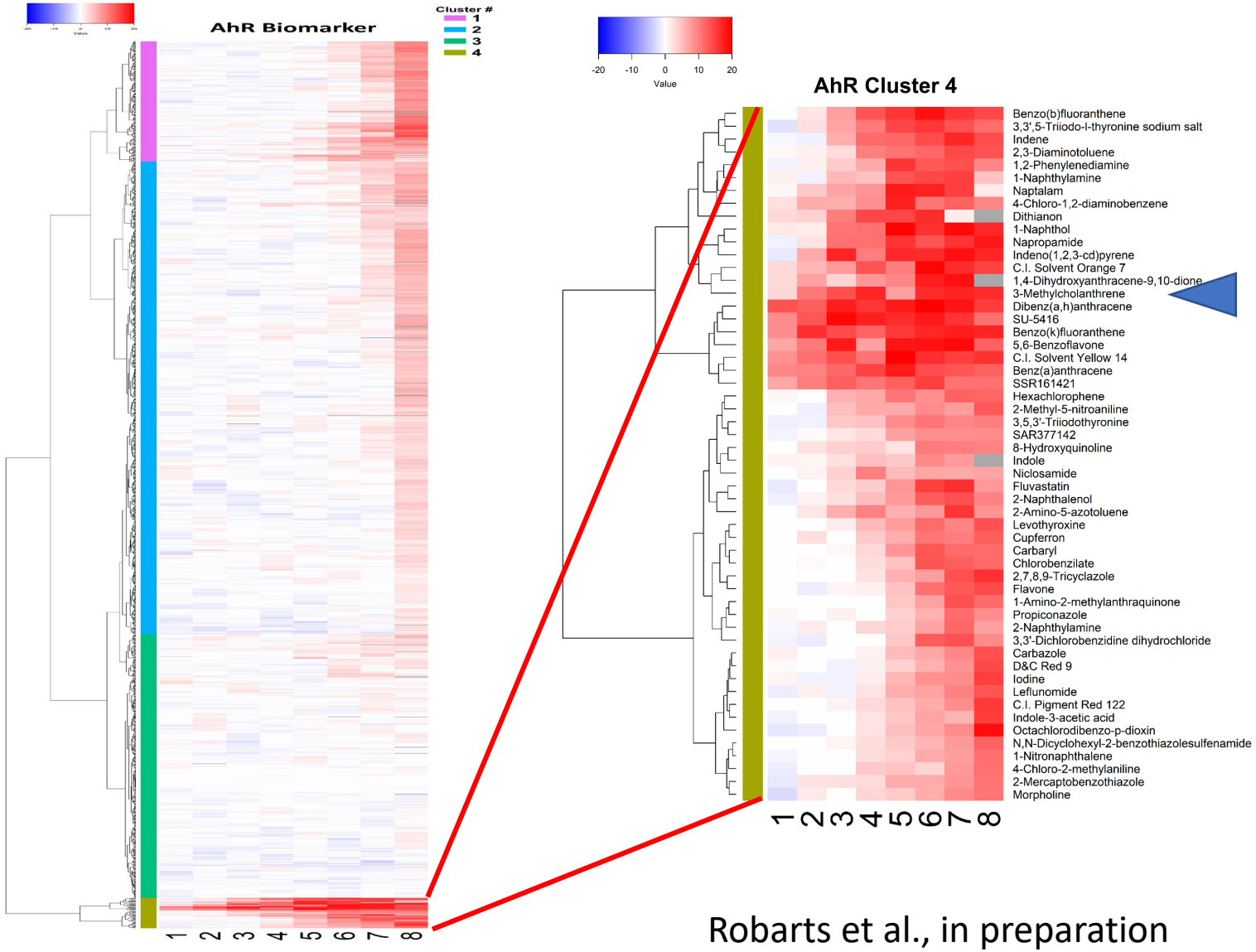


Robarts et al., in preparation

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

- 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 predictions to NCATS Tox21 AhR transactivation assay carried out in HepG2 cells
  - Sensitivity = 73%
  - Specificity = 59%
  - Balanced accuracy = 66%

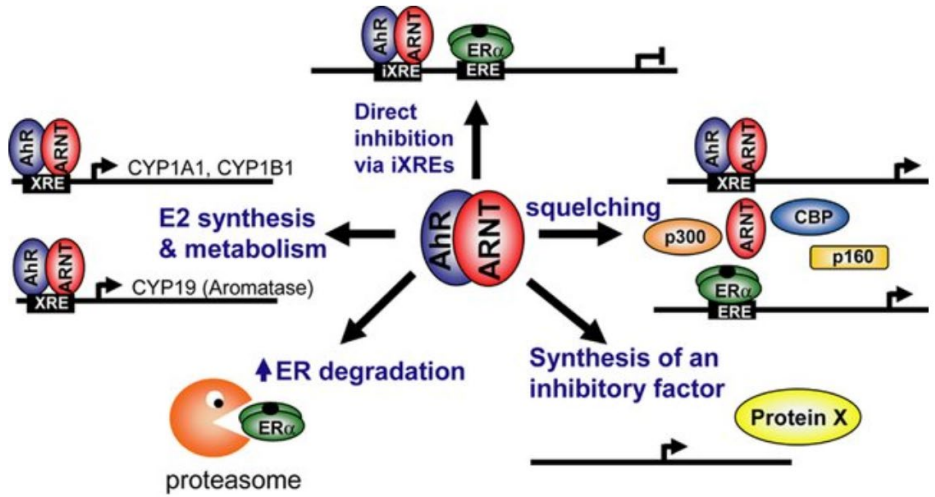
- Compared the ~12,800 profiles to the AhR biomarker



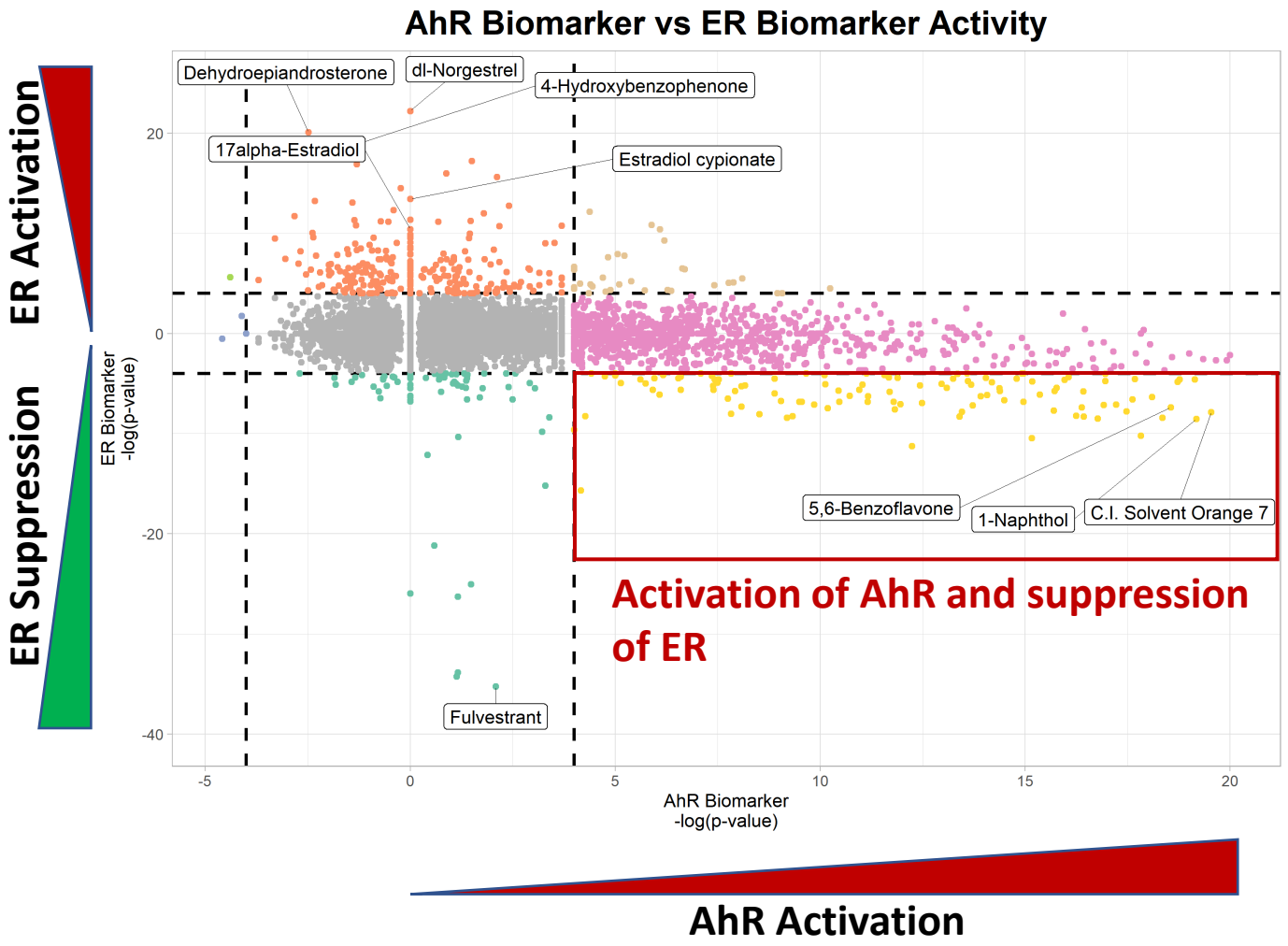
Robarts et al., in preparation

AhR activators suppress ER responses

- **Examined transcript changes in MCF-7 cells treated with ~1600 chemicals at 8 concentrations**
- **Compared the profiles to the estrogen receptor (ER) and aryl hydrocarbon receptor (AhR) biomarkers**



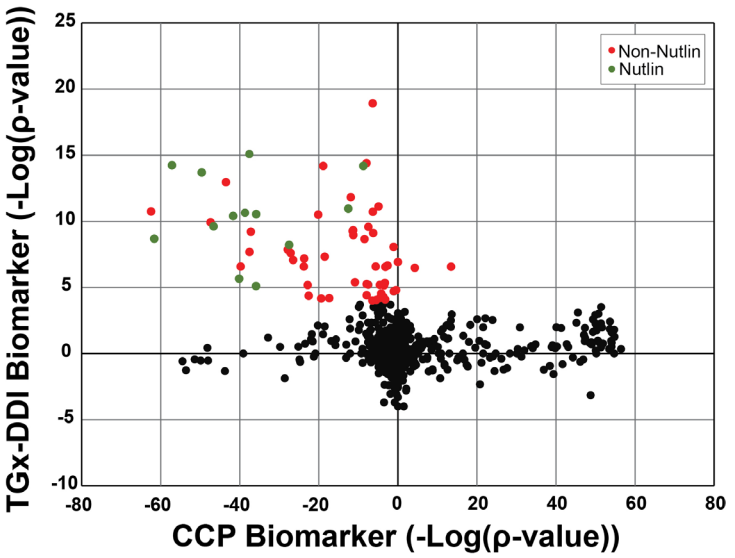
From Nuclear Receptor Signaling 4(1):e016

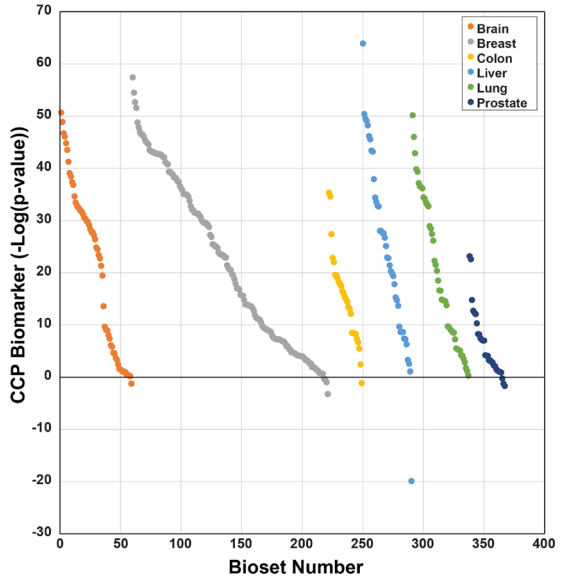


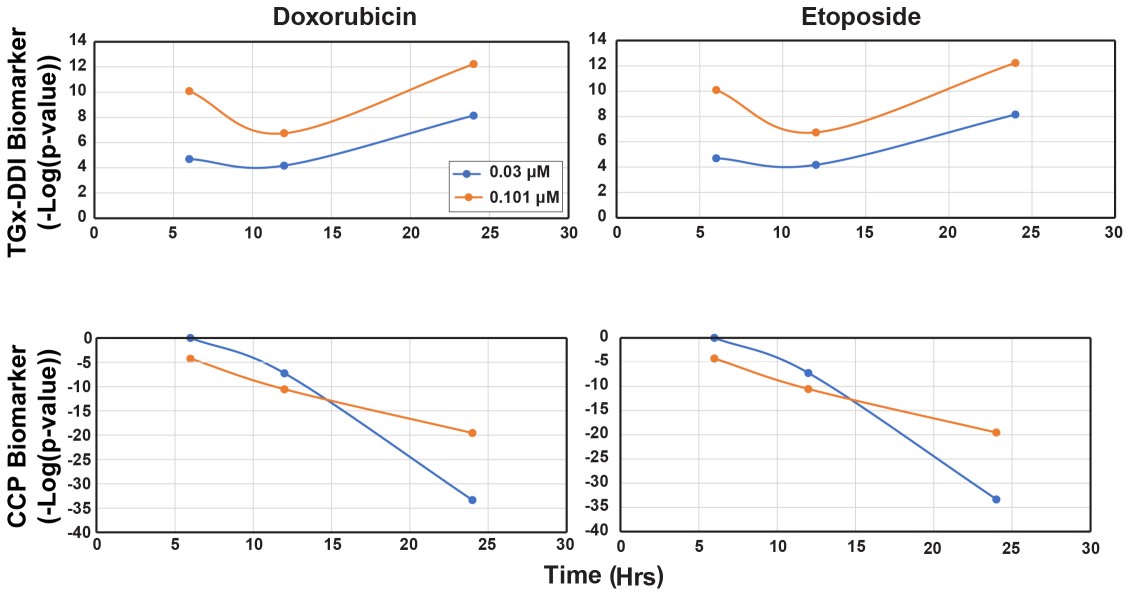
Robarts et al., in preparation

The Cell Cycle Progression Biomarker

- 34 genes identified as being involved in cell cycle progression in human prostate tumors
- Examined gene expression after 48 hrs of treatment with 6 estrogen receptor activators in MCF-7 cells
- Compared the CCP biomarker to a number of datasets to assess utility







Linking Estrogen Receptor Activation with Cell Proliferation

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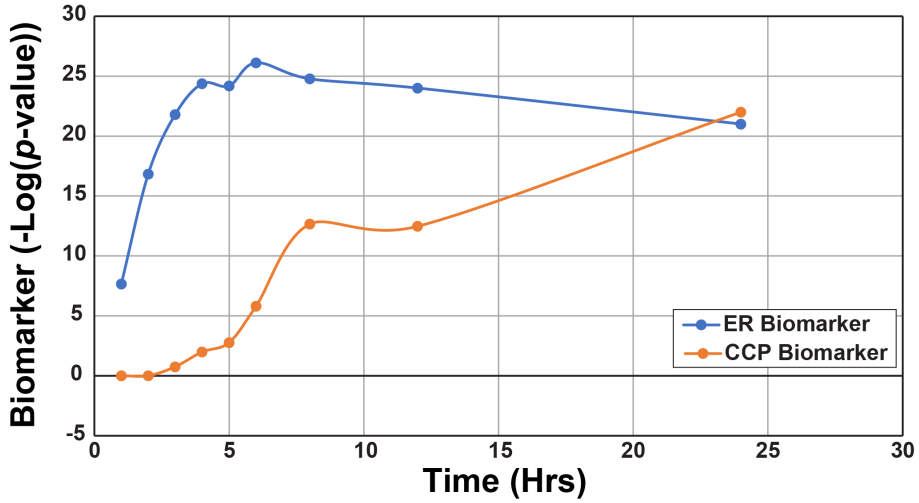
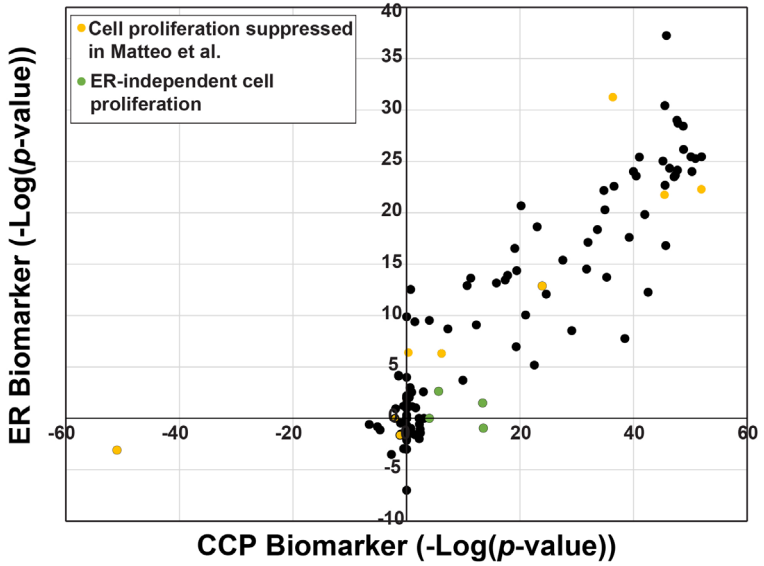
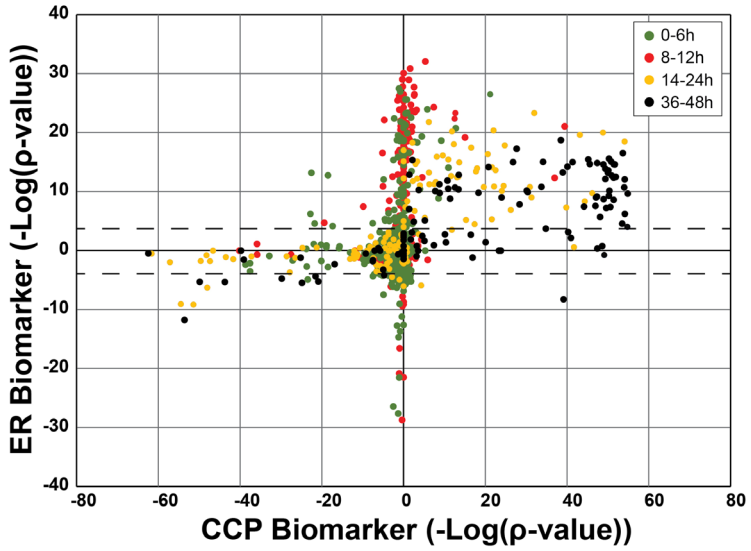
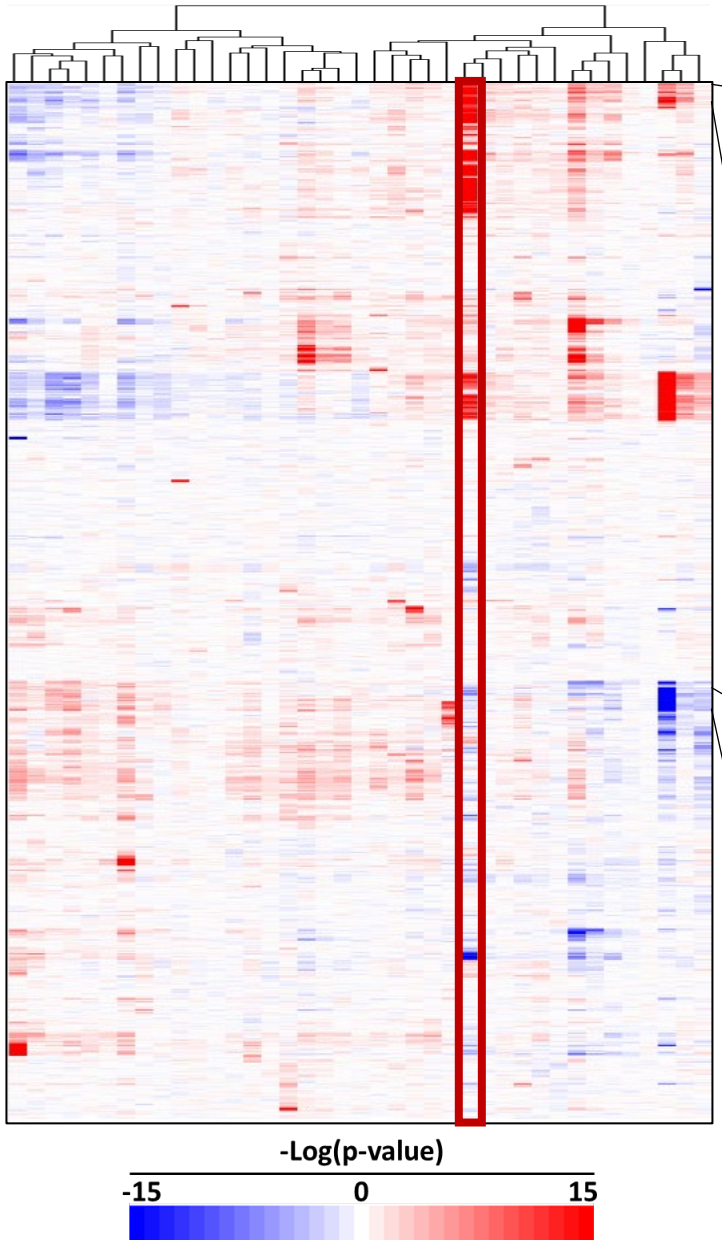


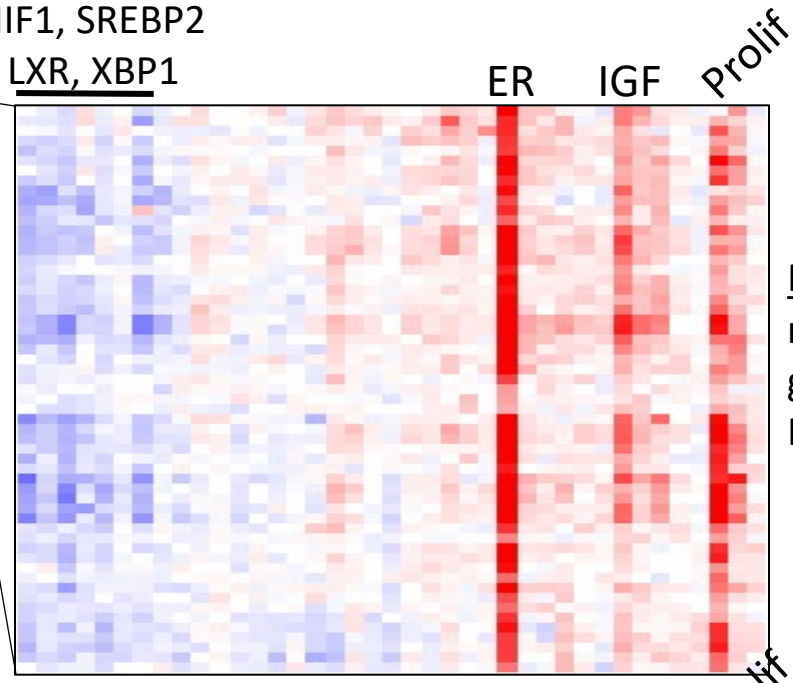




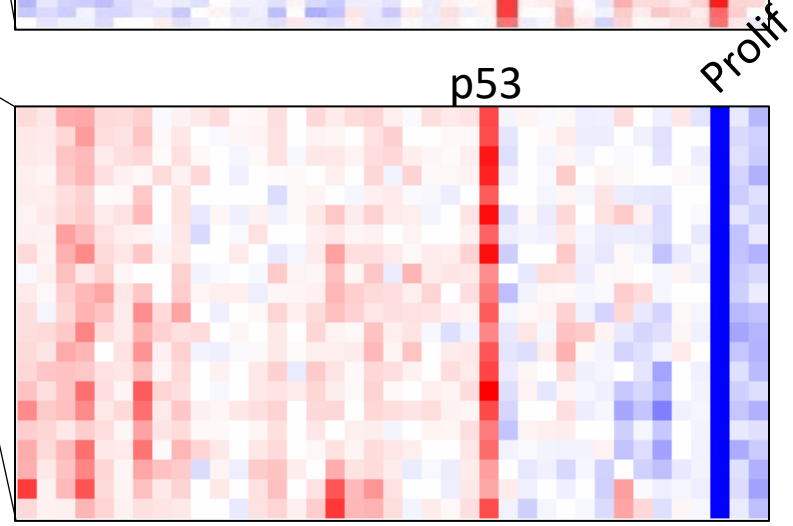
Behavior of Biomarkers in MCF-7 cells

- Examined relationships between 2165 microarray comparisons in MCF-7 cells across 39 biomarkers
- Includes chemicals, various stressors, cytokines
- Two-dimensional hierarchical complete linkage clustering





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



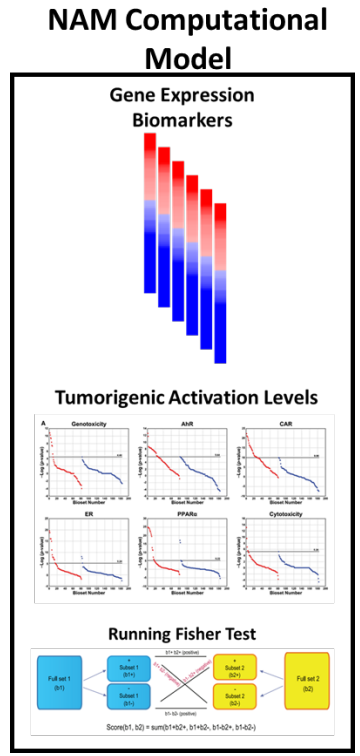
p53 activators: nutlin-3, 5-fluorouracil, doxorubicin

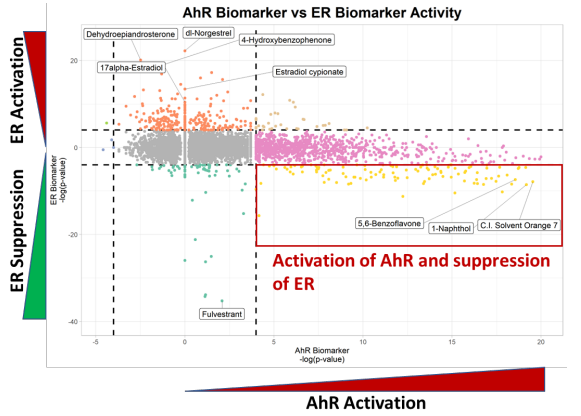
- Efforts are ongoing to integrate predictions into prioritization schemes and into the AOP network

Summary

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

- Biomarkers for Tier 1 screening in high throughput transcript profiling
  - Estrogen receptor biomarker
    - Used to identify MIE modulation
    - Potential for replacing HTS assays
    - Potential for replacing the uterotrophic assay
    - Uncovers interesting biology
      - Biomarker gene expression pattern determined by chemical structure
      - Identified AhR-ER interactions
  - Cell Cycle Proliferation Biomarker useful to identify conditions in which cell proliferation is induced





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