The impact of mixed estrogenic chemicals on non-malignant cell function

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Population-based Alternatives to Routine *In Vitro* Models

Random Periareolar Fine Needle Aspirates (RPFNA)

HRBECs High-Risk donor derived Breast Epithelial Cells

ARBECs Average-Risk donor derived Breast Epithelial Cells

☑ Study approved by CPMC Institutional Review Board

☑ Prior written consent obtained from donors

Rationale

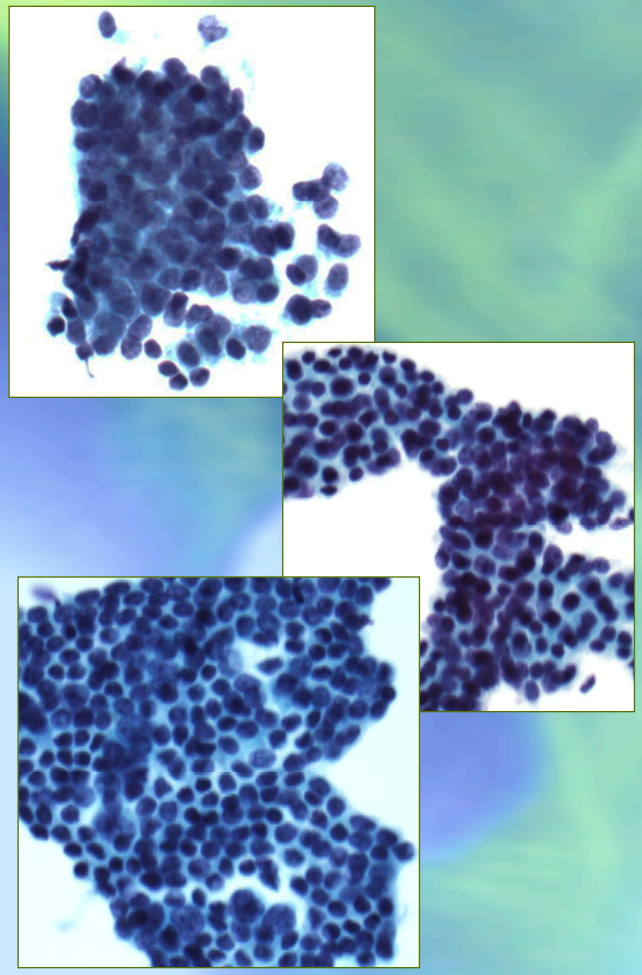
Overexposure to natural estrogens is strongly associated with breast cancer. Exposure to persistent synthetic estrogen mimics, also known as xenoestrogens (XEs) is thus potentially carcinogenic.

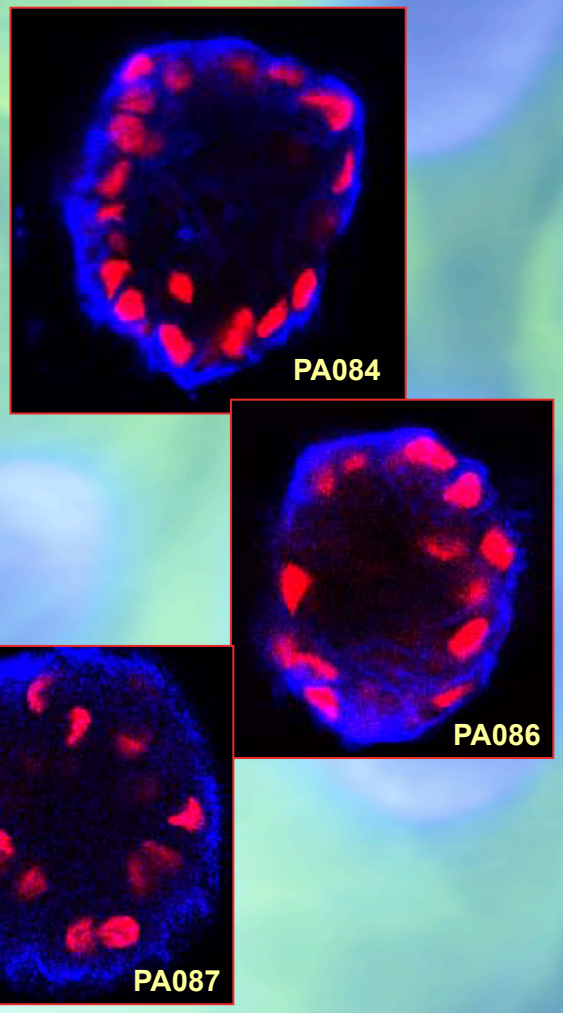
A causal role for XE exposure in breast cancer progression will be revealed by employing test systems representative of carcinogen-targeted healthy epithelial cells in the human breast - the cells it is hoped will not become malignant.

A finite *in vitro* life span of such healthy human cells is not a barrier for experimentation. Instead, by sampling a wide spectrum of individuals, the limitations of data collection from rare immortalized cancer cell lines will be surmounted.

Improvements in key parameters of breast carcinogen screening assays will advance breast cancer prevention.

RPFNA-derived non-malignant breast epithelial cells



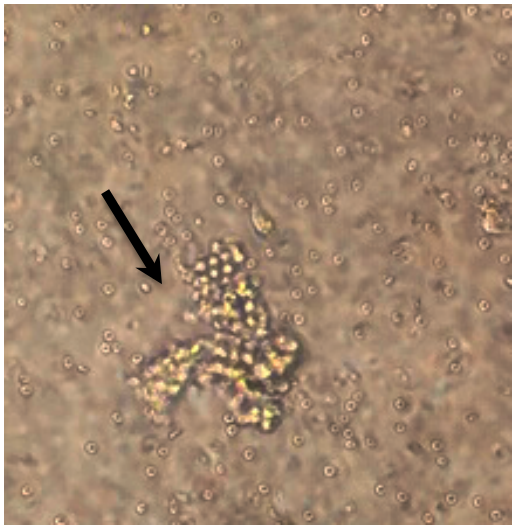


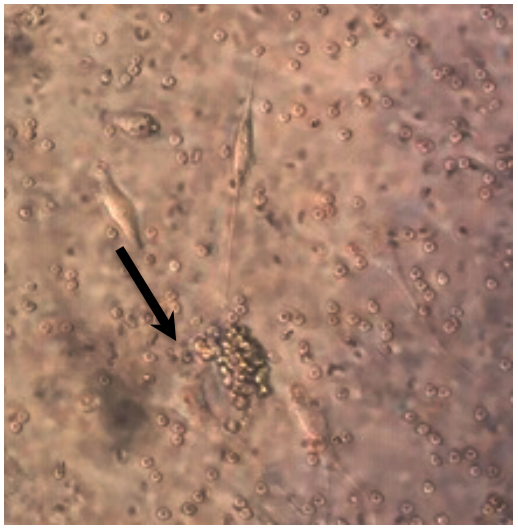
Cytopathology

3D phenotype *in vitro*

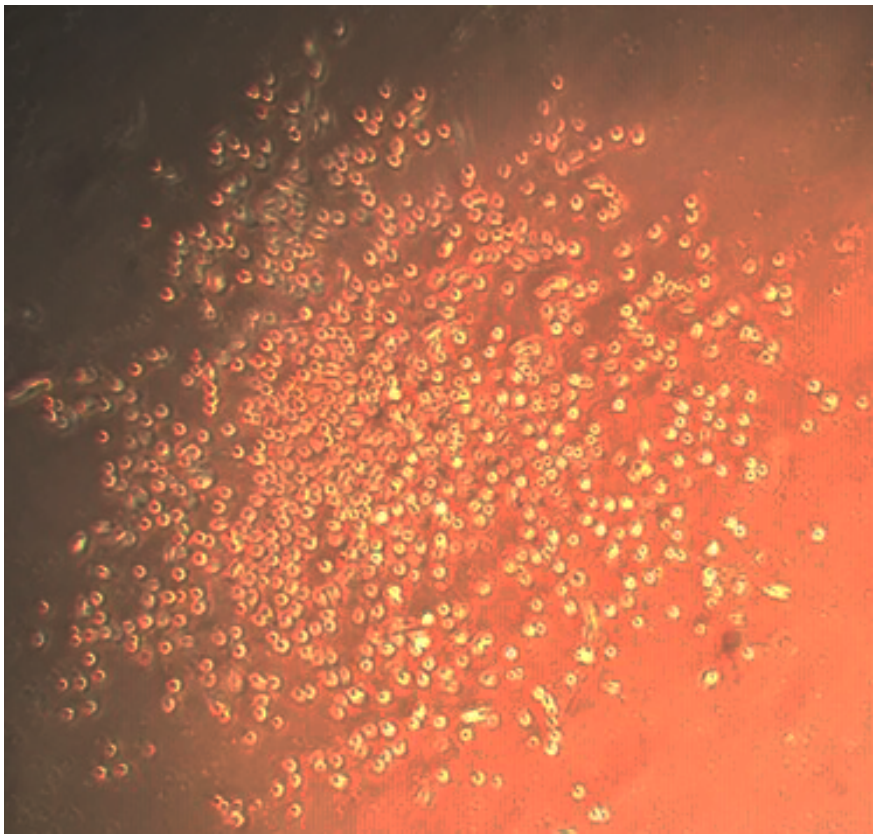
Expansion *in vitro*

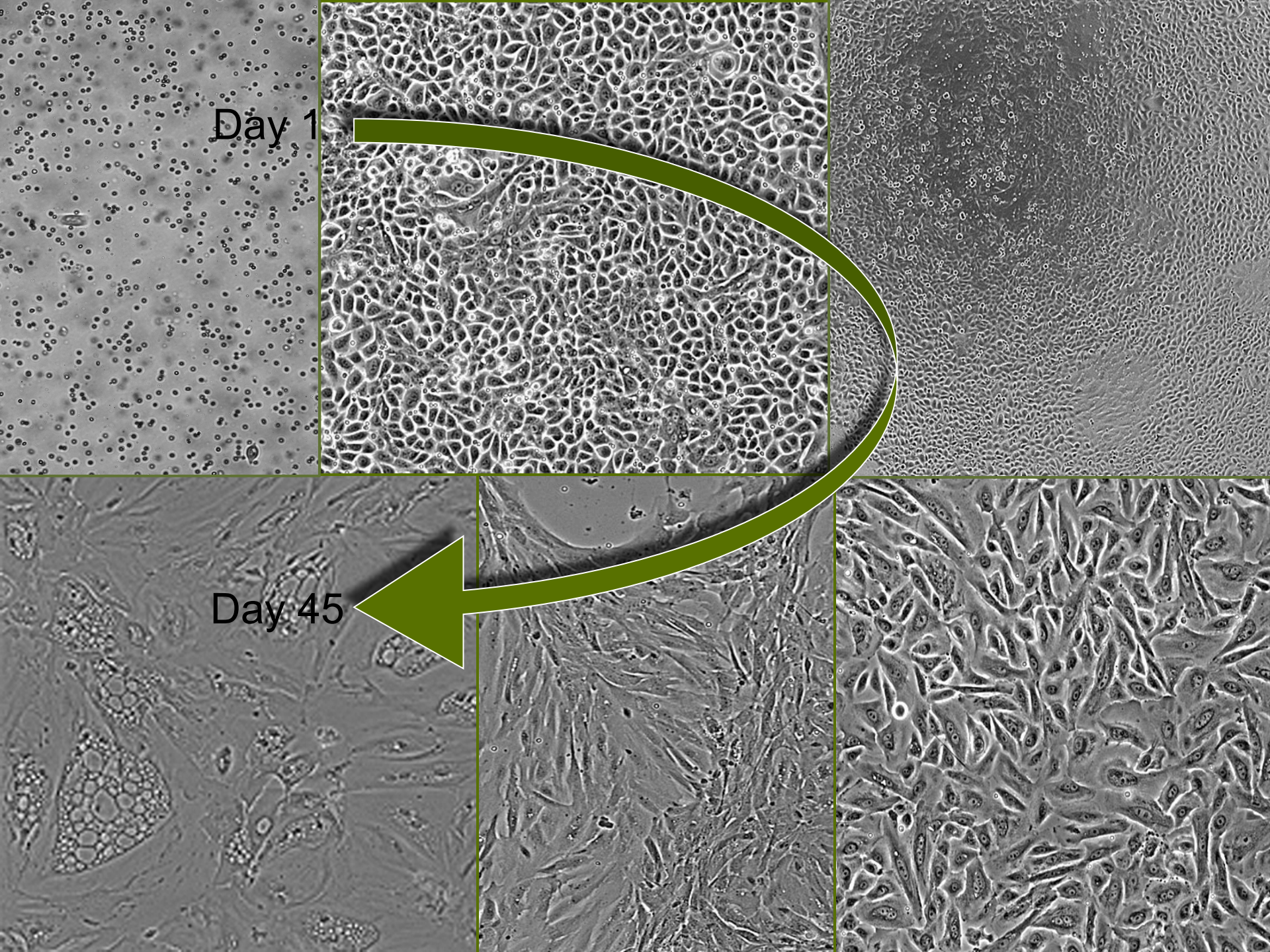
Fresh sample



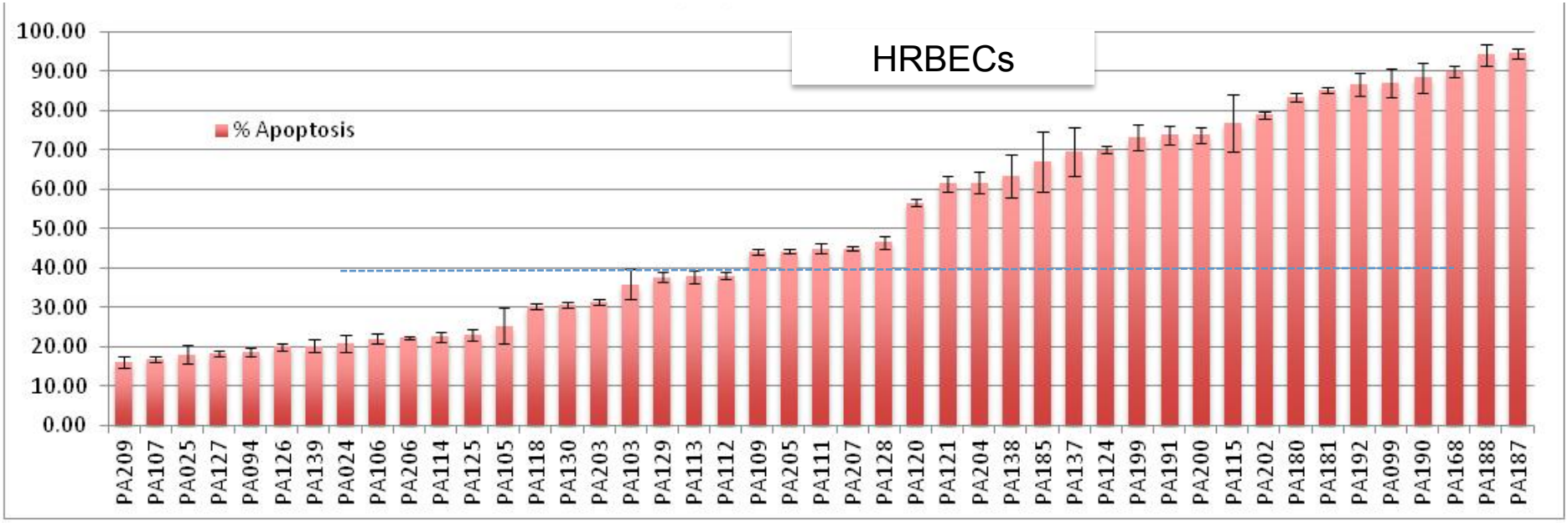


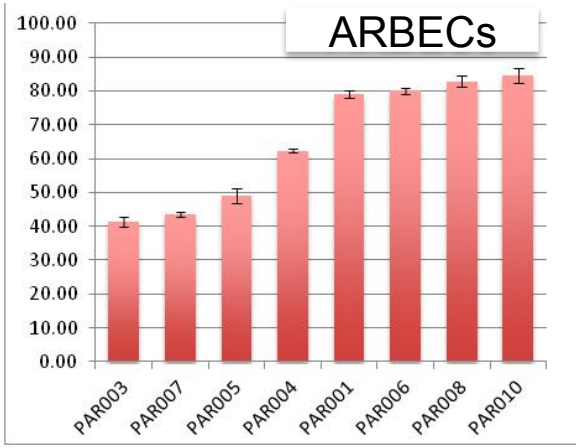
7-d *in vitro* colony



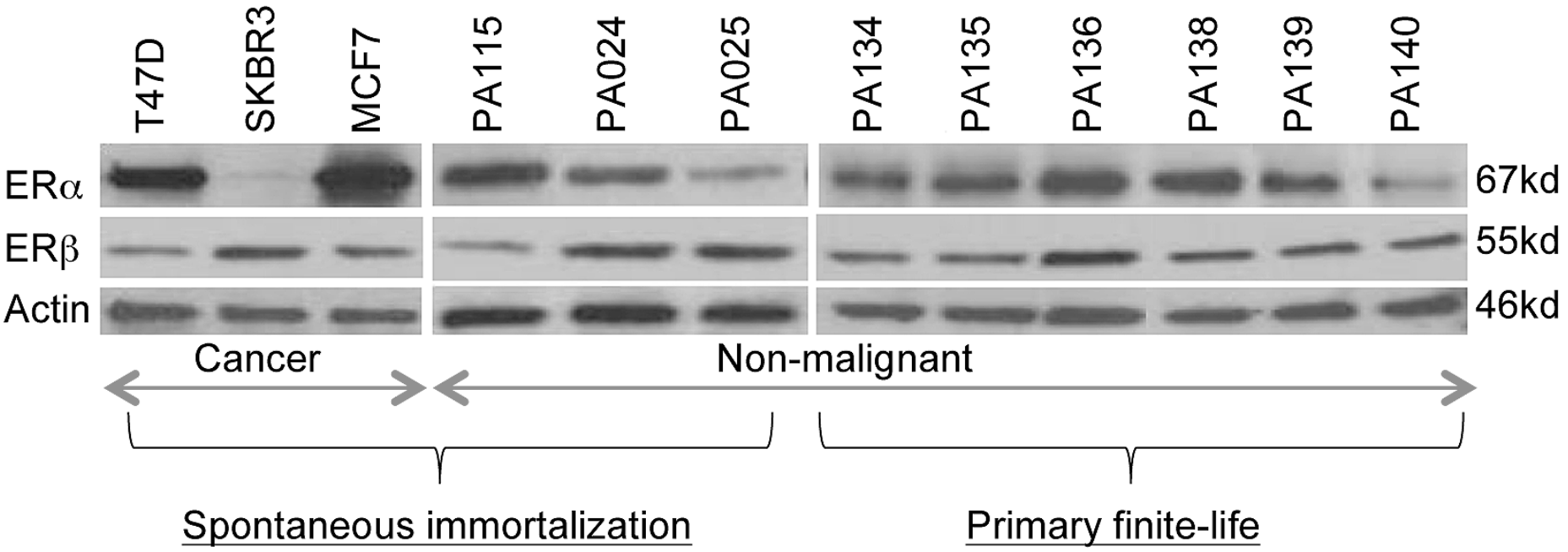

Day 1
Day 45

Functional heterogeneity





Maintenance of estrogen receptor (ER) expression



RPFNA Data Acquisition Flow Chart

1. Expansion of minimal numbers of live breast cells in samples collected from consented donors for *in vitro* chemical exposure.

2. Treatment with environmental chemicals of human relevance at a concentration range detected in body fluids and tissues.

3. Measurement of pathway functionality associated with known ‘hallmarks of cancer’.

4. Validation of exposure effects, singly and as mixtures, across additional population-based samples.

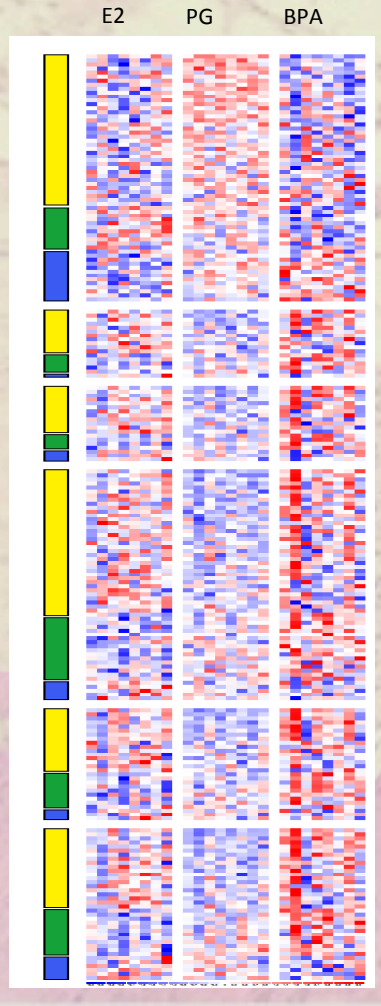
Further applications….

- Surrogate biomarkers of functional perturbations
- Reversal of chemically-induced perturbations

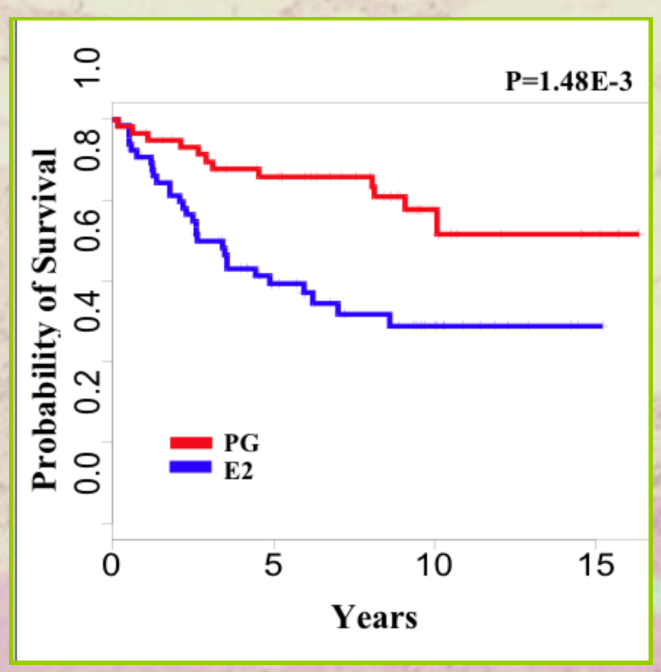
Priority Report Cancer Research 68: 2076-2080, 2008

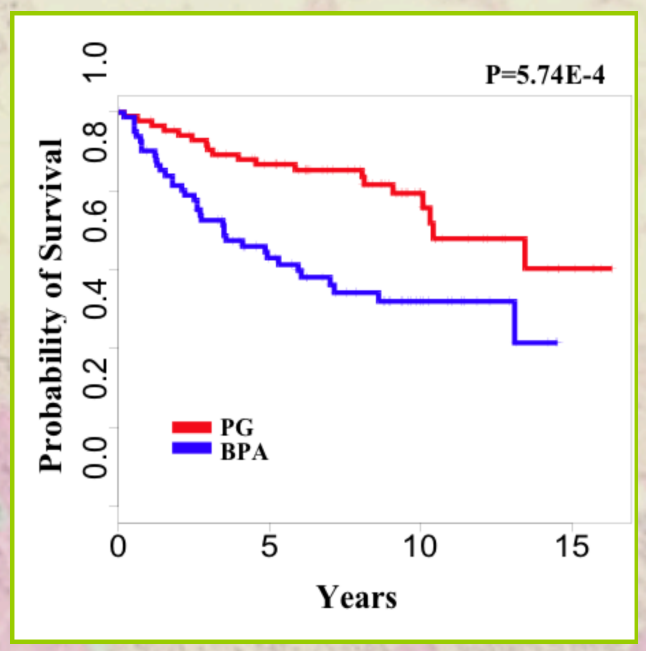
Bisphenol A Induces a Profile of Tumor Aggressiveness in High-Risk Cells from Breast Cancer Patients

Shanaz H. Dairkee,[1] Junhee Seok,[2] Stacey Champion,[1] Aejaz Sayeed,[1] Michael Mindrinos,[2] Wenzhong Xiao,[2] Ronald W. Davis,[2] and William H. Goodson[1]

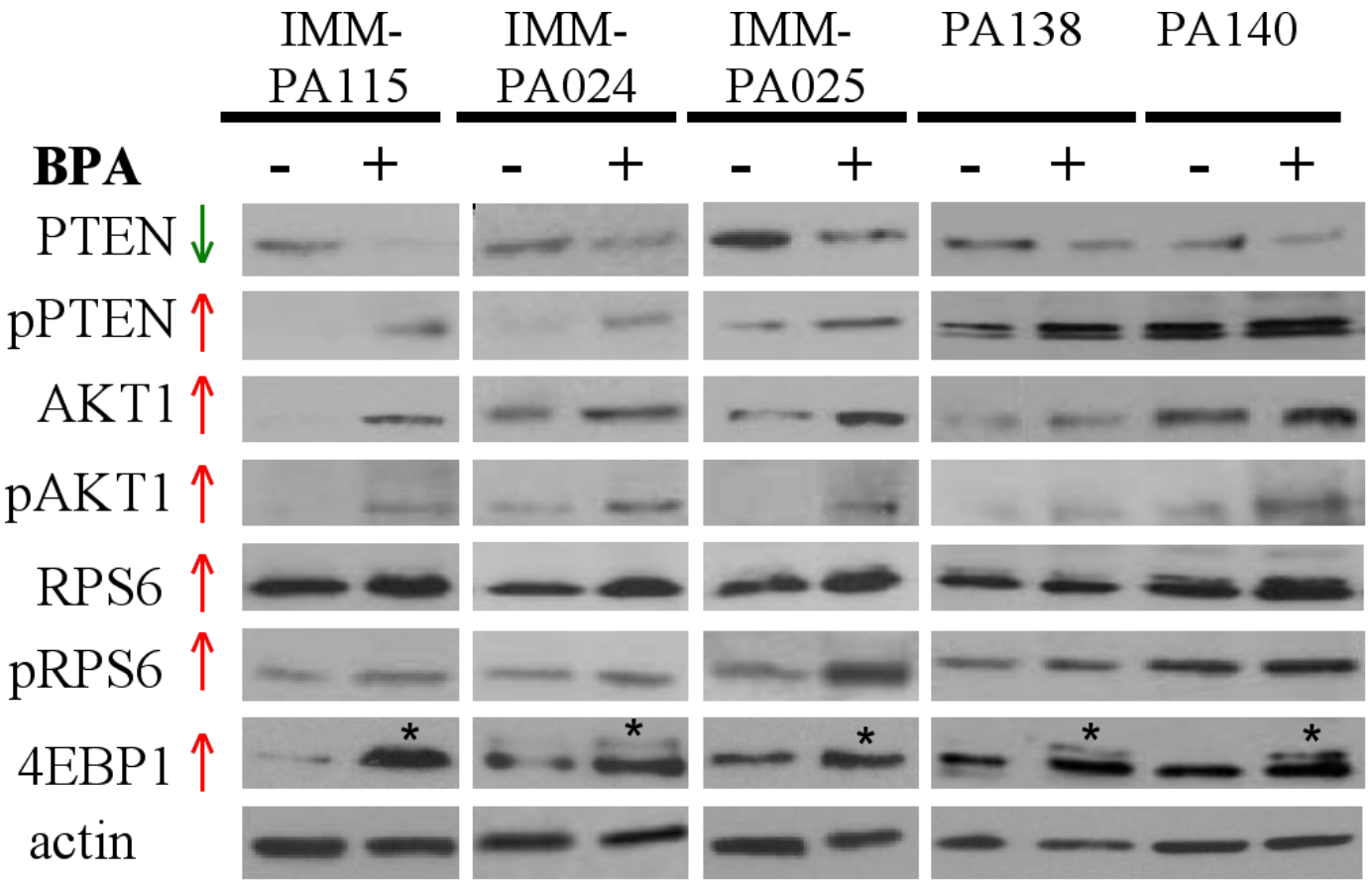


BPA signature and clinical outcome



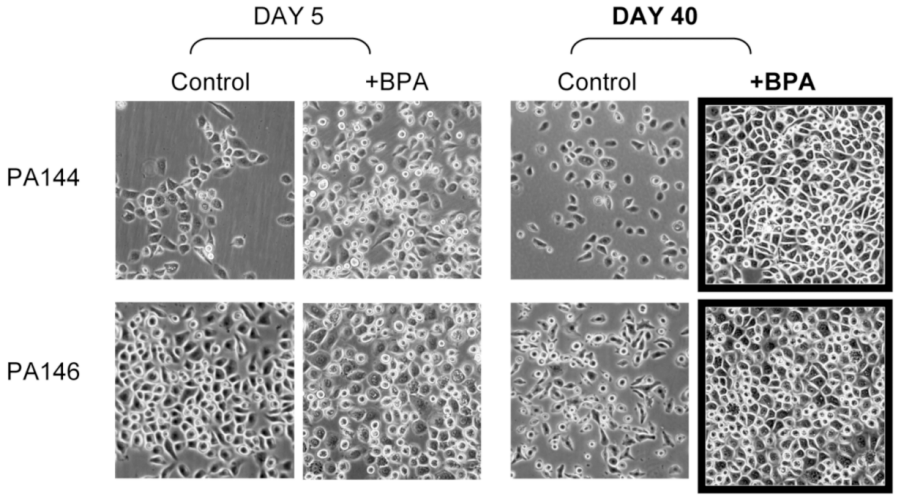


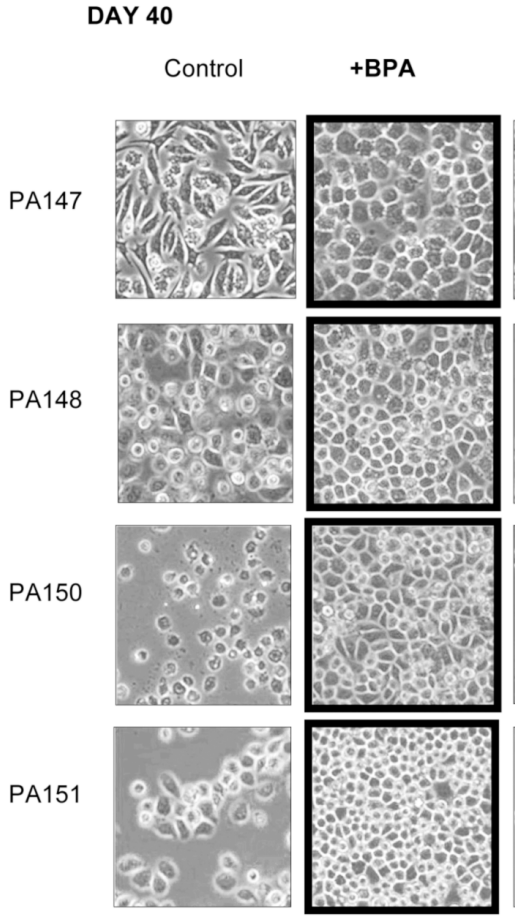
BPA deregulates mTOR pathway of cell survival in non-malignant breast cells



Dairkee et al., *Cancer Research* 68 (7):2076, 2008
Goodson et al., *Carcinogenesis* 32 (11):1724, 2011

BPA obliterates normal limit of cellular lifespan in non-malignant breast cells





Dairkee et al., *Carcinogenesis* 34 (3):703, 2013

Mixture treatment of non-malignant breast epithelial cells

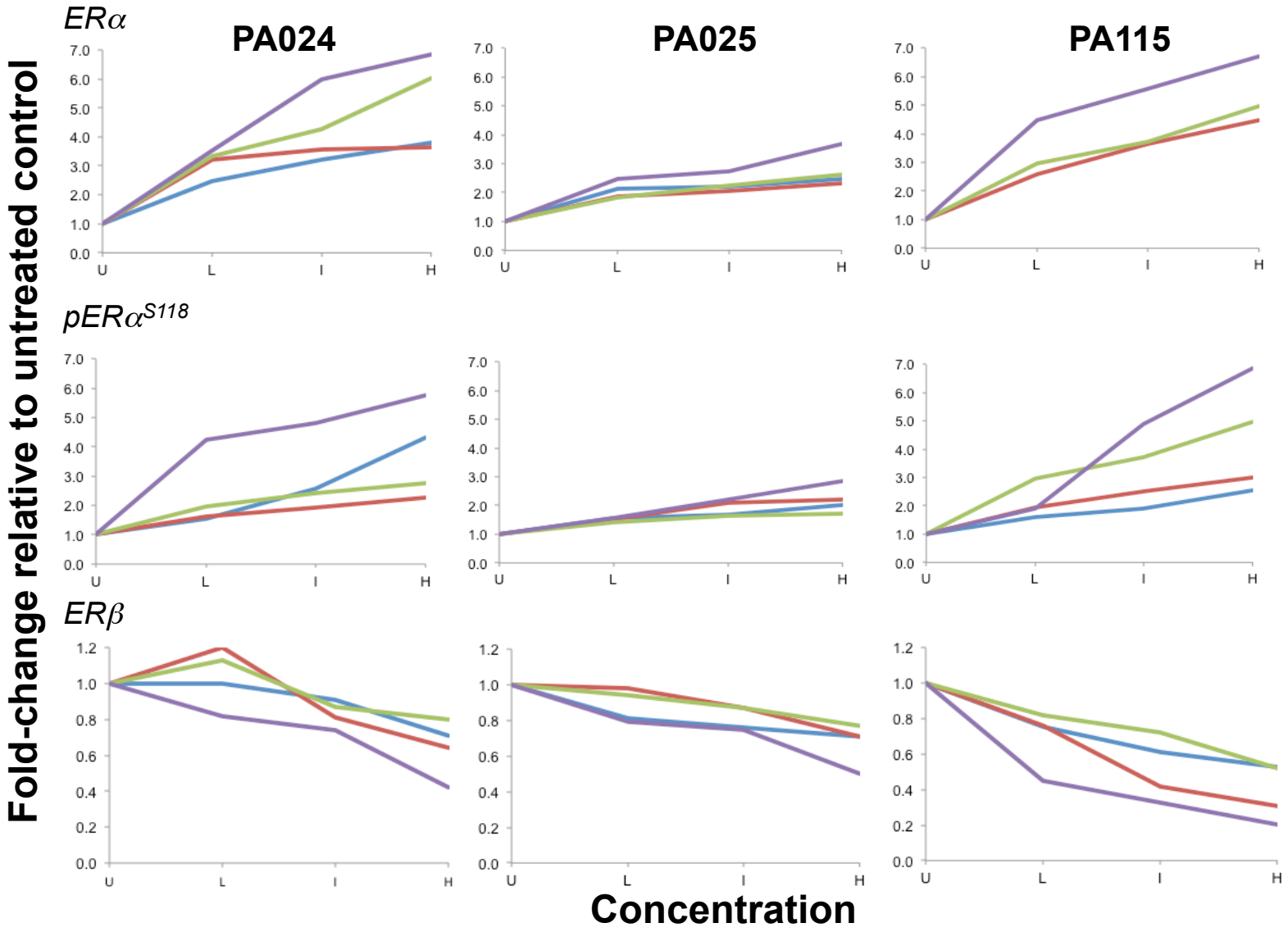
- Three high volume estrogenic chemicals: bisphenol A (BPA), methyl paraben (MP), and perfluorooctanoic acid (PFOA), were selected within the environmentally relevant concentration range of 1-10nM. Additionally, 10-fold higher exposure levels were also studied.

- We measured treatment effects upon total ERα, and ERβ based on their contrasting roles in cell cycle regulation. Additionally, we measured activated ERα phosphorylated at serine-118.

- Direct downstream consequences of effects on ER isoforms were assayed as the S-phase fraction of the cell cycle, and the proportion of cells that evaded experimentally induced apoptosis.

Mixture vs. single components

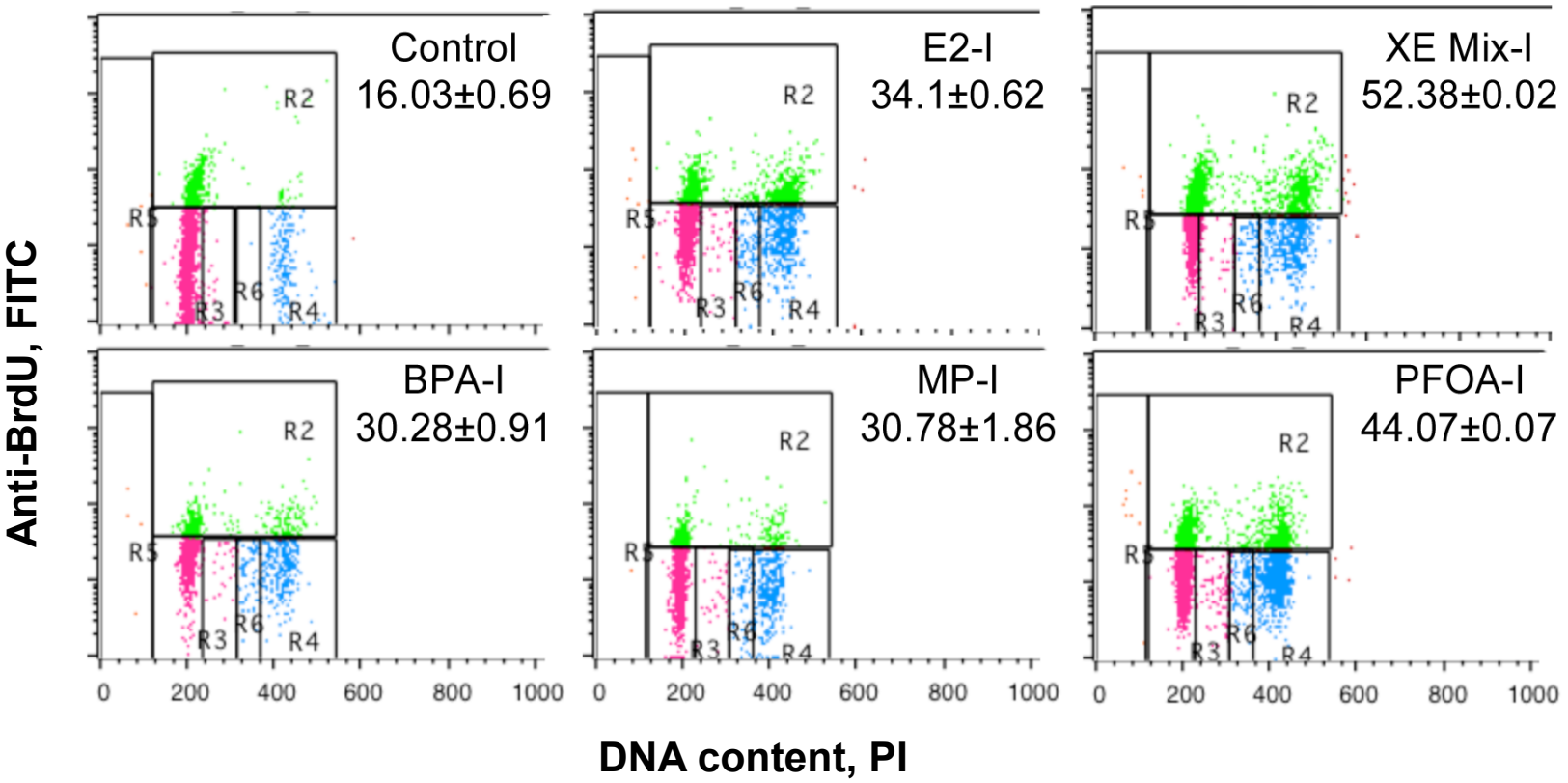
Modulation of ER isoforms



Dairkee et al., *Toxicological Sciences* 165:131-144, 2018

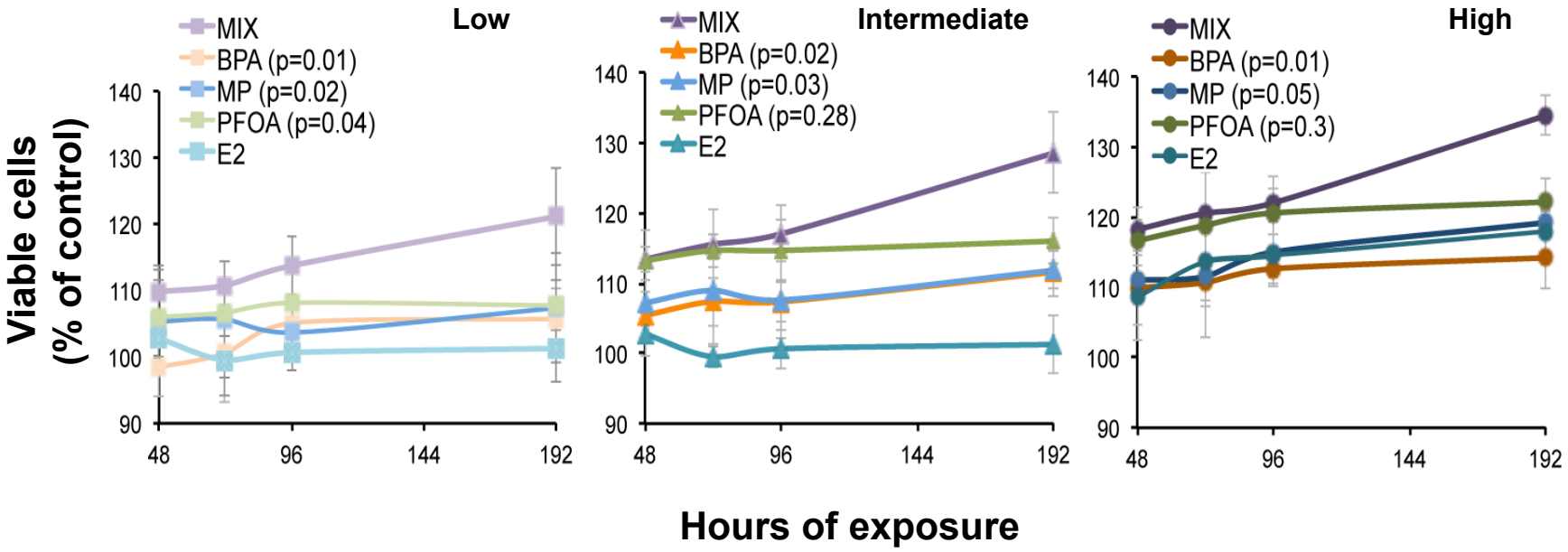
Mixture vs. single components

S-phase induction



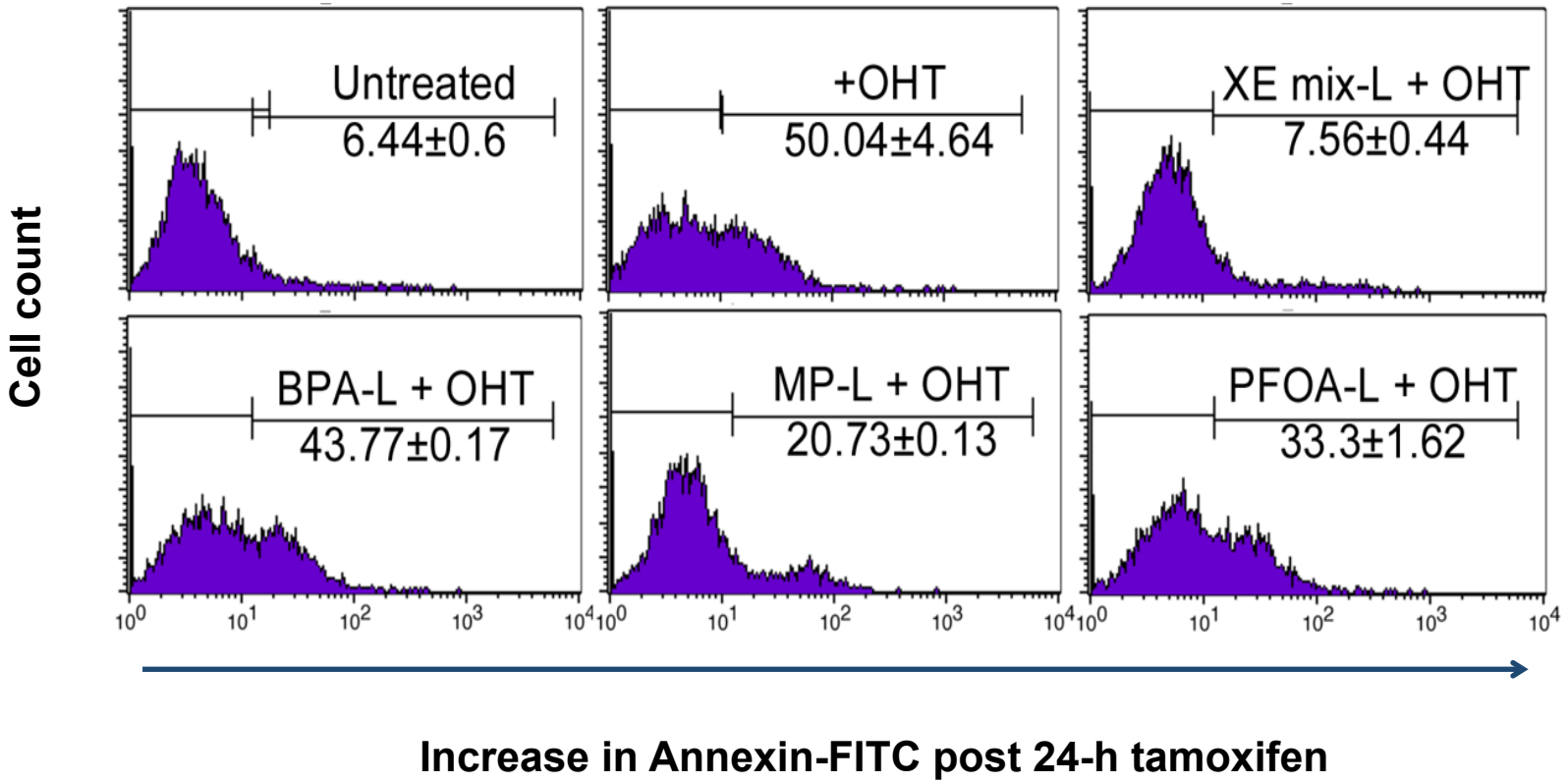
Mixture vs. single components

Increased rate of cell proliferation

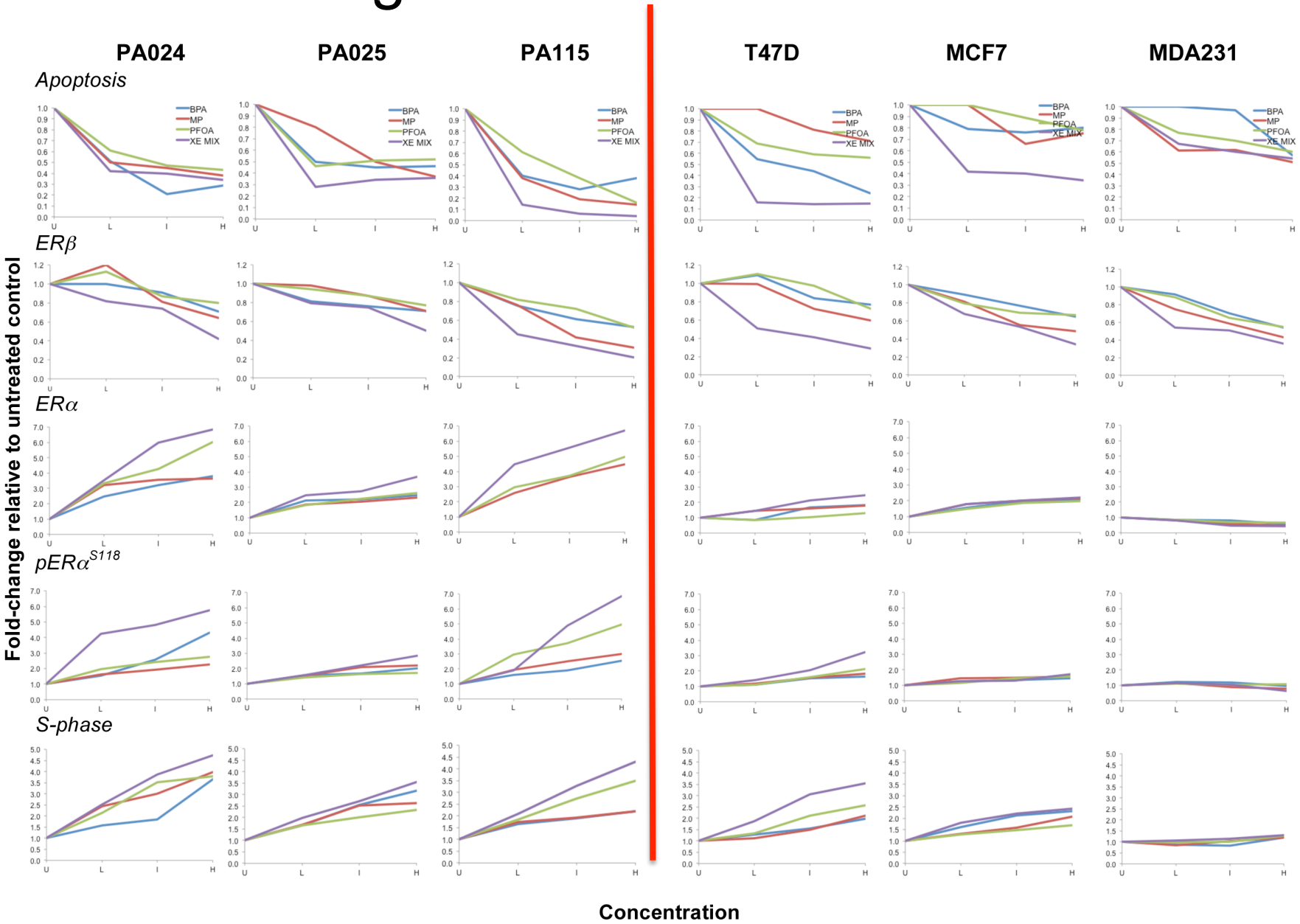


Mixture vs. single components

Programmed cell death evasion



Differential mixture effects on non-malignant vs. breast cancer cells



Conclusions

- The RPFNA-derived non-malignant breast cell model is as close as is ethically possible to carcinogen-targeted cells within human breast tissue.

- Some functional endpoints are more readily perturbed in chemically-exposed benign cells than in malignant cells. Testing cancer cell lines alone can miss important dysfunctional events.

- Unlike generalized one-size-fits-all screening schemes, RPFNA samples allow a direct test of population variability in regard to complex issues, such as perturbations induced by chemical mixtures.

Colleagues

Bill Goodson, M.D.

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