



# BioSeek - ToxCast Phase I Project Update

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EPA Chemical Prioritization Community of Practice  
Monthly Meeting

January 24, 2008

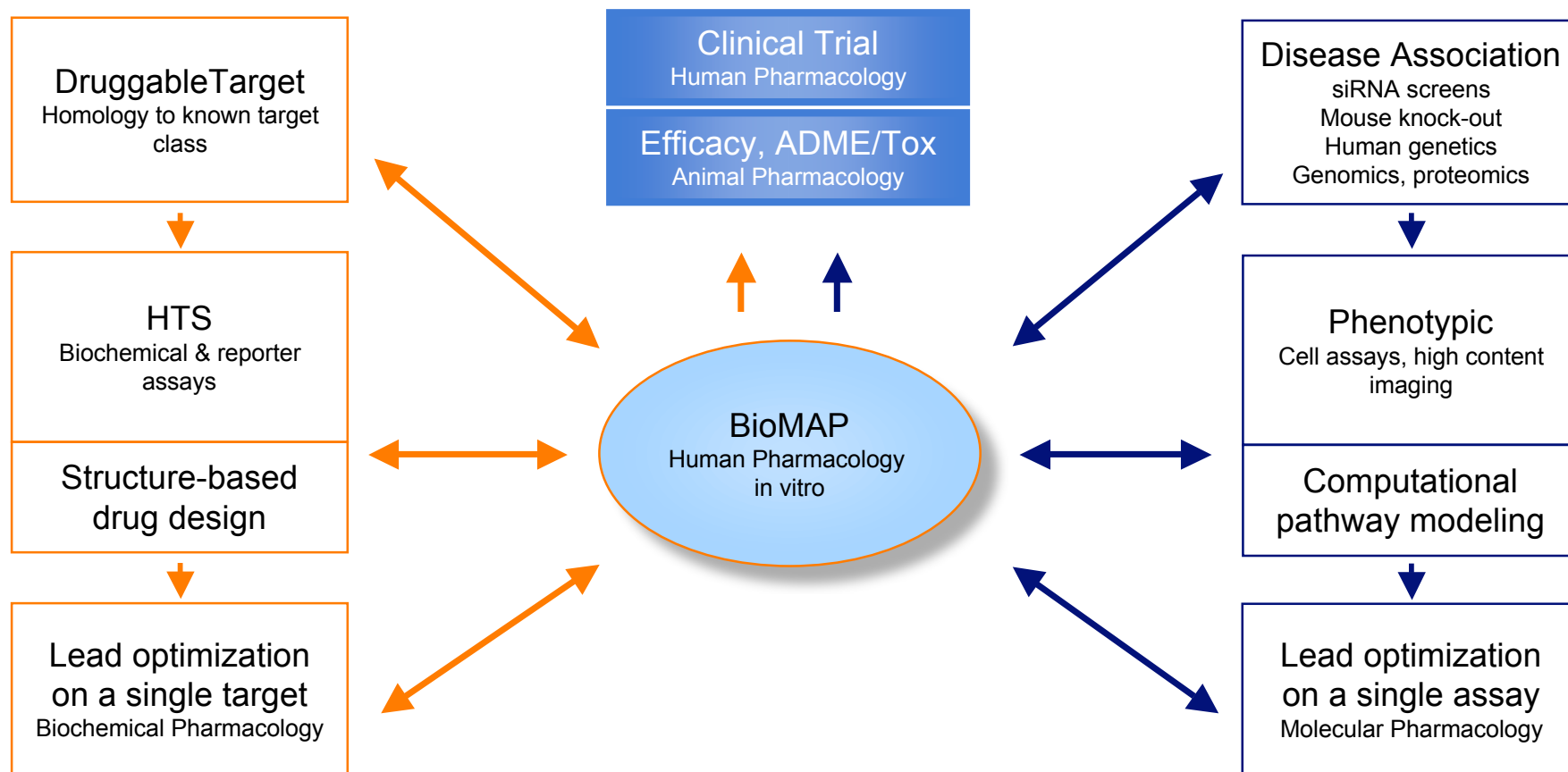
# Agenda

**Introduction to BioSeek's BioMAP Technology**

**Summary of results - Proof-of-Concept study**

**Next steps**

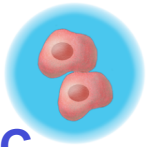
# Translating Chemistry into Human Pharmacology

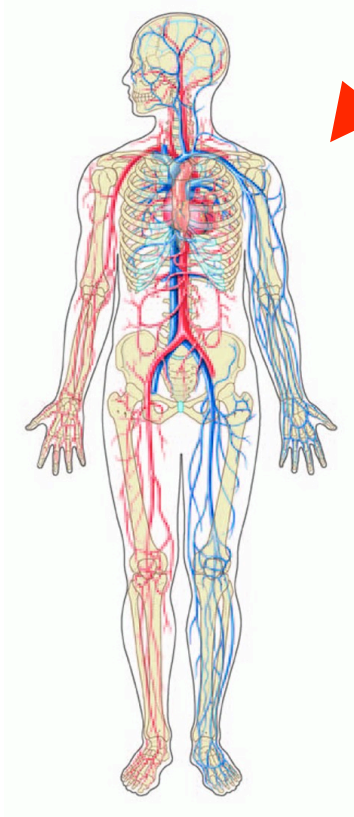


***With BioMAP, pharmacological properties of compounds are addressed at each step, de-risking critical decision points***

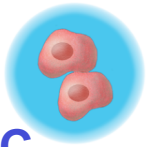


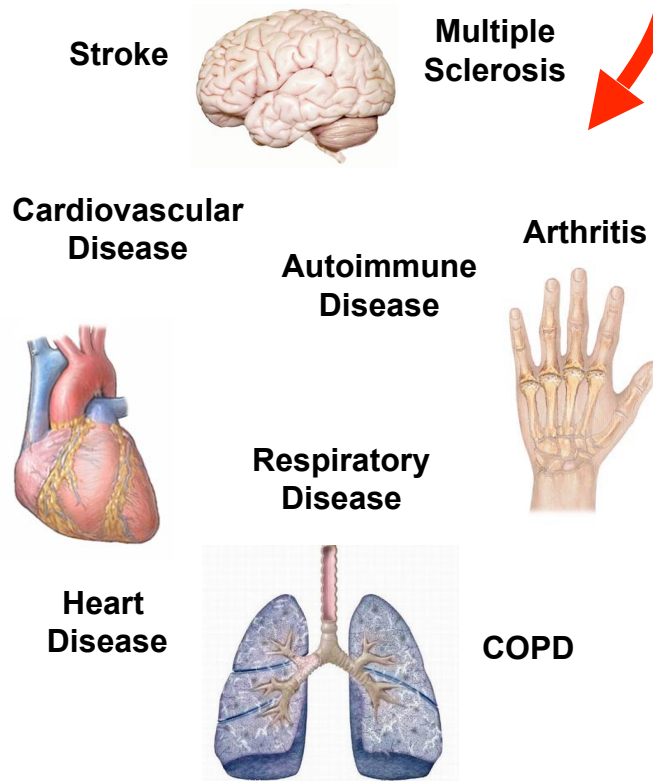
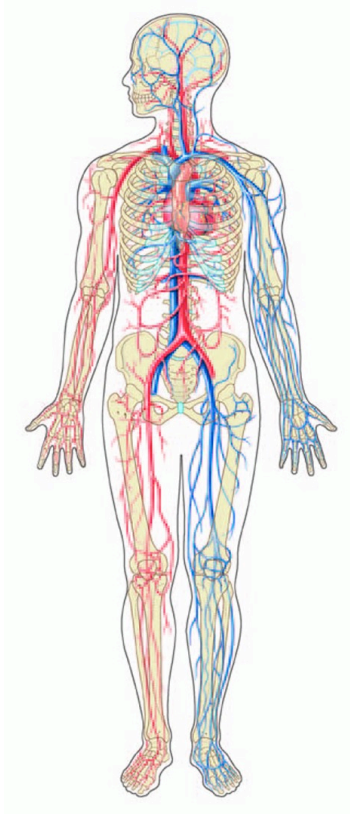
# Example Assay System - Vascular Inflammation

System	Cell Type	Tissue Setting	Biomarkers
 3C	Endothelial Cells	Chronic Th1 Inflammation IL-1 $\beta$ + TNF $\alpha$ + IFN $\gamma$	MCP-1, VCAM, CD141, CD142, ICAM, E-selectin, uPAR, IL-8, MIG, HLA-DR

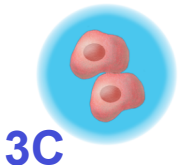


# Example Assay System - Vascular Inflammation

System	Cell Type	Tissue Setting	Biomarkers
 <p>3C</p>	Endothelial Cells	<div style="border: 2px solid red; padding: 5px;">                     Chronic Th1 Inflammation  <math>IL-1\beta + TNF\alpha + IFN\gamma</math> </div>	MCP-1, VCAM, CD141, CD142, ICAM, E-selectin, uPAR, IL-8, MIG, HLA-DR



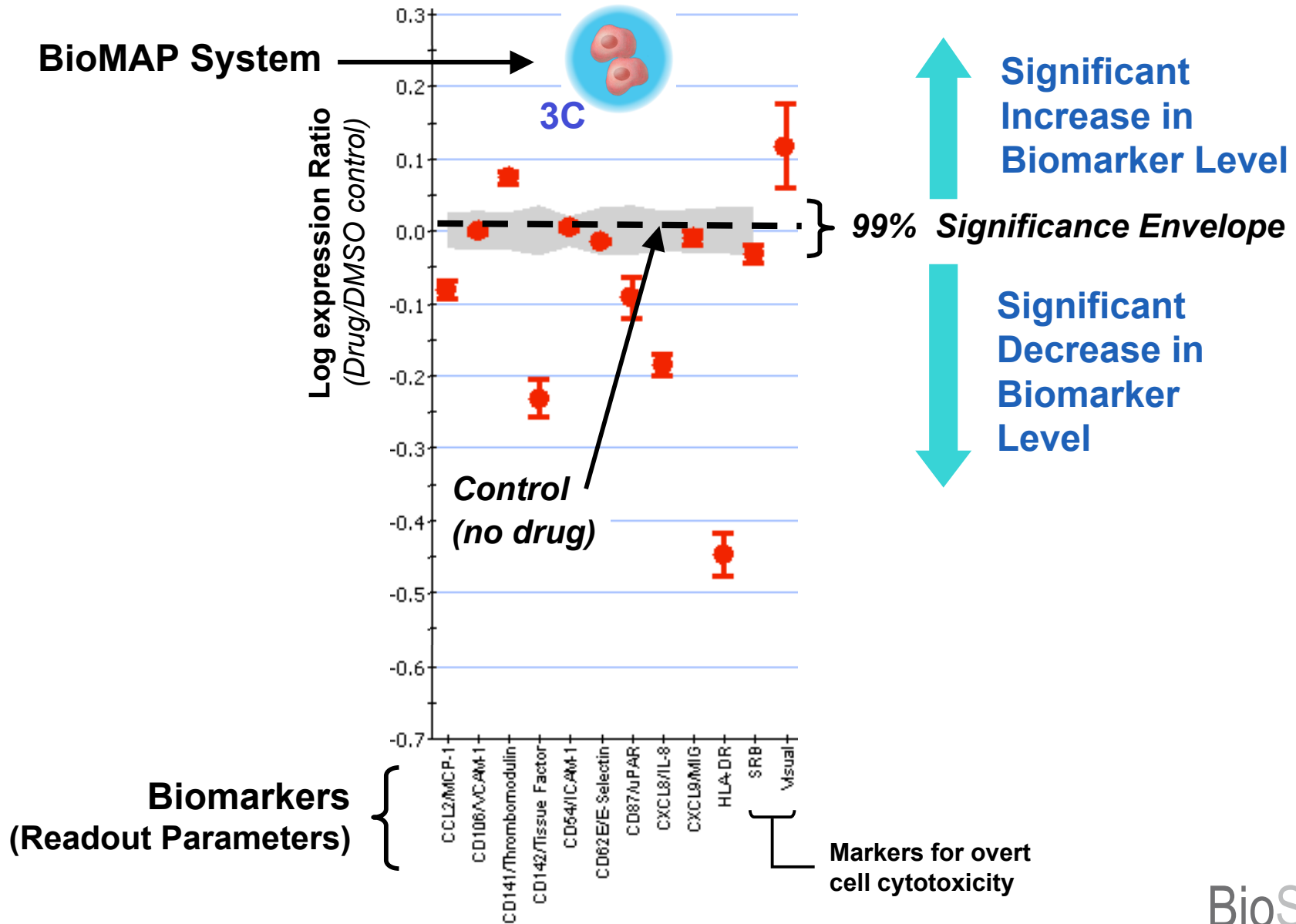
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Biomarker	Biological Process
MCP-1	Vascular inflammation, monocyte, T cell recruitment
VCAM	Chronic inflammation, redox stress; monocyte, T cell recruitment
CD141/Thrombomodulin	Fibrinolysis, thrombosis (-)
CD142/Tissue Factor	Angiogenesis, coagulation, thrombosis
ICAM	Acute and chronic inflammation; leukocyte adhesion and recruitment
E-Selectin	Leukocyte adhesion, neutrophil recruitment, skin T cell recruitment
CD87/uPAR	Cell migration, proliferation, angiogenesis
IL-8	Acute inflammation, neutrophil recruitment, angiogenesis
MIG	Chronic inflammation, T cell recruitment, angiogenesis (-)
HLA-DR	Th1 immune responses, infection, antigen presentation

# BioMAP Profiling: Example Profile

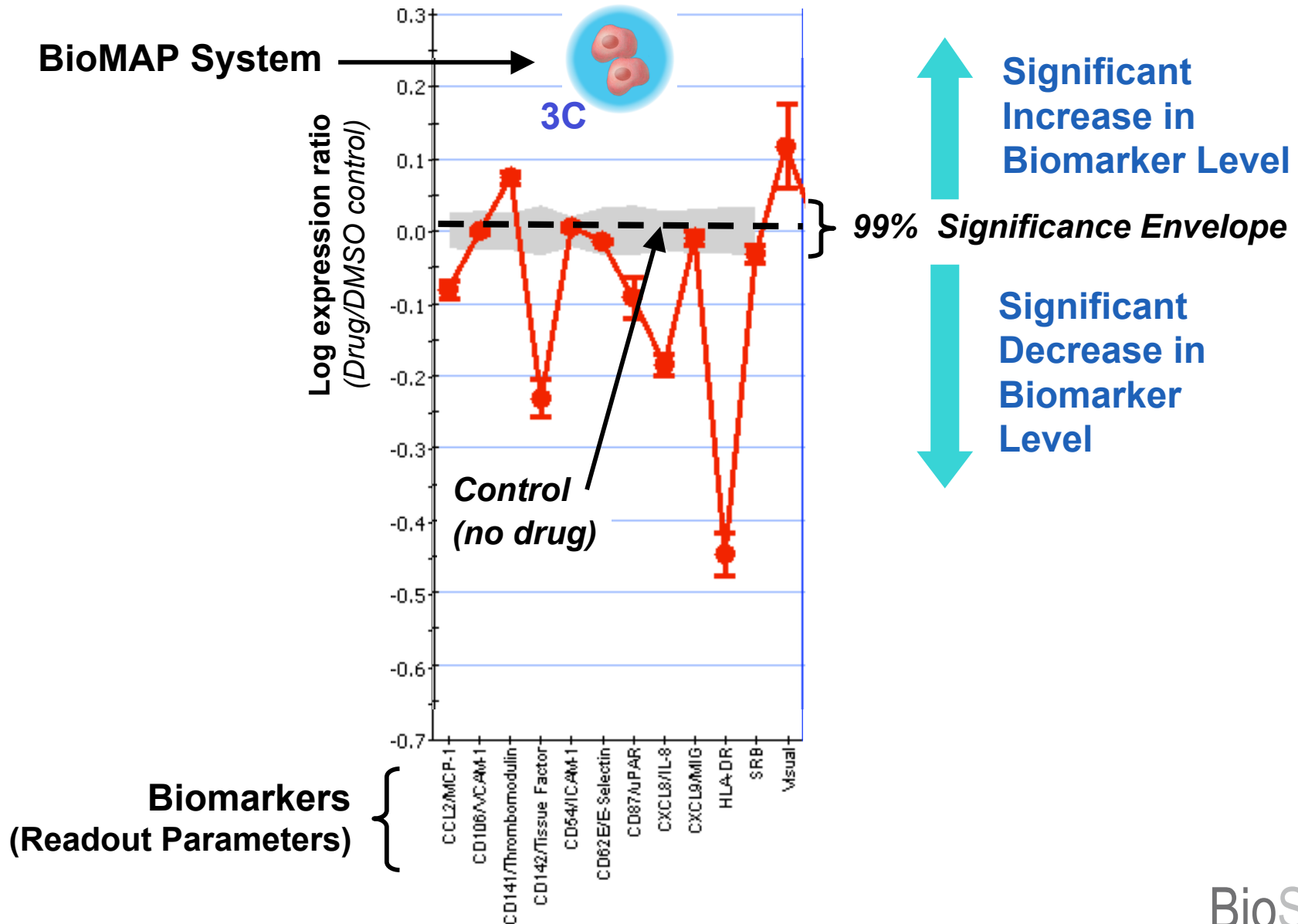
*Biomarkers affected by reference p38 MAPK Inhibitor*





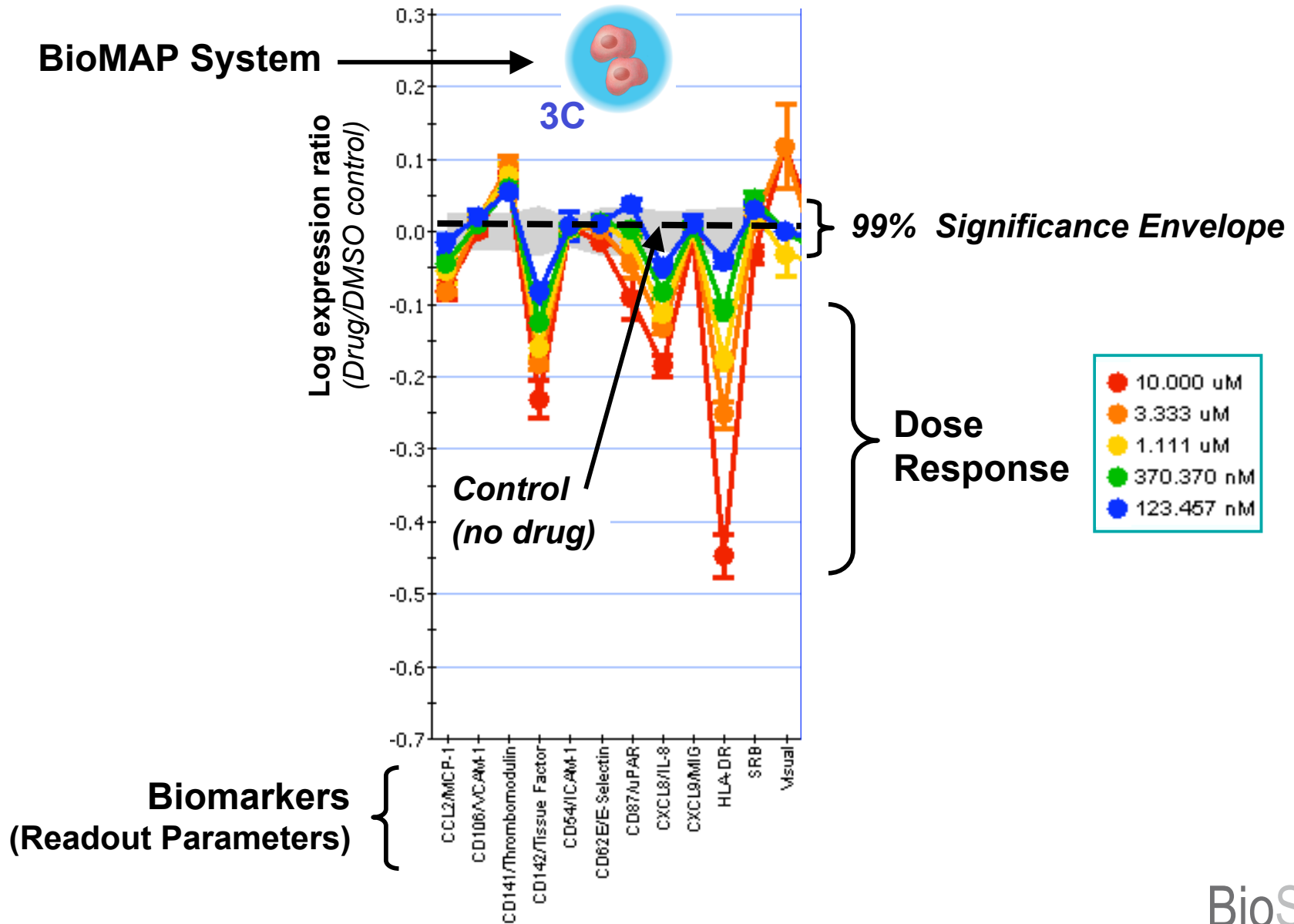
# BioMAP Profiling: Example Profile

Pattern of biomarker activities creates a “profile”



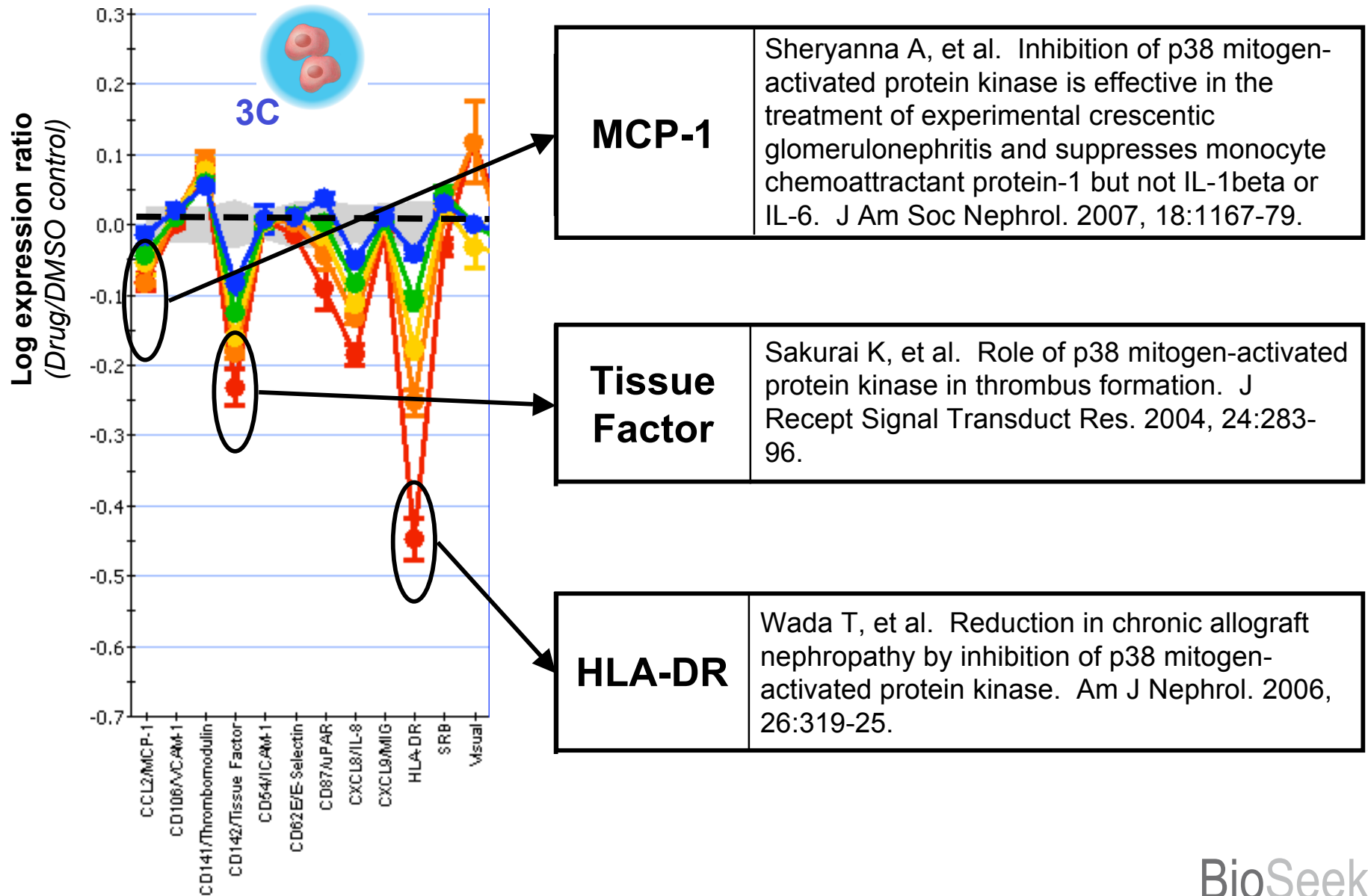
# BioMAP Profiling: Example Profile

Multiple dose profile of reference p38 MAPK Inhibitor



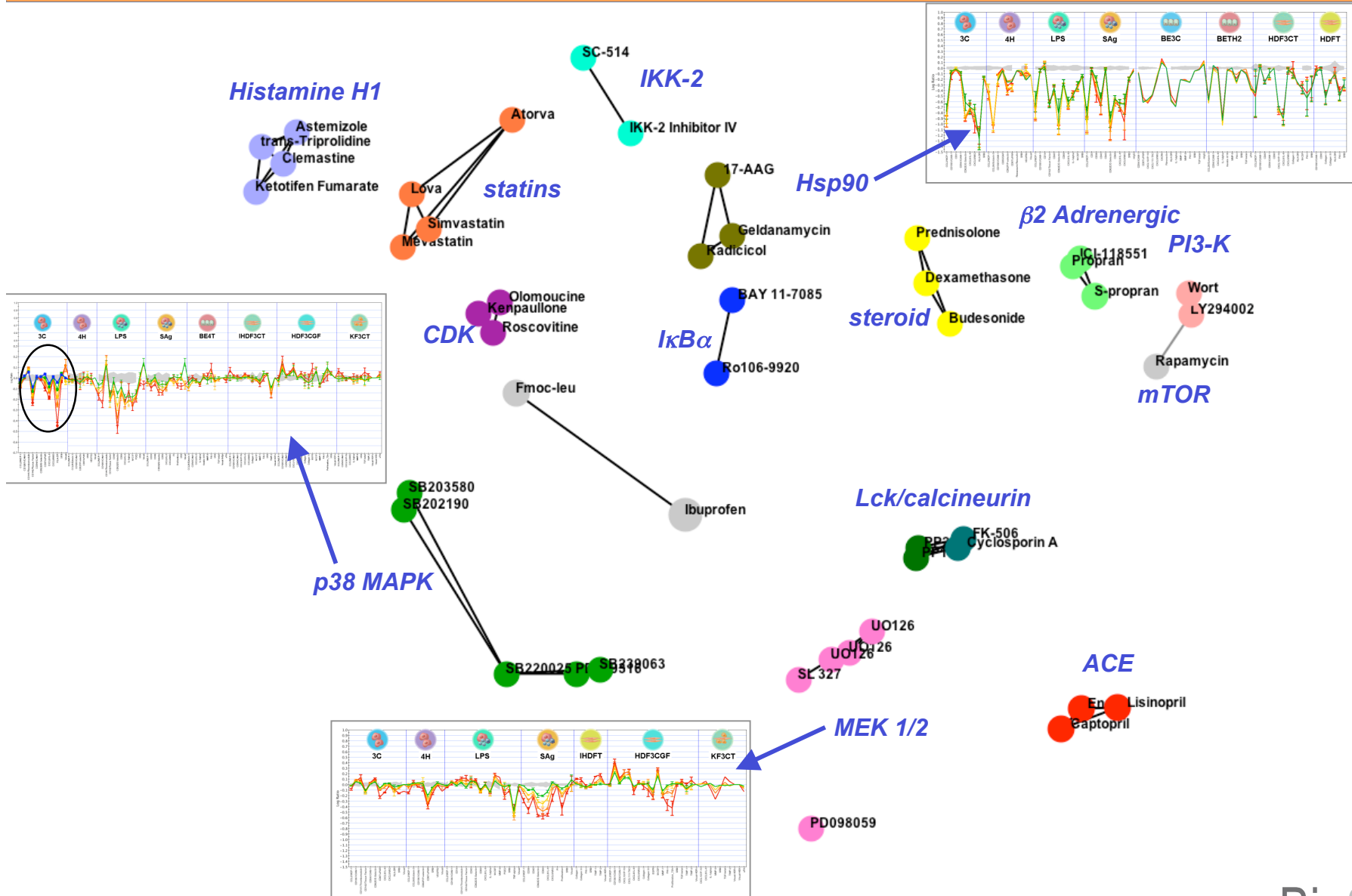
# BioMAP Profiling: Example Profile

Validation of activities identified for reference p38 Inhibitor



# BioMAP Classifies Drugs By Mechanism of Action

## Multidimensional Scaling - Function Similarity Map

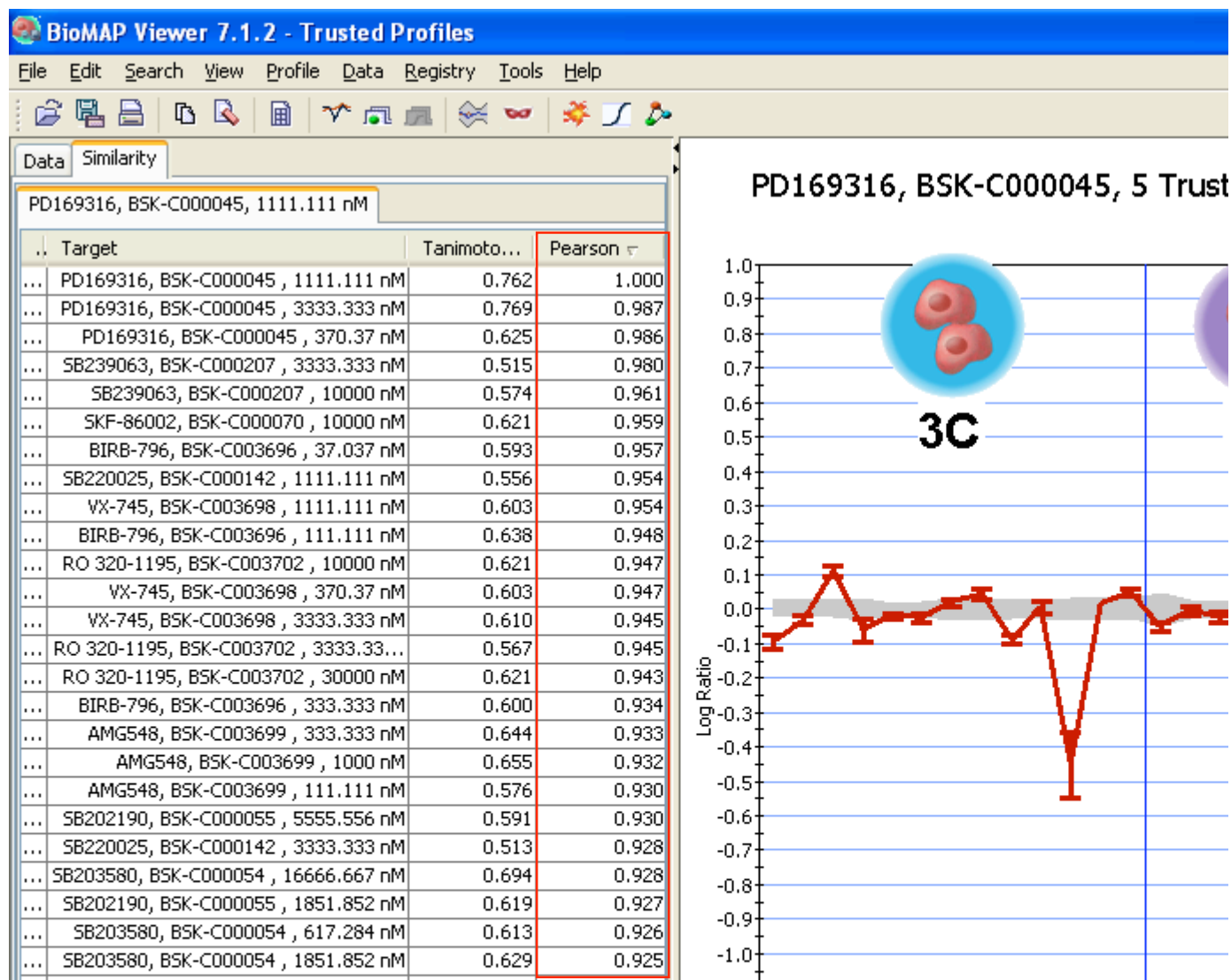


# Similarity Search for Mechanism of Action

## BioMAP Database

**Test compound:**  
**PD169316**  
 p38 MAPK inhibitor

**Top matches:**  
 other  
 p38 MAPK  
 inhibitors

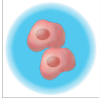
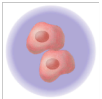
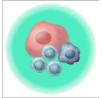


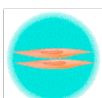
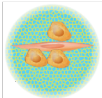
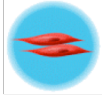


# BioSeek - EPA ToxCast Project

## BioSeek - EPA Proof-of Concept Project

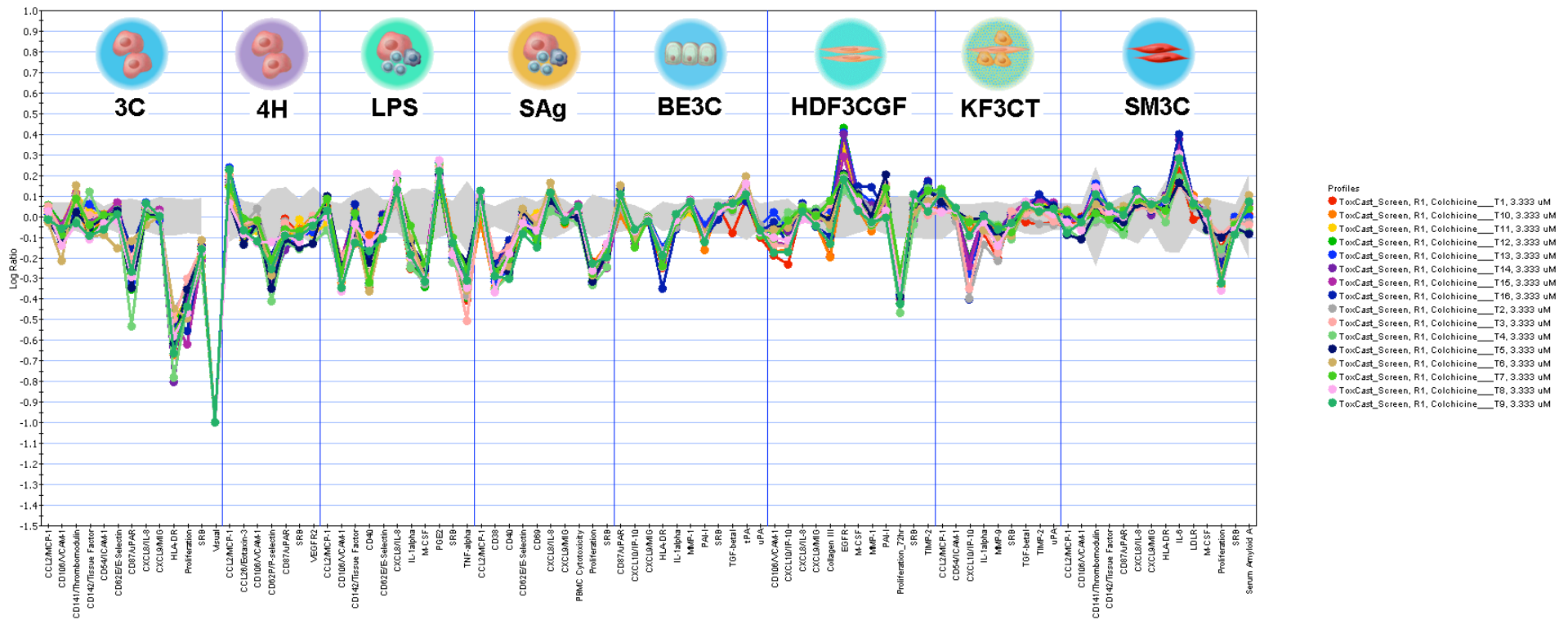
- **320 compounds, 4 concentrations each**
- **8 BioMAP Systems, 7-14 readouts (endpoints) per System**
- **Total: 87 readouts per compound**
- **Single well, screening format**
- **~112,000 datapoints (348 datapoints per compound)**

# BioMAP Systems for EPA ToxCast

▶ System	▶ Cell Types	Environment	Readout Parameters
3C 	Endothelial cells	IL-1 $\beta$ +TNF- $\alpha$ +IFN- $\gamma$	MCP-1, VCAM-1, ICAM-1, Thrombomodulin, Tissue Factor, E-selectin, uPAR, IL-8, MIG, HLA-DR, Proliferation, Vis., SRB (13)
4H 	Endothelial cells	IL-4+histamine	VEGFR2, P-selectin, VCAM-1, uPAR, Eotaxin-3, MCP-1, SRB (7)
LPS 	Peripheral blood mononuclear cells + Endothelial cells	TLR4	CD40, VCAM-1, Tissue Factor, MCP-1, E-selectin, IL-1 $\alpha$ , IL-8, M-CSF, TNF- $\alpha$ , PGE2, SRB (11)
SAg 	Peripheral blood mononuclear cells + Endothelial cells	TCR	MCP-1, CD38, CD40, CD69, E-selectin, IL-8, MIG, PBMC Cytotox., SRB, Proliferation (10)
BE3C 	Bronchial epithelial cells	IL-1 $\beta$ +TNF- $\alpha$ +IFN- $\gamma$	uPAR, IP-10, MIG, HLA-DR, IL-1 $\alpha$ , MMP-1, PAI-1, SRB, TGF- $\beta$ 1, tPA, uPA (11)
HDF3CGF 	Fibroblasts	IL-1 $\beta$ +TNF- $\alpha$ +IFN- $\gamma$ +bFGF+EGF+PDGF-BB	VCAM-1, IP-10, IL-8, MIG, Collagen III, M-CSF, MMP-1, PAI-1, Proliferation, TIMP-1, EGFR, SRB (12)
KF3CT 	Keratinocytes + Fibroblasts	IL-1 $\beta$ +TNF- $\alpha$ +IFN- $\gamma$ +TGF- $\beta$	MCP-1, ICAM-1, IP-10, IL-1 $\alpha$ , MMP-9, TGF- $\beta$ 1, TIMP-2, uPA, SRB (9)
SM3C 	Vascular smooth muscle cells	IL-1 $\beta$ +TNF- $\alpha$ +IFN- $\gamma$	MCP-1, VCAM-1, Thrombomodulin, Tissue Factor, IL-6, LDLR, SAA, uPAR, IL-8, MIG, HLA-DR, M-CSF, Prolif., SRB (14)

# Reproducibility of BioMAP Profiles

## Positive Control (Colchicine) Replicates

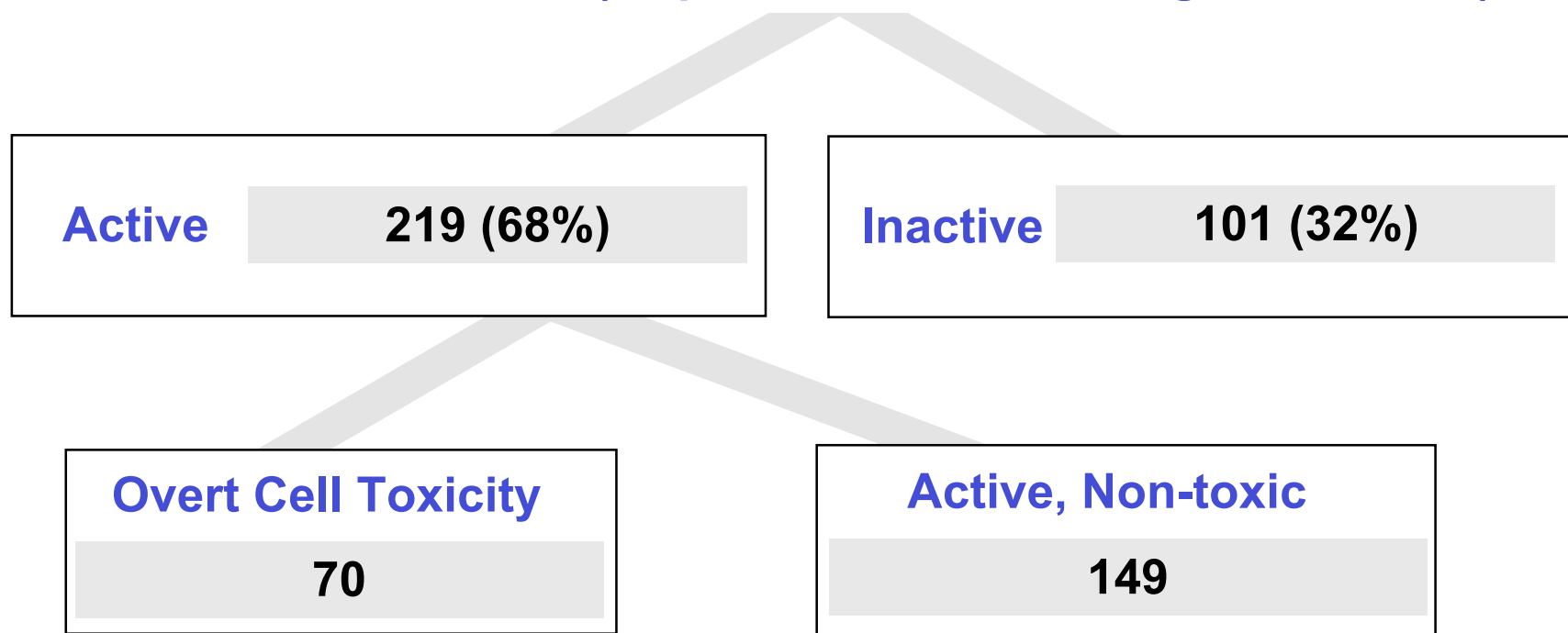


- **Overlay of BioMAP profiles of positive controls (colchicine)**
- **Each replicate represents a separate plate (template)**
- **99% Significance envelope is shown (grey shading)**



# Overall Summary

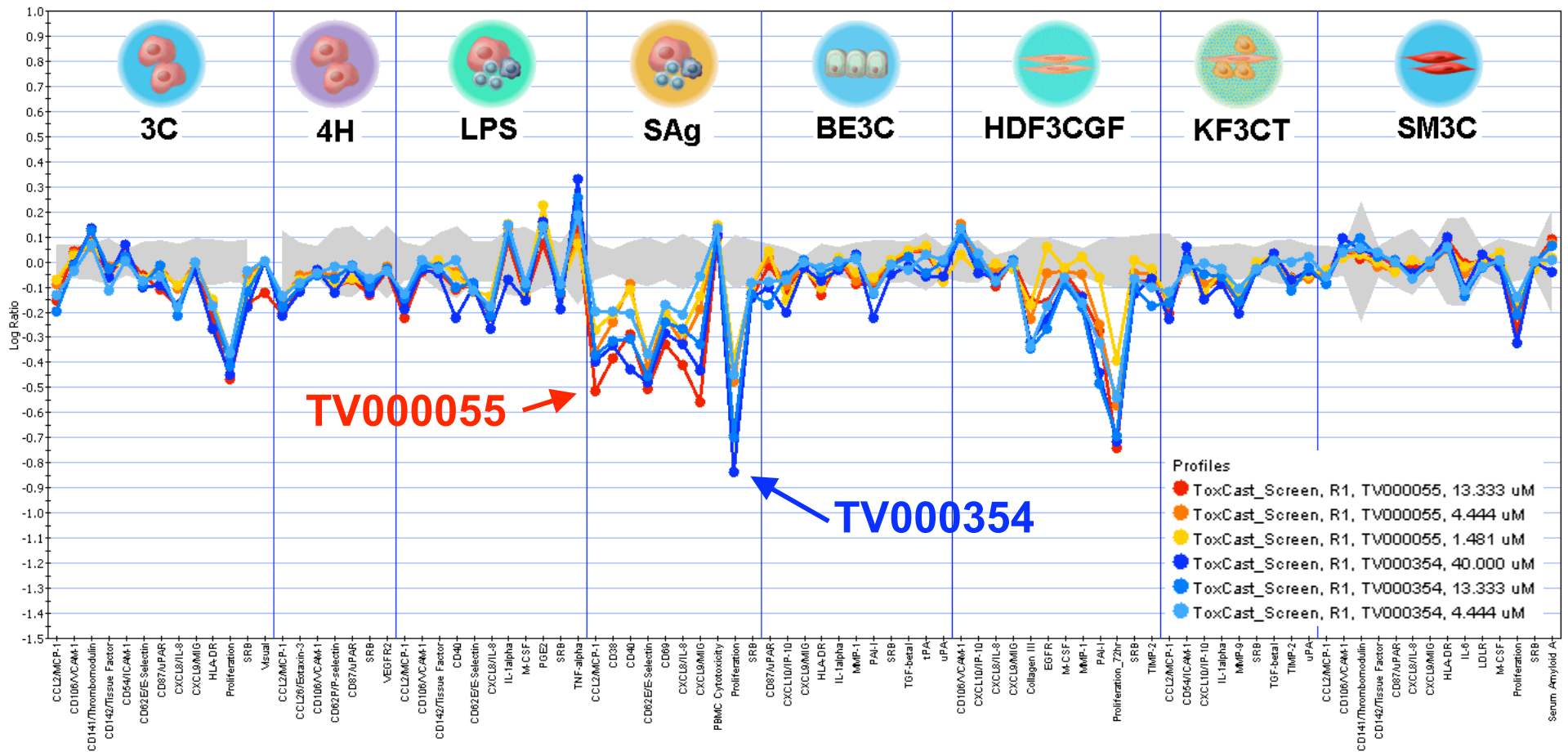
320 Compounds → 8 BioMAP Systems, 4 concentrations  
(87 parameters, screening format, n=1)





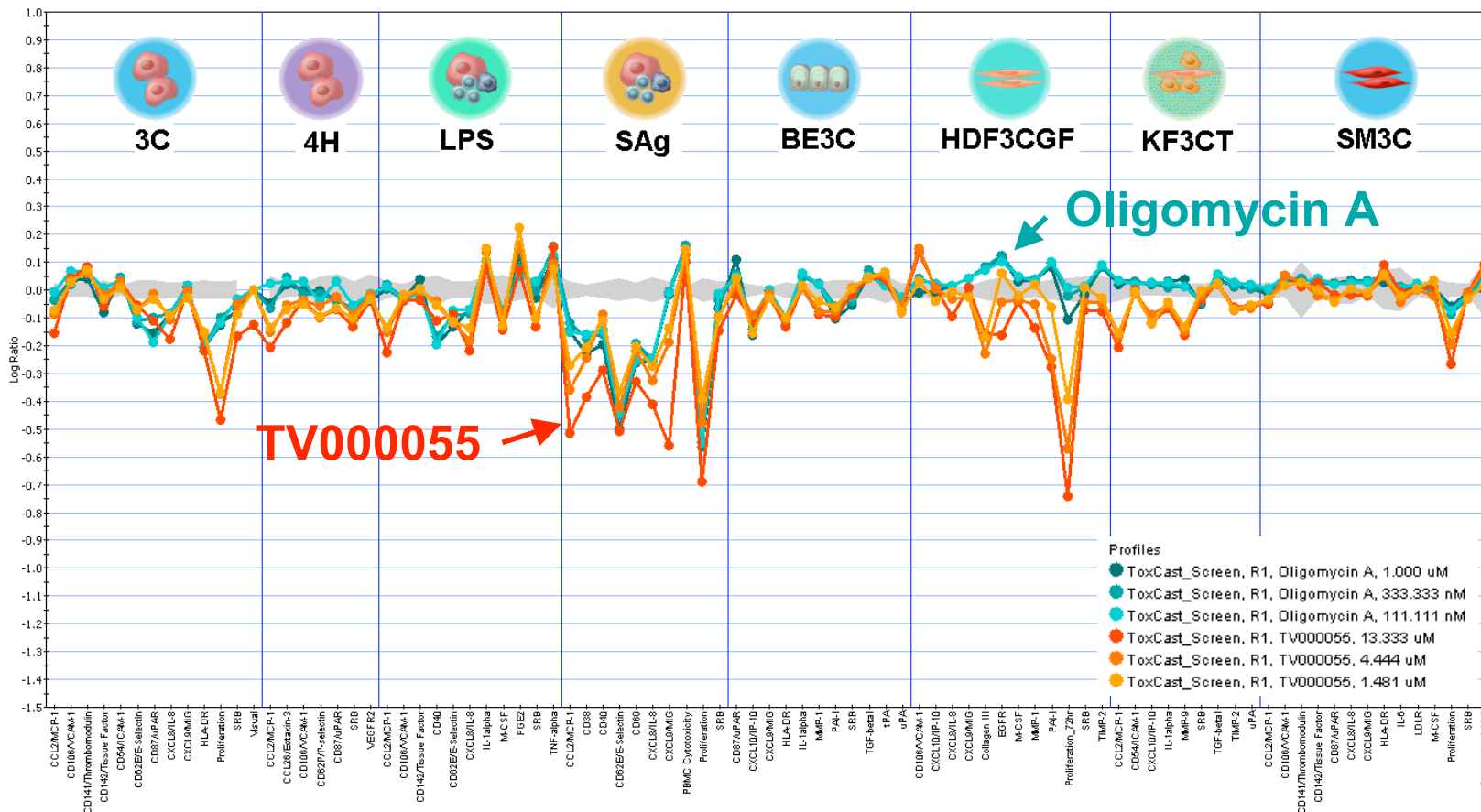


# TV00055 and TV000354



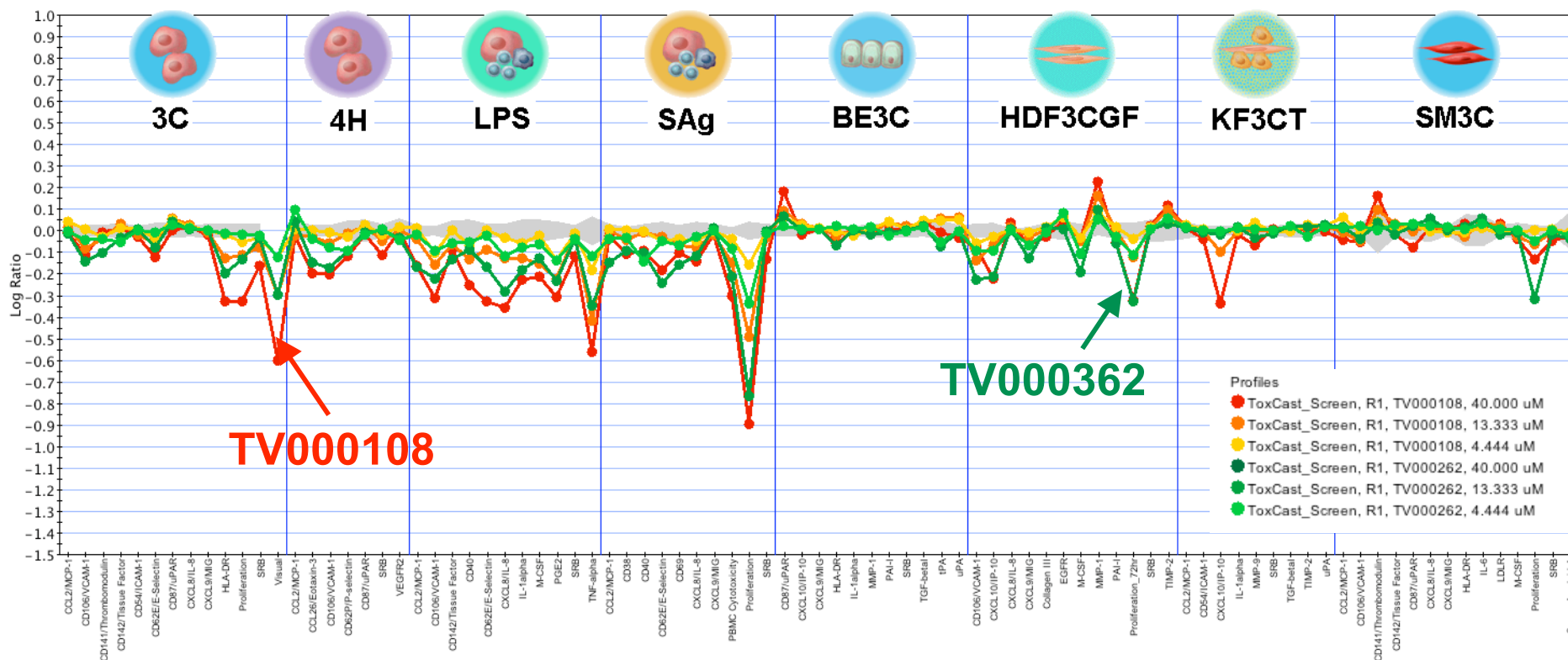
- **BioMAP Profiles of compounds that cluster in the MDS graph are highly similar to one another**

# BioMAP Profiles of Oligomycin A and TV000055



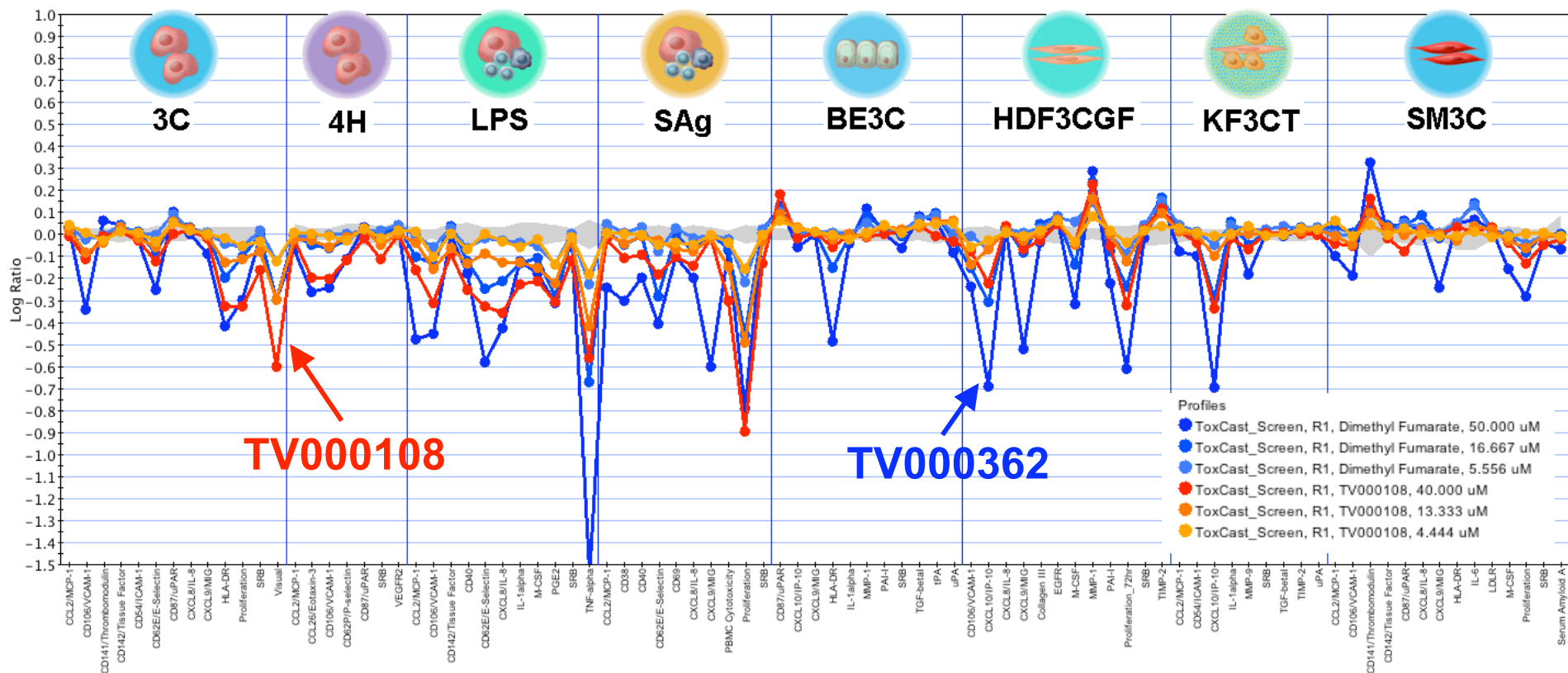
- Oligomycin A is an inhibitor of mitochondrial ATPase
- Similarity suggests inhibition of mitochondrial function by TV000055
  - (TV000055 is most similar to Complex I inhibitors)

# TV000108 and TV000362



- **BioMAP Profiles of compounds that cluster in the MDS graph are highly similar to one another**

# BioMAP Profiles of Dimethyl Fumarate and TV000108



- **Dimethyl Fumarate is an inhibitor of NFkappaB translocation**

# Classification of Compounds

## *Example Mechanism Classes*

- **Mitochondria energy inhibition**
  - Electron transport chain inhibitors
- **ER stress (unfolded protein response)**
  - Proteasome inhibition
  - DNA damage
    - Chlorambucil (alkylating agent)
  - Inhibition of translation
    - Cycloheximide
- **Microtubule function inhibition**
  - Colchicine, vincristine, paclitaxel



# Classification of Compounds

## *In Progress*

<u>Mechanisms</u>	<u># Compounds</u>
Mitochondrial Dysfunction	41
ER Stress	23
Microtubule Function Inhibition	7
PI3 Kinase Inhibition	9
cAMP Elevation	12

## Next Steps

- **Decode compound IDs**
- **Complete similarity analyses**
- **Correlate BioMAP data with other data types**
- **Build classifiers for specific mechanism classes**

