DiscoveRx

Simple Solutions for Complex Biology

BioMAP Systems: Phenotypic drug profiling and benchmarking through human primary cell systems

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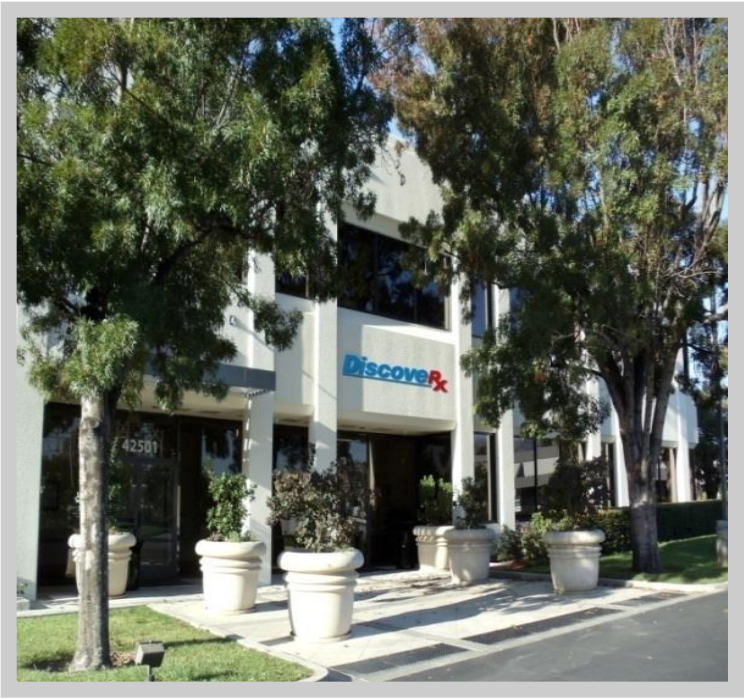
DiscoveRx – At a Glance



Founded in 2001

Acquired KINOMEscan and BioSeek

- **Innovative**
  - Premier provider of innovative assays and services for drug discovery researchers
- **Comprehensive**
  - >1,200 proprietary assays
  - Primary cell models for disease biology
  - Product & service offering
- **Proven Expertise**
  - Technology development
  - Drug discovery
  - Screening and profiling

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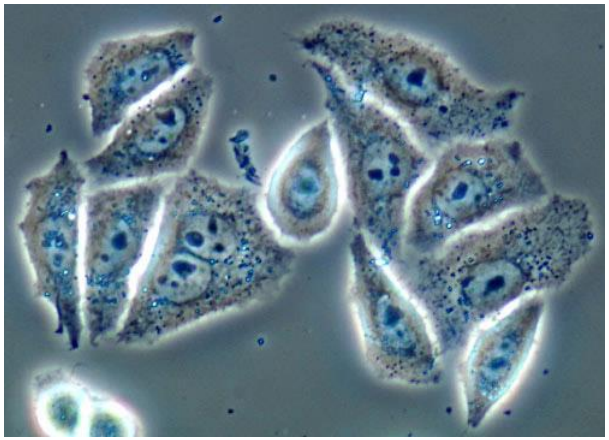
Common Models for Drug Discovery



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The Value of the BioMAP Platform

- **BioMAP captures valuable clinically relevant information on the following key properties of a compound or biologic:**
  - **Therapeutic Efficacy**
  - **Off-target Biology**
  - **Mechanism of Action**
- **No other screening approach (genomic, proteomic, biochemical, cell-based, phenotypic,…) can reliably assess these key attributes in a single screen.**

Key BioMAP® Applications

BioMAP® can be used for:

- Prediction of compound mechanism of action
- Target validation in human primary cells
- Benchmarking to clinical standards of care
- Comparison to approved drugs, failed drugs, tool compounds
- Safety assessment
- Testing drug combinations
- Drug repositioning
- Due diligence for in-licensing

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BioMAP® Service Offerings

Service	Description	Research Stage
Diversity Plus	• 12 systems , 148 readouts, • 4 doses / compound / singlicate	• Project teams in Lead Op or with late stage compounds
Predictive Tox	• 8 systems, 87 readouts, • 2 or 4 doses / compound / singlicate	• Preclinical Safety Groups • All Therapeutic Areas
BioMAP System ELECT	• Choose one or more of the >40 validated BioMAP Systems • All readouts in that system • 4 doses/ compound / triplicate	• Project teams in Lead Op • Follow-up from Diversity Plus
BioMAP System ELECT Oncology	• 2 New Systems, 40 readouts • 4 doses/ compound / triplicate	• Oncology researchers in Lead Op or with late stage compounds
BioMAP EC50 ELECT	• Choose one or more of the >40 validated BioMAP Systems • Select any readouts from a system • 7 doses/ compound / triplicate	• Project teams in Lead Op • Follow-up from Diversity Plus , Predictive Tox, or System ELECT
T Cell Autoimmune	• 4 systems, 52 readouts • 4 doses / compound / triplicate	• Autoimmune disease, Cancer Immunotherapy researchers

BioMAP® Service Offerings

Service	Description
Fibrosis Panel	• 3 systems, 54 readouts • 4 conc. / compound / singlicate
ComboELECT	• Any system or Panel • 4x4 matrix combination of 2 drugs • Drugs alone (4 concentrations) and matrixed at every conc.
BioMAP System ELECT	• Choose one or more of the 42 validated BioMAP Systems • All readouts in that system • 4 doses/ compound / triplicate
Reference Compound Data Packages	• Profile plots, activity annotation of biological significance • Excel data tables

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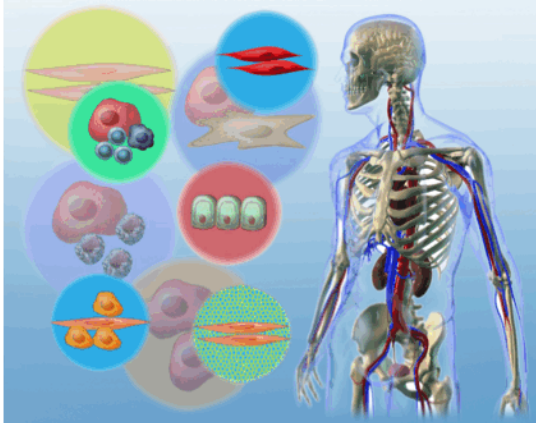
BioMAP® Technology Platform

BioMAP Assay Systems

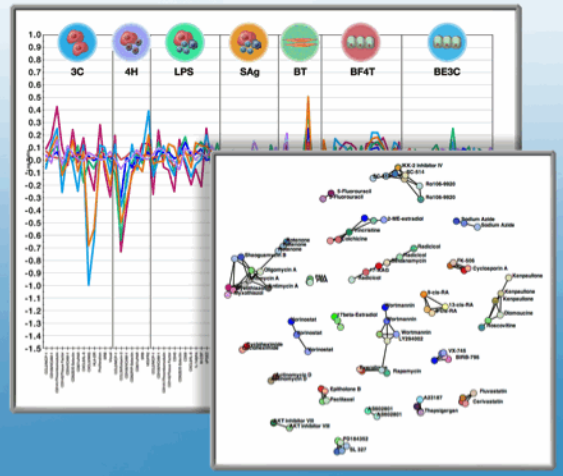


Human primary cells
Disease-models
30+ systems

Reference Profile Database

Biomarker responses to drugs
are stored in the database
>3500 drugs

Predictive Informatics Tools



Specialized informatics tools are
used to predict clinical outcomes

Human Biology Integrated into a Robust, Scalable Platform

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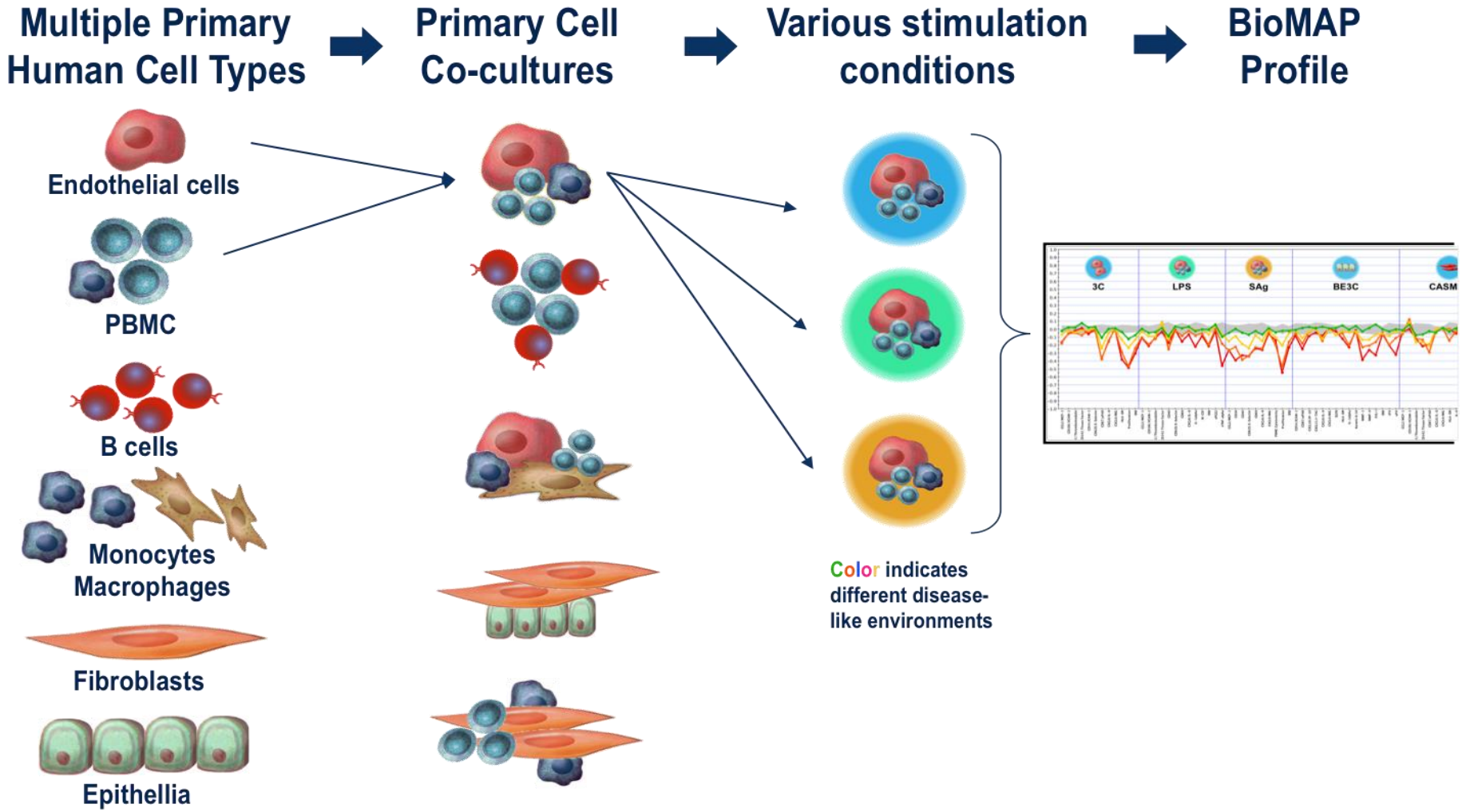
BioMAP® Systems
Management of Technical Variability

- **Cell Sourcing**
  - Primary cell banks derived from healthy, pathogen-free, drug-free donors
- **"Pre-screening" of primary cell banks**
  - Screen donor cells to ensure no "pre-stimulation" has occurred
  - Screen with positive control compounds to ensure donor cell responsiveness
- **Cell Pooling approach** - validated by data from 100's of historic pools
- **Stringent BioMAP Assay Conditions**
  - QC/QA Assays are included on every plate of every screen
  - Most readouts are 24 hr after treatment
- **Data Management** – Log fold-change ratios in Biomarker Levels are reported

BioMAP® Systems Model Complex Biology



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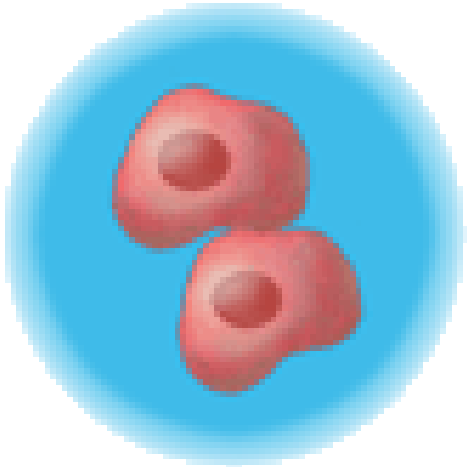
Why Primary Human Cells?

- Primary human cells
  - Freshly isolated from tissues
  - Not adapted to life on plastic
  - Remember “where they came from”



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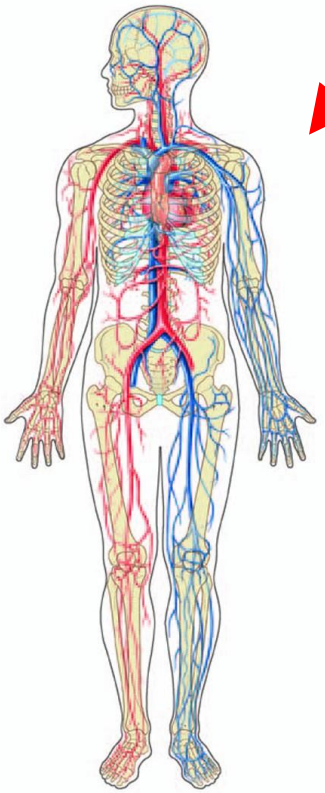
BioMAP® 3C System

System	Cell Type	Tissue Setting	Biomarkers
3C	Endothelial Cells	Chronic Th1 Inflammation IL-1β + TNFα + IFNγ	MCP-1, VCAM, CD141, CD142, ICAM, E-selectin, uPAR, IL-8, MIG, HLA-DR



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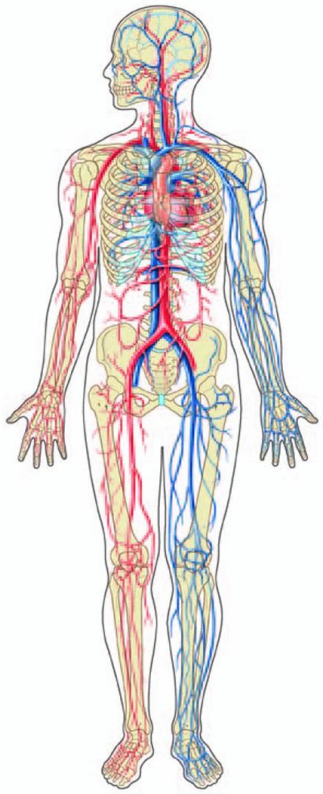
Chronic Inflammation Model

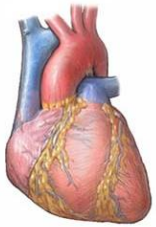
System	Cell Type	Tissue Setting	Biomarkers
3C	Endothelial Cells	Chronic Th1 Inflammation IL-1β + TNFα + IFNγ	MCP-1, VCAM, CD141, CD142, ICAM, E-selectin, uPAR, IL-8, MIG, HLA-DR



Stroke

Multiple Sclerosis

Cardiovascular Disease

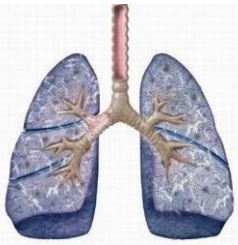


Autoimmune Disease

Arthritis

Respiratory Disease

Heart Disease



COPD

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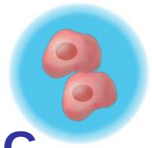
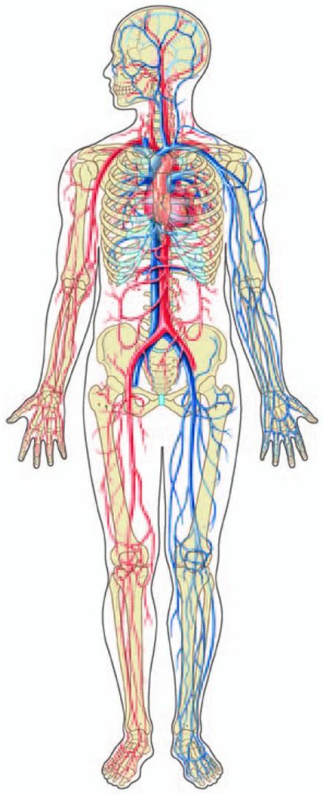
Biomarker Readouts Are Disease Relevant

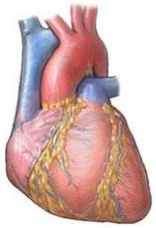
System	Cell Type	Tissue Setting	Biomarkers
 3C	Endothelial Cells	Chronic Th1 Inflammation IL-1β + TNFα + IFNγ	MCP-1, VCAM, CD141, CD142, ICAM, E-selectin, uPAR, IL-8, MIG, HLA-DR



Stroke

Multiple Sclerosis

Cardiovascular Disease



Autoimmune Disease

Arthritis

Respiratory Disease

Heart Disease

COPD

NCBI PubMed A service of the U.S. and the www.pubmed.gov

1: Atherosclerosis. 2005 Dec;183(2):301-7. Epub 2005 Apr 14.

Plasma MCP-1 level and risk for peripheral arterial disease and incident coronary heart disease: Atherosclerosis Risk in Communities study.

Hoogeveen RC, Morrison A, Boerwinkle E, Miles JS, Rhodes CE, Sharrett AR, Ballantyne CM.

Section of Atherosclerosis and Lipoprotein Research, Department of Medicine, Baylor College of Medicine and Center for Cardiovascular Disease Prevention, Methodist DeBakey Heart Center, 6565 Fannin, M.S. F-701, Houston, TX 77030, USA. ronh@bcm.tmc.edu

Monocyte chemoattractant protein-1 (MCP-1), which mediates the recruitment of monocytes, has been suggested to play a role in atherosclerosis. Because the correlation between circulating MCP-1 and cardiovascular risk has not been thoroughly investigated, we determined the relationship between MCP-1 level and peripheral arterial disease (PAD) or coronary heart disease (CHD). In the Atherosclerosis Risk in Communities (ARIC) study, 209 cases with lower extremity PAD and 412 cases with incident CHD were compared with 733 and 709 subjects without PAD and CHD, respectively. Mean plasma MCP-1 levels were significantly higher in PAD cases (468.7 versus 416.5 pg/mL in non-cases). MCP-1 levels correlated significantly with other inflammatory markers in comparison subjects. Logistic regression analyses showed a significant association of MCP-1 with PAD, independent of traditional CHD risk factors, with an odds ratio of 2.14 (95% CI, 1.28-3.60) for the highest MCP-1 tertile compared with the lowest. Incident CHD risk increased significantly per 1 standard deviation (S.D.) difference in MCP-1 level independent of other cardiovascular risk factors, including inflammatory markers. These data show that MCP-1 is associated with atherosclerotic disease in two vascular beds and suggest that MCP-1 may be a novel target for atherosclerosis therapy.

PMID: 16285993 [PubMed - indexed for MEDLINE]

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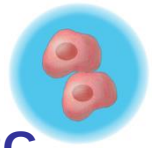
Biomarkers Cover Diverse Biological Processes

System	Cell Type	Tissue Setting	Biomarkers
 3C	Endothelial Cells	Chronic Th1 Inflammation IL-1β + TNFα + IFNγ	MCP-1, VCAM, CD141, CD142, ICAM, E-selectin, uPAR, IL-8, MIG, HLA-DR

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

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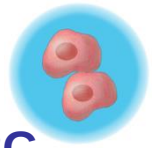
Biomarkers Cover Diverse Clinical Indications

System	Cell Type	Tissue Setting	Biomarkers
 3C	Endothelial Cells	Chronic Th1 Inflammation IL-1β + TNFα + IFNγ	MCP-1, VCAM, CD141, CD142, ICAM, E-selectin, uPAR, IL-8, MIG, HLA-DR

Biomarker	Clinical Indications (Biomarker or Risk Factor)
MCP-1	Acute coronary syndrome, Atherosclerosis, Diabetes, Heart disease
VCAM	Atherosclerosis, Multiple sclerosis, Rheumatoid arthritis, Diabetes
CD141/Thrombomodulin	Coronary artery disease, Lupus, Stroke, Vasculitis
CD142/Tissue Factor	Coronary Artery Disease, Stroke, Myocardial Infarction
ICAM	Infection, Acute inflammation, Multiple sclerosis
E-Selectin	Psoriasis, Diabetes, Cancer
CD87/uPAR	Atherosclerosis, Cancer, COPD, Restenosis
IL-8	COPD, Lung cancer (NSCLC), Rheumatoid arthritis, Ulcerative colitis
MIG	Transplant rejection, Pulmonary sarcoidosis
HLA-DR	Rheumatoid arthritis, Lupus, Multiple sclerosis, Transplant rejection

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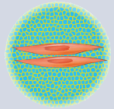
Test Variables – Diversity Plus

 **BF4T**	Bronchial epithelial cells + Dermal fibroblasts	Asthma, Allergy, Fibrosis, Lung	MCP-1, Eotaxin-3, VCAM-1, ICAM-1, CD90, IL-8, IL-1α, Keratin 8/18, MMP-1, MMP-3, MMP-9, PAI-1, SRB, tPA, uPA
 **HDF3CGF**	Dermal Fibroblasts	Fibrosis, Chronic Inflammation	MCP-1, VCAM-1, ICAM-1, Collagen I, Collagen III, IP-10, I-TAC, IL-8, MIG, EGFR, M-CSF, MMP-1, PAI-1, Proliferation, SRB, TIMP-1, TIMP-2
 **KF3CT**	Keratinocytes + Dermal fibroblasts	Psoriasis, Dermatitis, Skin	MCP-1, ICAM-1, IP-10, IL-8, MIG, IL-1α, MMP-9, PAI-1, SRB, TIMP-2, uPA
 **CASM3C**	Coronary artery smooth muscle cells	Cardiovascular Inflammation, Restenosis	MCP-1, VCAM-1, TM, TF, uPAR, IL-8, MIG, HLA-DR, IL-6, LDLR, M-CSF, PAI-1, Proliferation, SAA, SRB
 **MyoF**	Lung fibroblasts	Fibrosis, Chronic Inflammation	α-SMA, bFGF, VCAM-1, Collagen I, Collagen III, Collagen IV, IL-8, Decorin, MMP-1, PAI-1, SRB, TIMP-1
 **IMphg**	Venular endothelial cells + macrophages	Cardiovascular Inflammation, Restenosis, Chronic Inflammation	MCP-1, MIP-1α, VCAM-1, CD40, E-selectin, CD69, IL-8, IL-1α, M-CSF, sIL-10, SRB, SRB-Mphg

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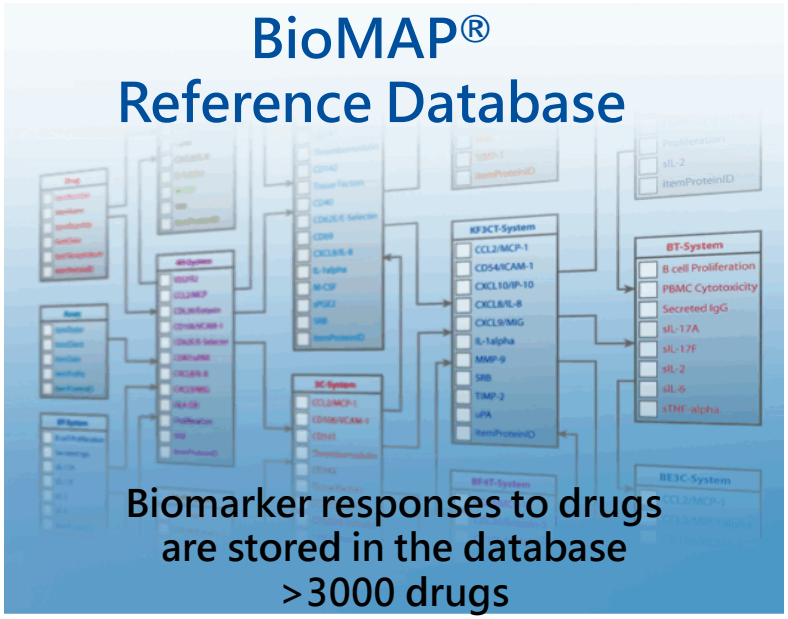
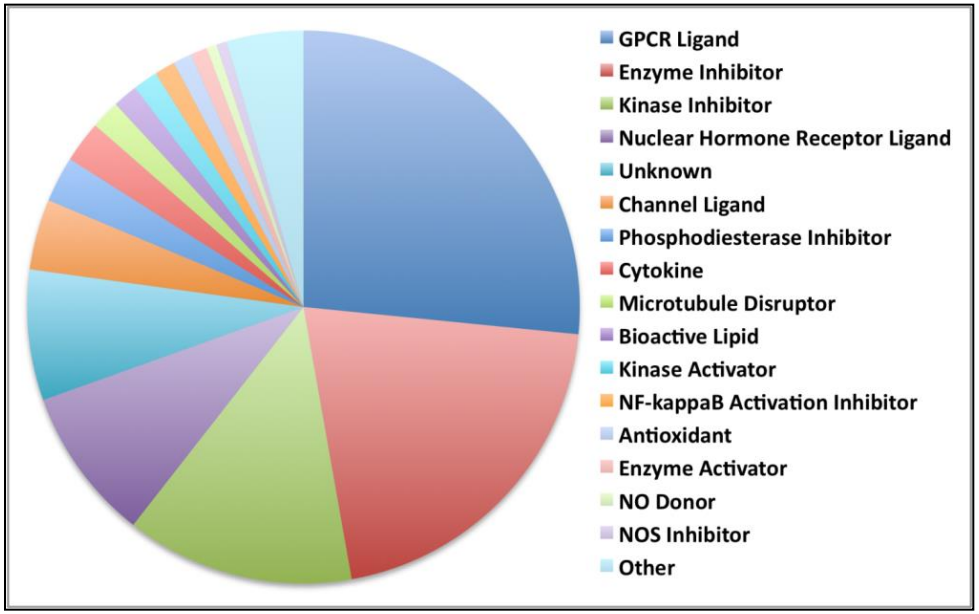
BioMAP Database





Greater than 3,000 molecules

- **Drugs** – Clinical stage, approved, and failed
- **Experimental Chemicals** - Research tool compounds, environmental chemicals, nanomaterials
- **Biologics** – Antibodies, cytokines, factors, peptides, soluble receptors

Aggressively expanding our database

- **Compound Acquisition Strategy**
  - Direct Purchase (1400 FDA approved drugs in progress)
  - Partnerships (SGC)
  - Customer donations (CSB)
  - Consortium
  - EPA

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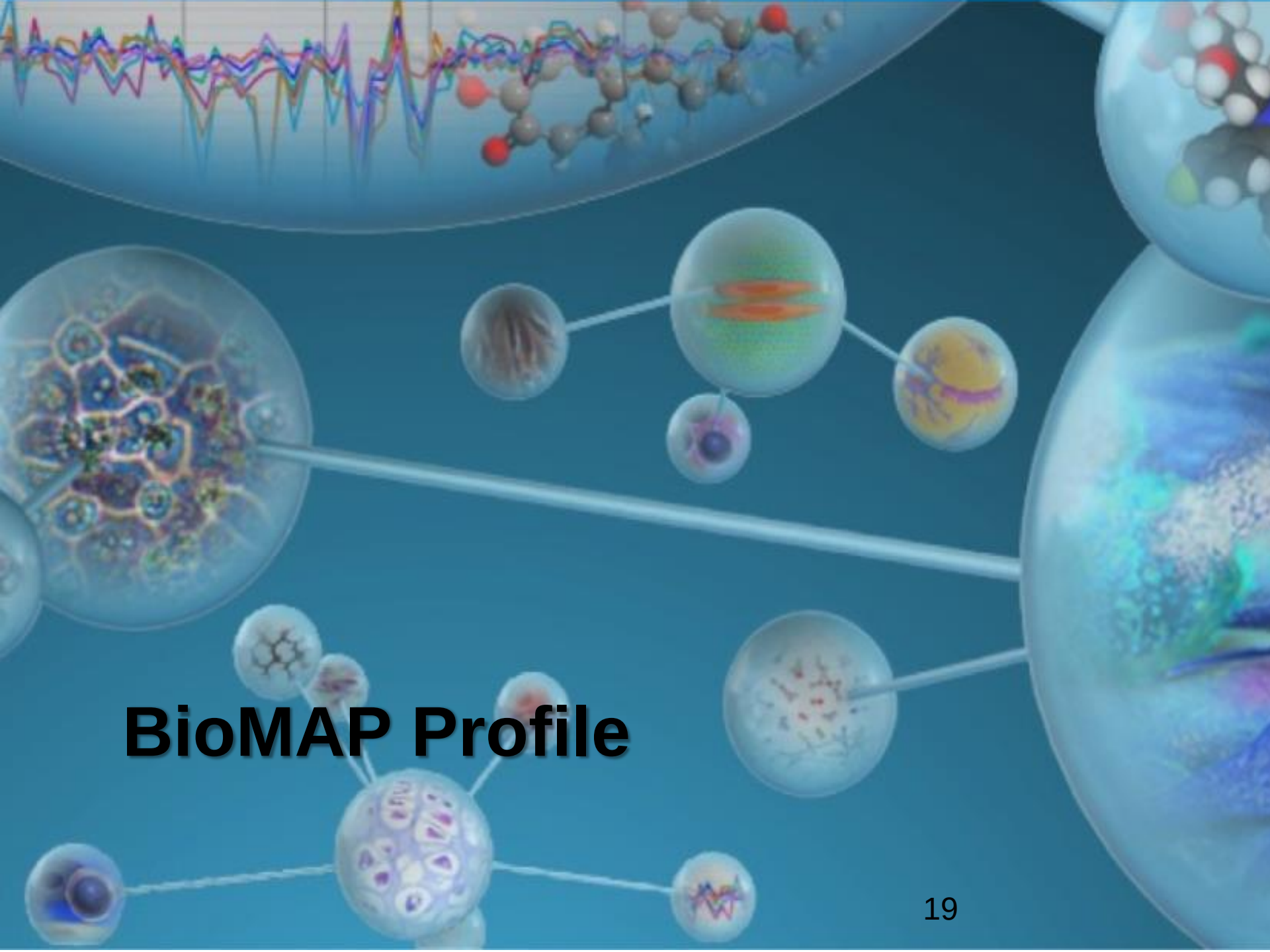
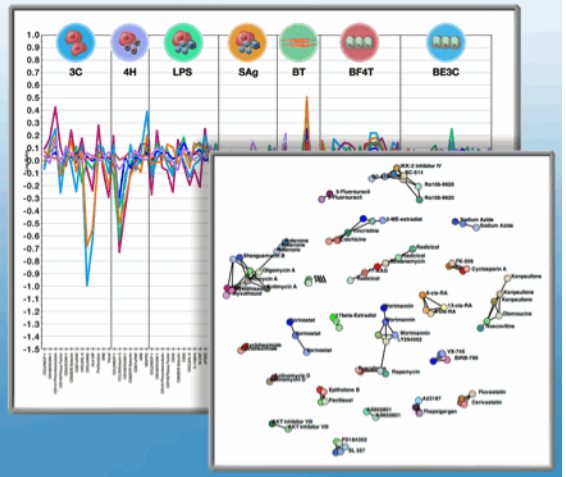

BioMAP Profile

BioMAP® Predictive Tools & Analysis

Predictive Informatics Tools



Custom informatics tools are used to predict outcomes

- Benchmarking
  - Head to head comparisons
- Similarity Search
  - Unsupervised search of the database
- Mechanism Models
  - Computational predictive models

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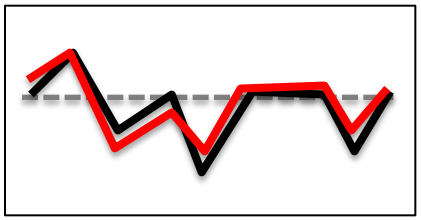
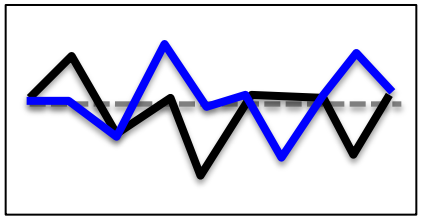
Similarity Analysis of BioMAP Profiles



- Highly correlated → Similar
  - Pearson's correlation of ***r*** > 0.7



- Low correlation → Not similar
  - Pearson's correlation of ***r*** < 0.7

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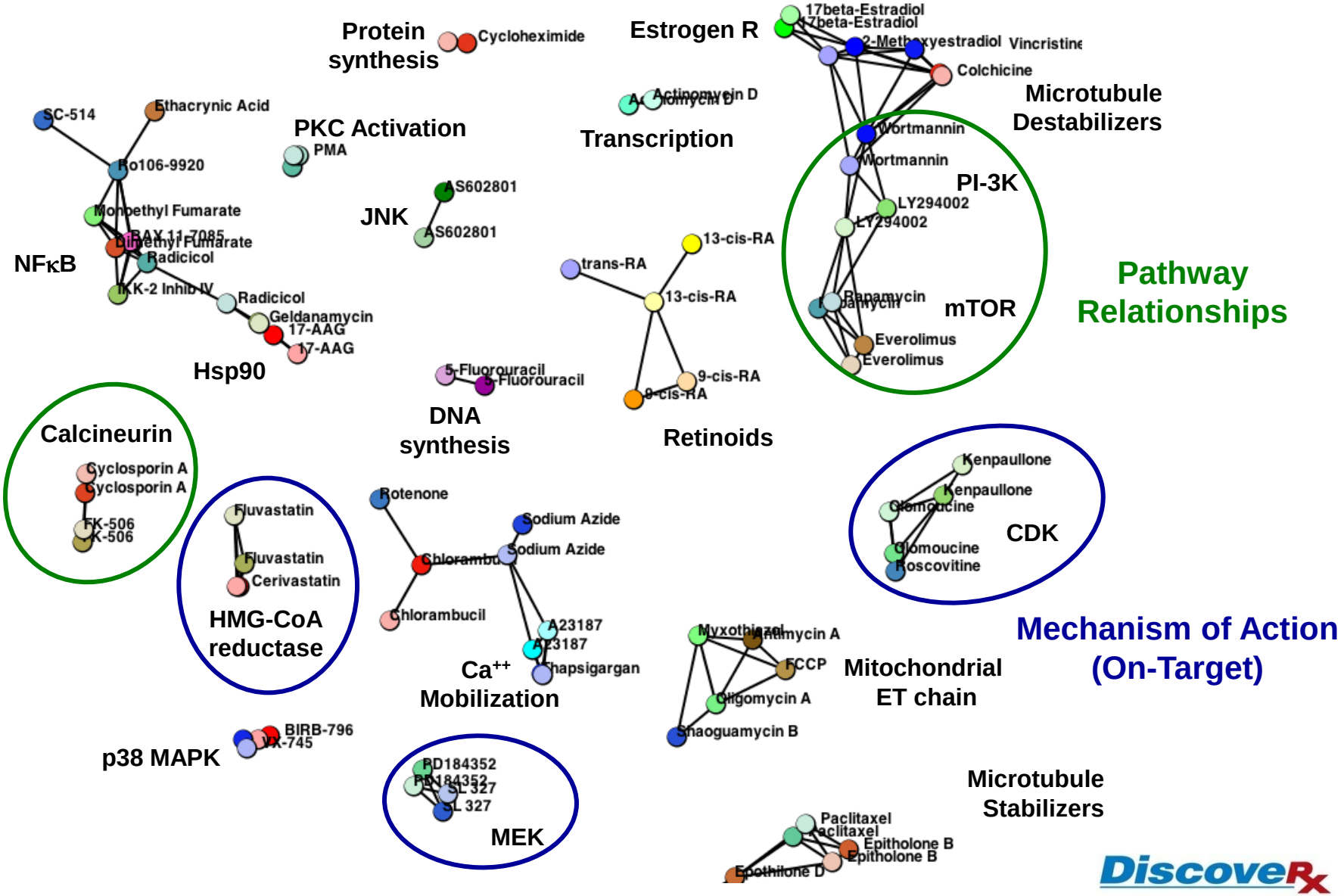
BioMAP® Analysis Reveals Mechanism of Action

Pairwise Correlation of BioMAP Reveals Functional Similarities



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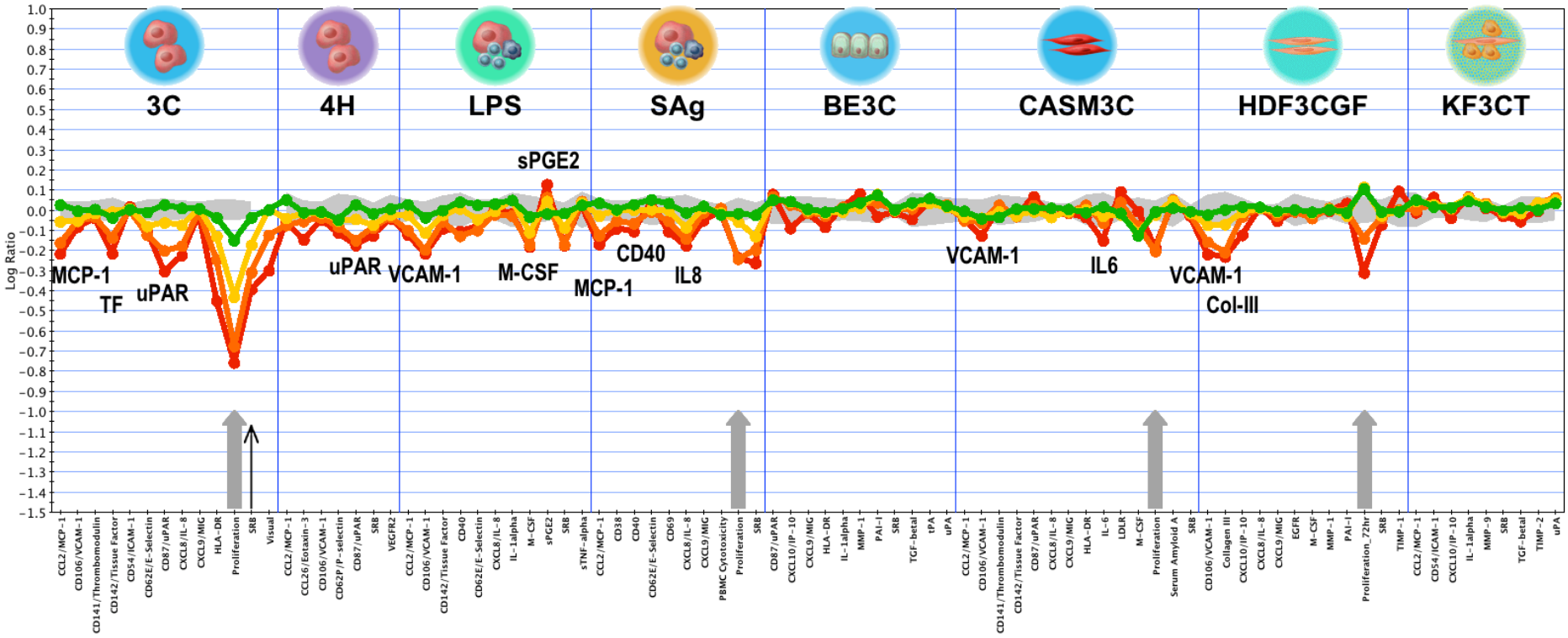
Typical Profiling Report

1. Annotated BioMAP Profiles



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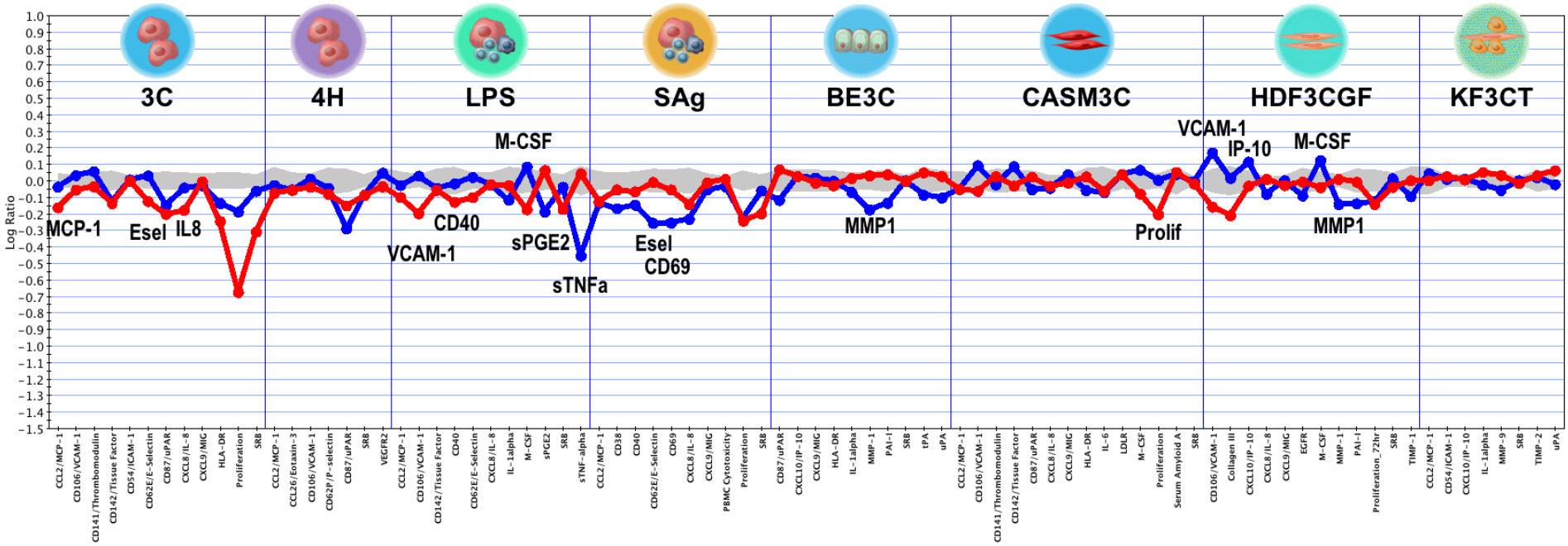
Profiling Report

2. Benchmarking



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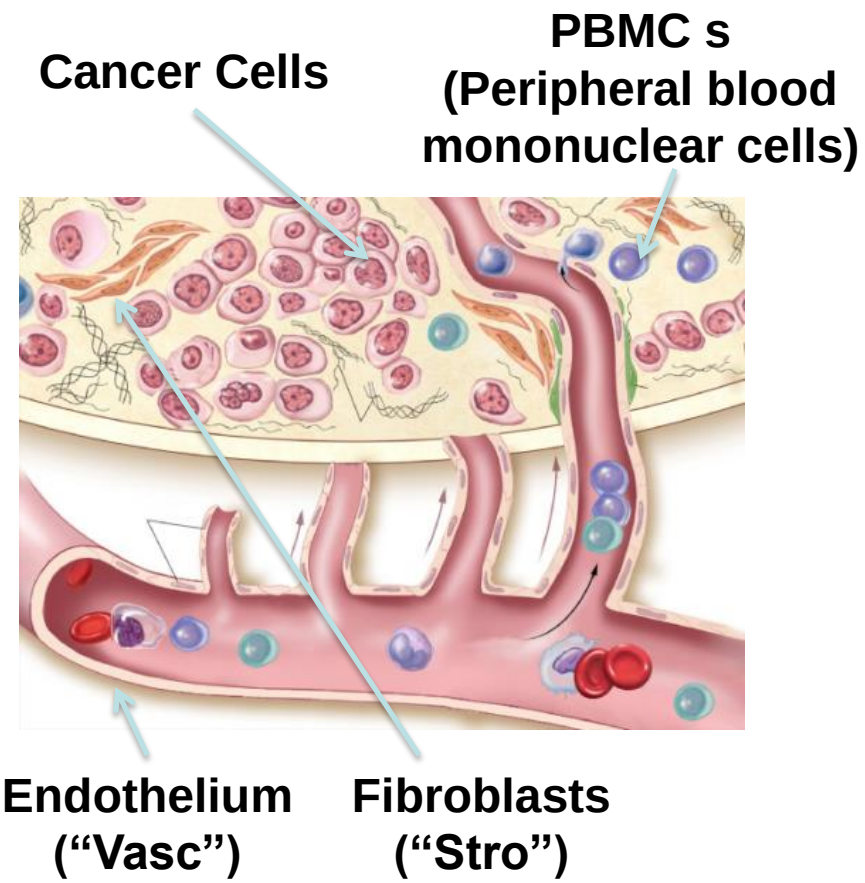
Benchmark Compounds for Oncology Systems



Agent	Mechanism of Action (MOA)
Rapamycin	mTor inhibitor
17-AAG	Hsp 90 inhibitor
Vorinostat	HDAC inhibitor
SB431542	TGF-beta1 inhibitor
Oligomycin	ATPase inhibitor
Paclitaxel	microtubule stabilizer
IC87114	PI-3K delta inhibitor
Colchicine	microtubule destabilizer
GSK-1120212	MEK inhibitor
LY294002	Pan PI-3K inhibitor
Daunorubicin	Intercalates DNA
Topotecan	DNA topoisomerase I inhibitor
Erlotinib	EGFR inhibitor
Nutlin-3	MDM2/p53 interaction inhibitor
Vemurafenib	PLX-4032, B-Raf V600E inhibitor
GDC-0879	B-Raf, ERK inhibitor
AZD2281	PARP-1, -2 inhibitor
AZD6244	MEK inhibitor
BIRB-796	p38 MAPK inhibitor

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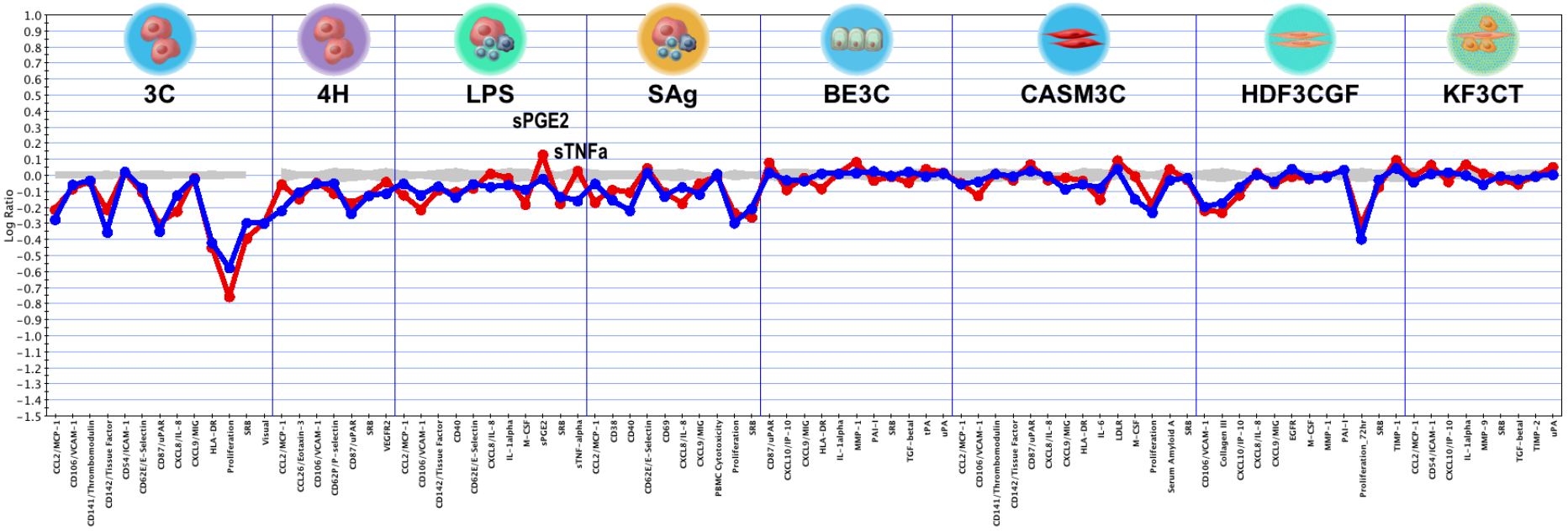
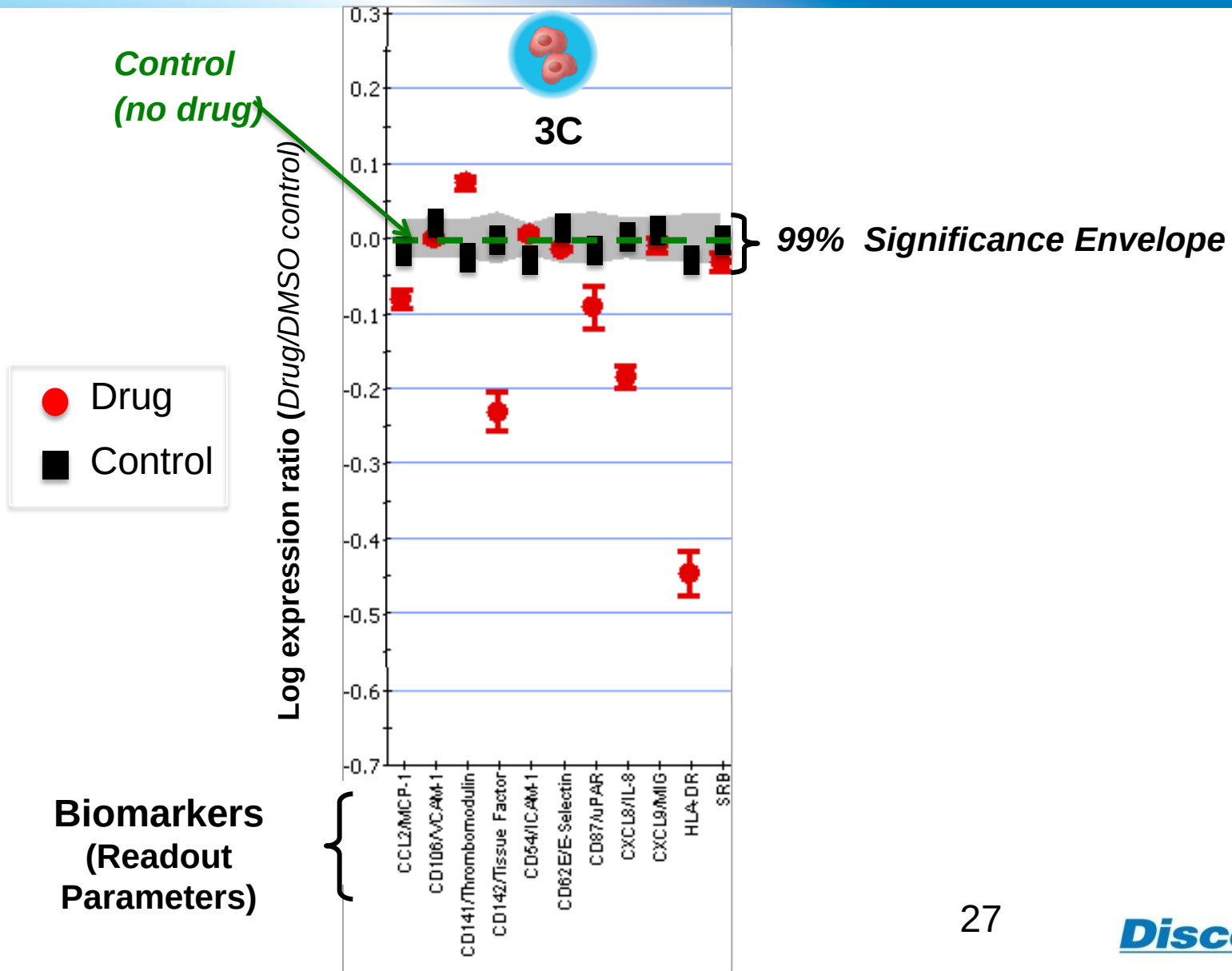
Profiling Report

3. Similarity Search



BioMAP Profile: Up Close



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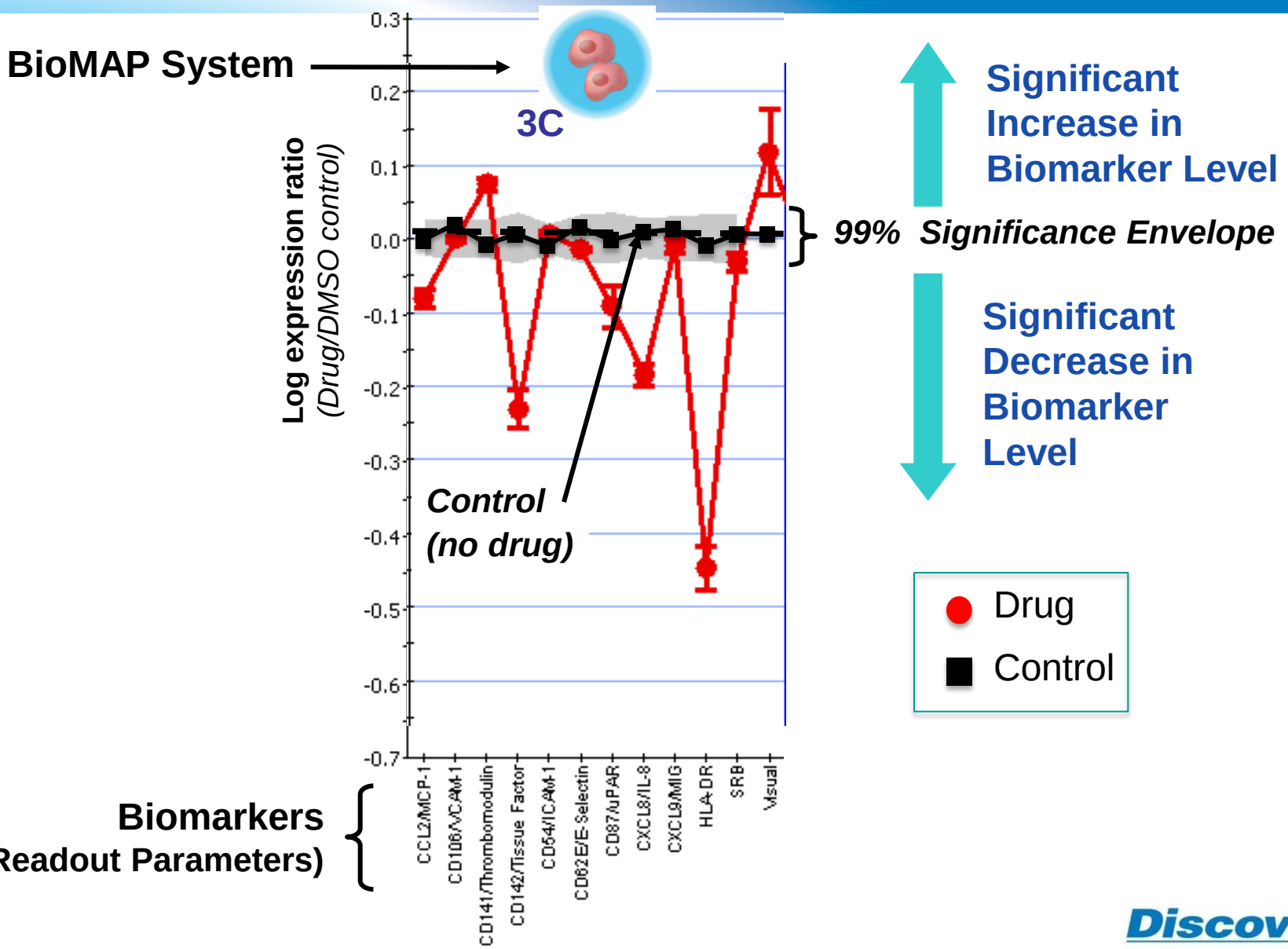
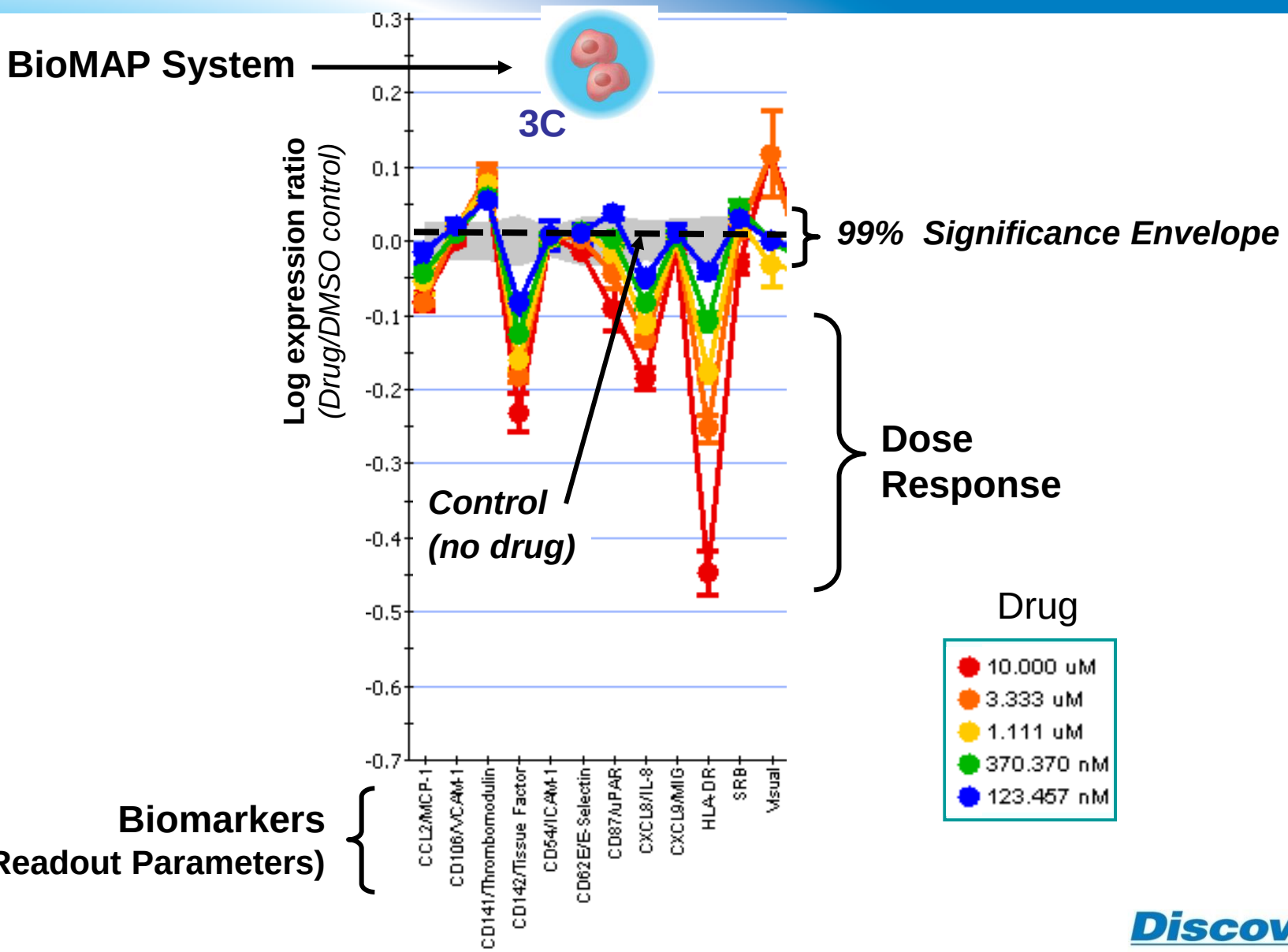
Drugs Effect Biomarker Levels In BioMAP Systems

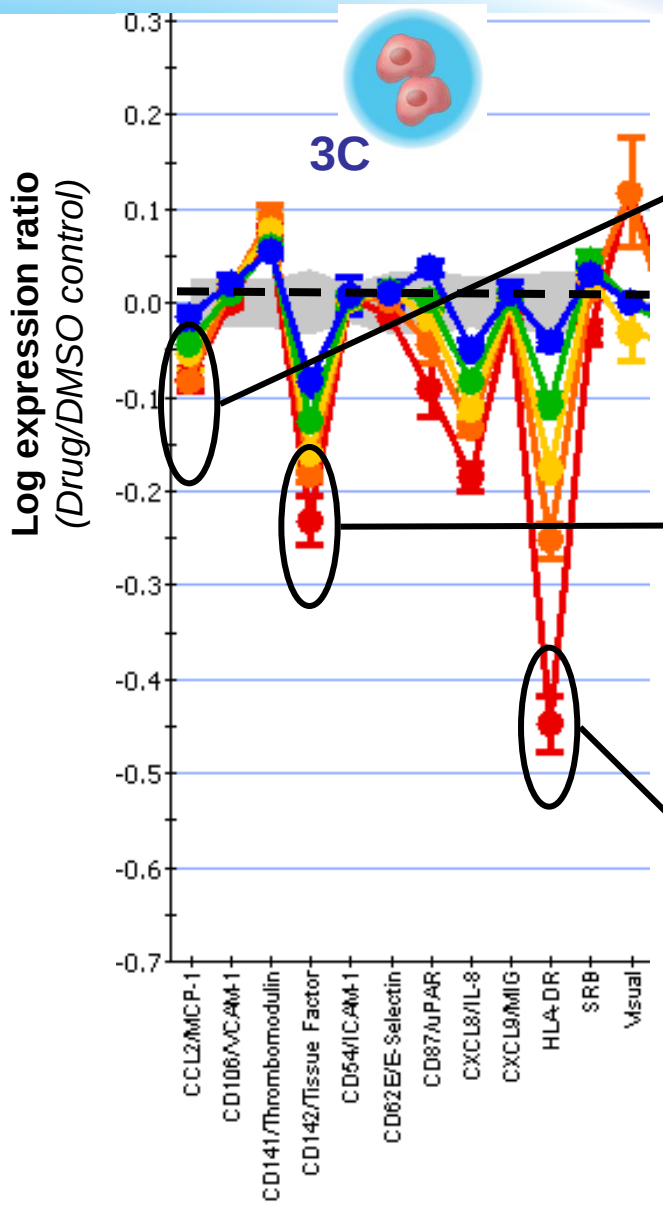
Example: p38 MAPK inhibitor



BioMAP® Profile of a p38 MAP Kinase Inhibitor



Biomarkers Provide Insight on Biological Processes (p38 MAPK Inhibitor Example)



MCP-1	**Monocyte recruitment into inflammatory tissues**
Tissue Factor	**Platelet activation and thrombosis**
HLA-DR	**Antigen presentation and T cell activation**

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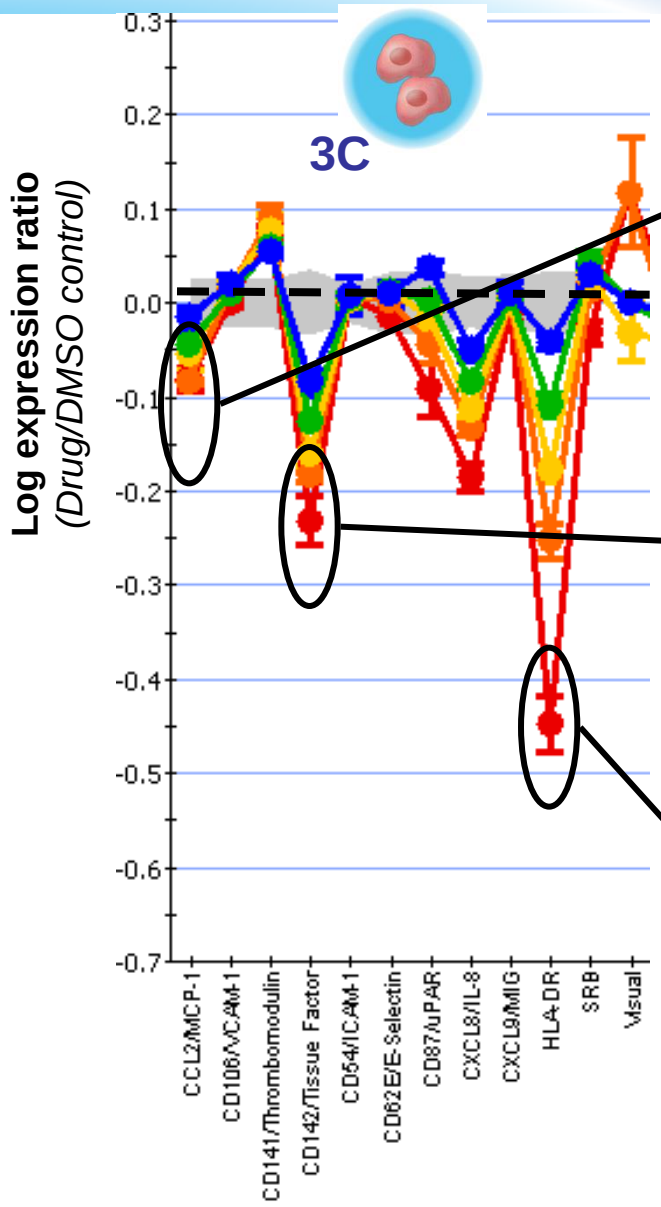
Biomarkers Provide Insight on Disease Indications (p38 MAPK Inhibitor Example)



MCP-1	**Acute coronary syndrome, Atherosclerosis, Diabetes, Heart Disease**
Tissue Factor	**Coronary artery disease, Myocardial infarction, Stroke**
HLA-DR	**Rheumatoid arthritis, Lupus, Multiple Sclerosis, Transplant rejection**

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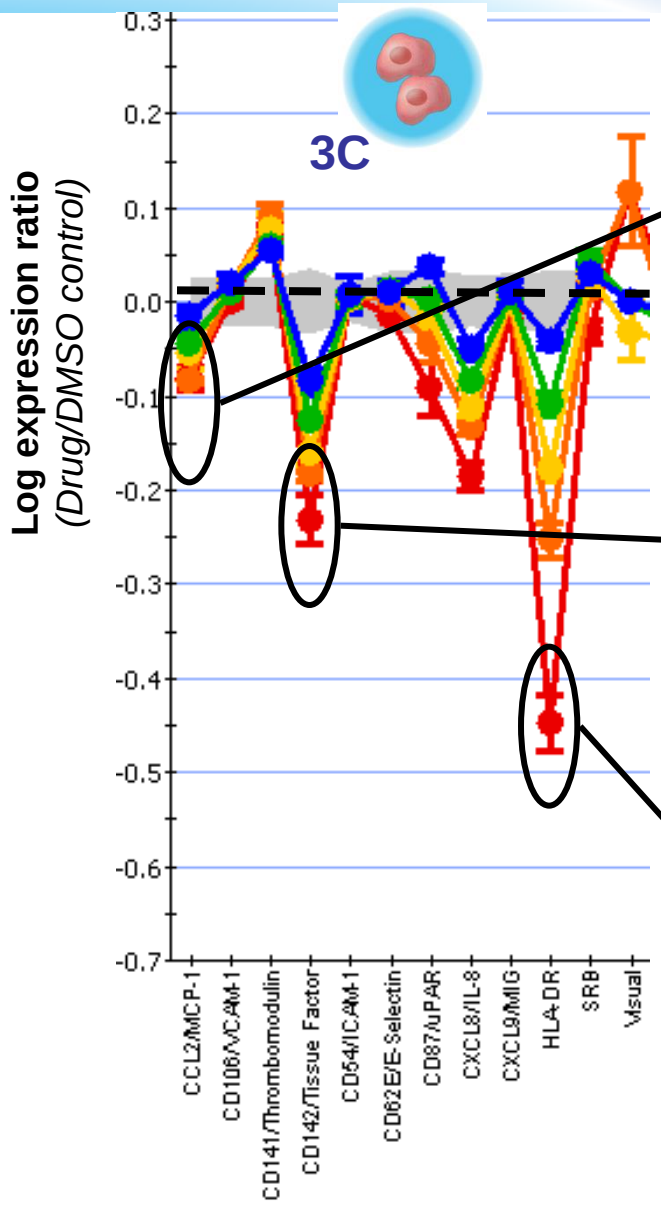
In Vivo Confirmation of BioMAP® Biomarkers

Example: p38 MAPK Inhibitor



MCP-1	Sheryanna A, et al. Inhibition of p38 mitogen-activated protein kinase is effective in the treatment of experimental crescentic glomerulonephritis and suppresses monocyte chemoattractant protein-1 but not IL-1beta or IL-6. J Am Soc Nephrol. 2007, 18:1167-79.
Tissue Factor	Sakurai K, et al. Role of p38 mitogen-activated protein kinase in thrombus formation. J Recept Signal Transduct Res. 2004, 24:283-96.
HLA-DR	Wada T, et al. Reduction in chronic allograft nephropathy by inhibition of p38 mitogen-activated protein kinase. Am J Nephrol. 2006, 26:319-25.

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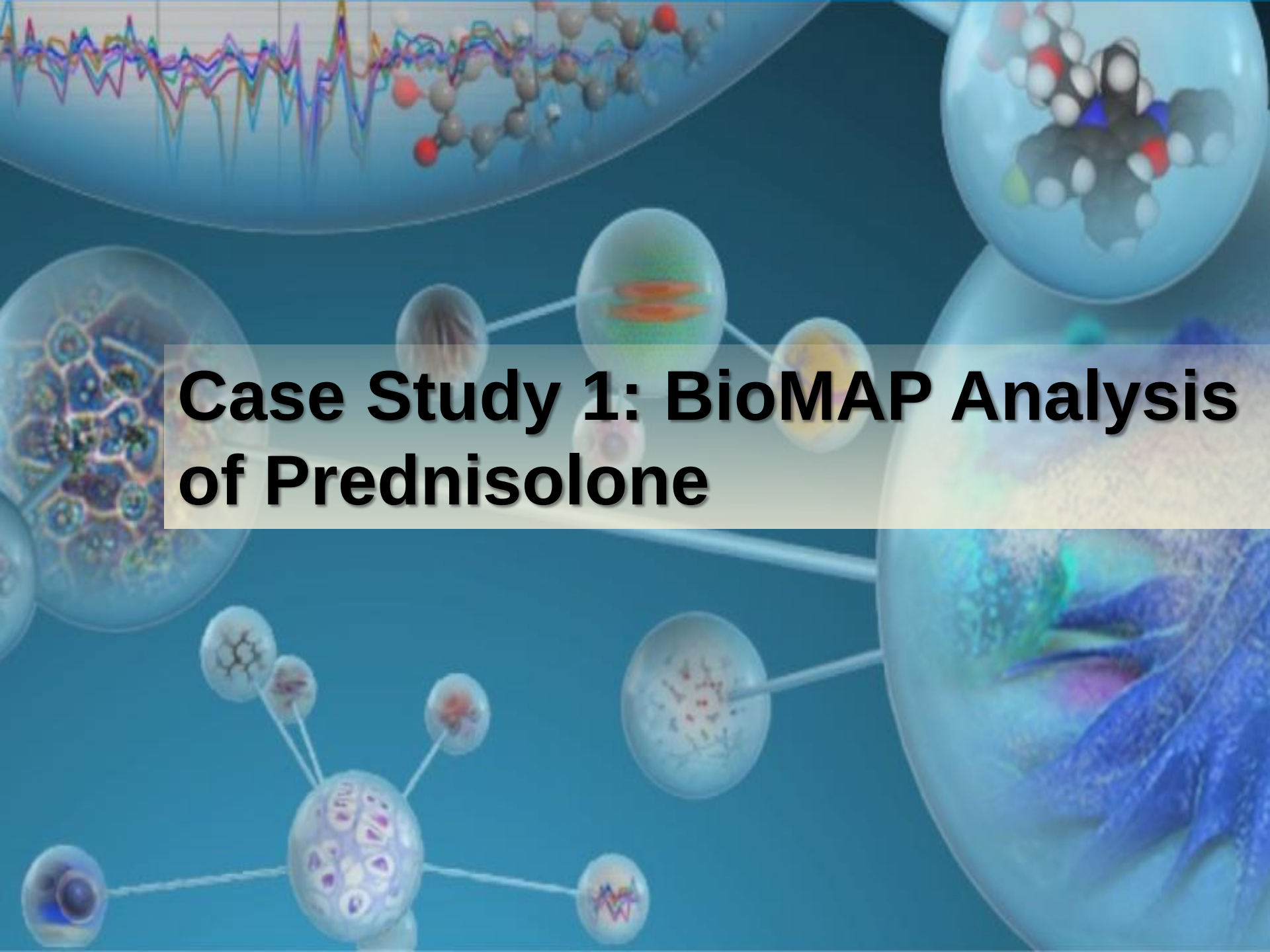
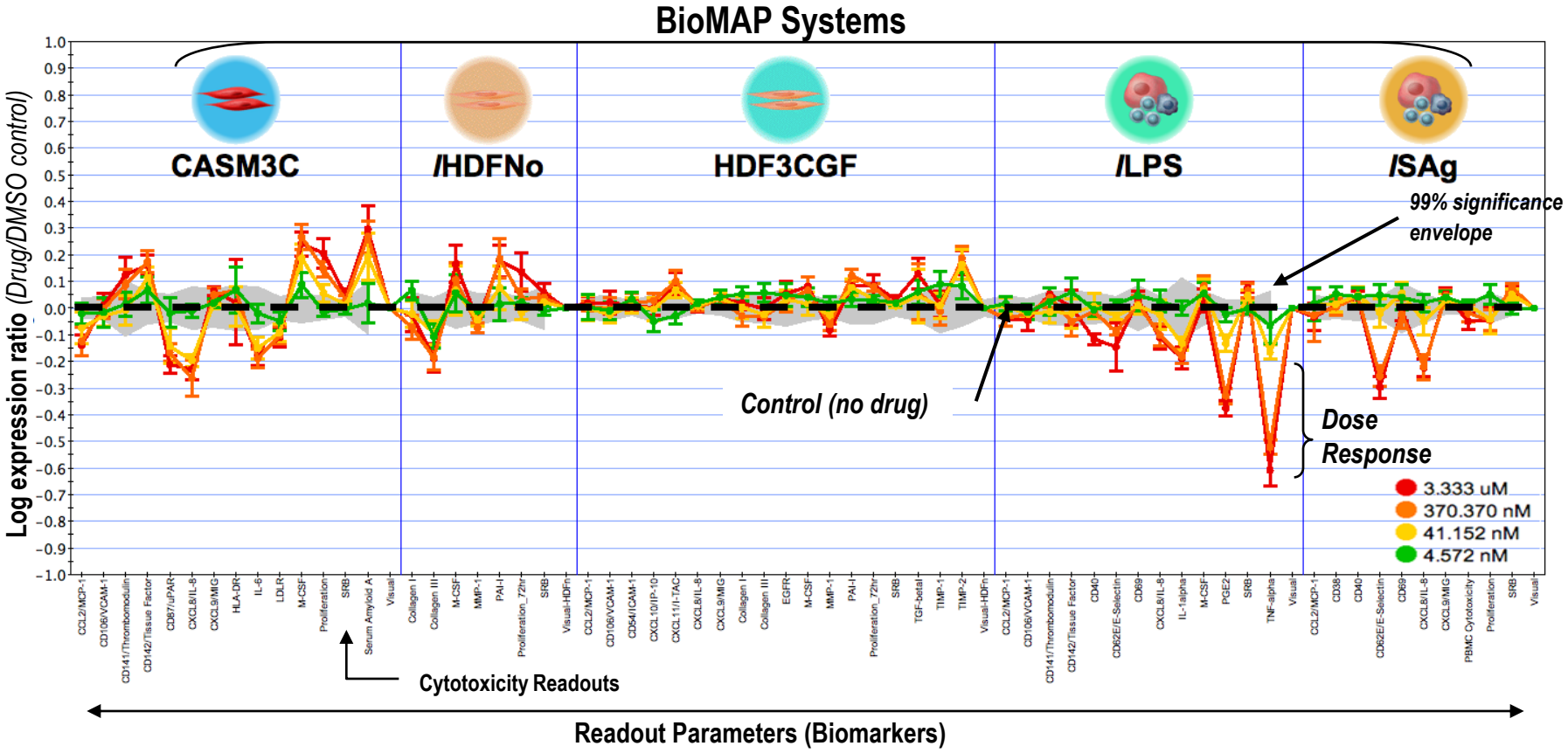


Case Study 1: BioMAP Analysis of Prednisolone

Profile for Prednisolone



Every compound, every analyte has a unique signature

Profiles retain shape over multiple concentrations

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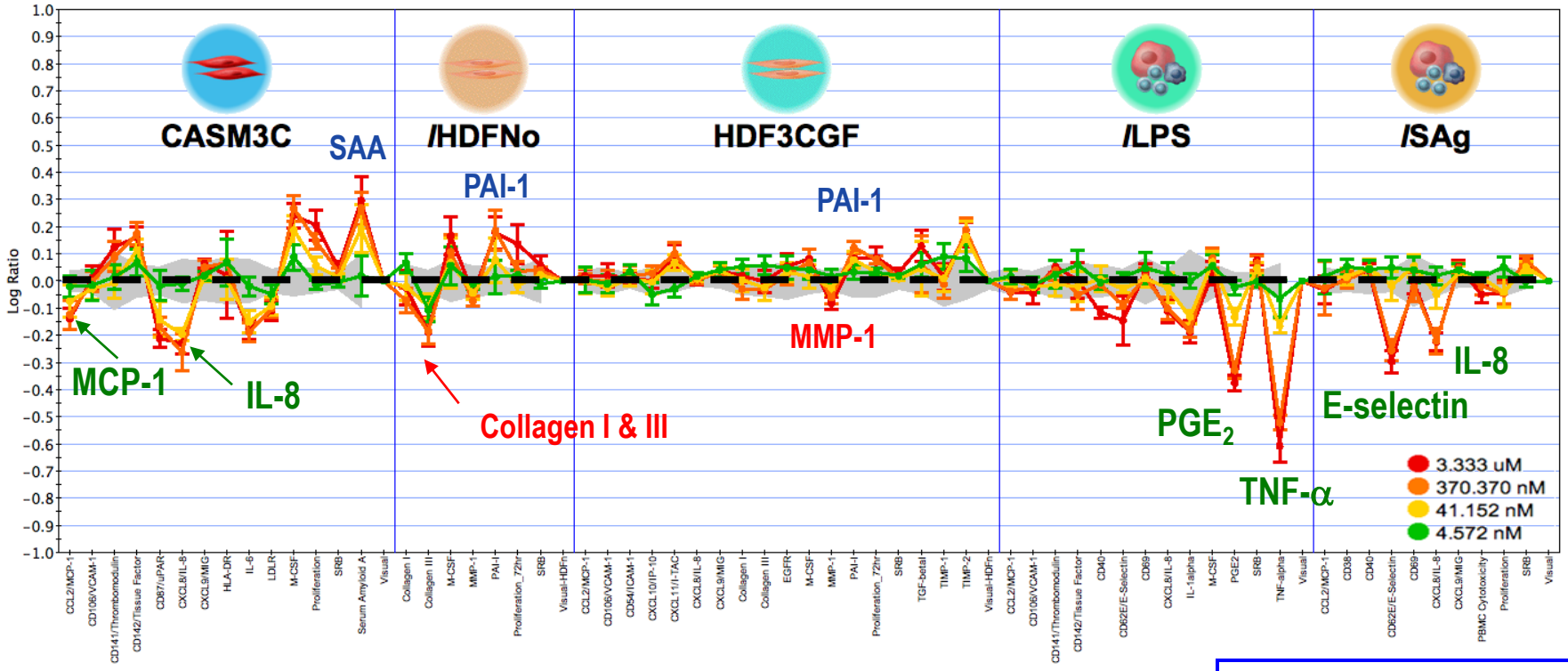
BioMAP Profile of Prednisolone: What it Means?



MCP-1, IL-8, E-sel. decrease
Leukocyte recruitment
Many, e.g. Jilma et al., 2000

PGE_2 decrease
Pain and Sweling
Sebaldt et al., 1990

Collagen I, III decrease
Skin Atrophy
Autio et al., 1994

PAI-1, SAA increase
Cardivascular Indications
Sartori et al., 1999
Fyfe et al., 1997

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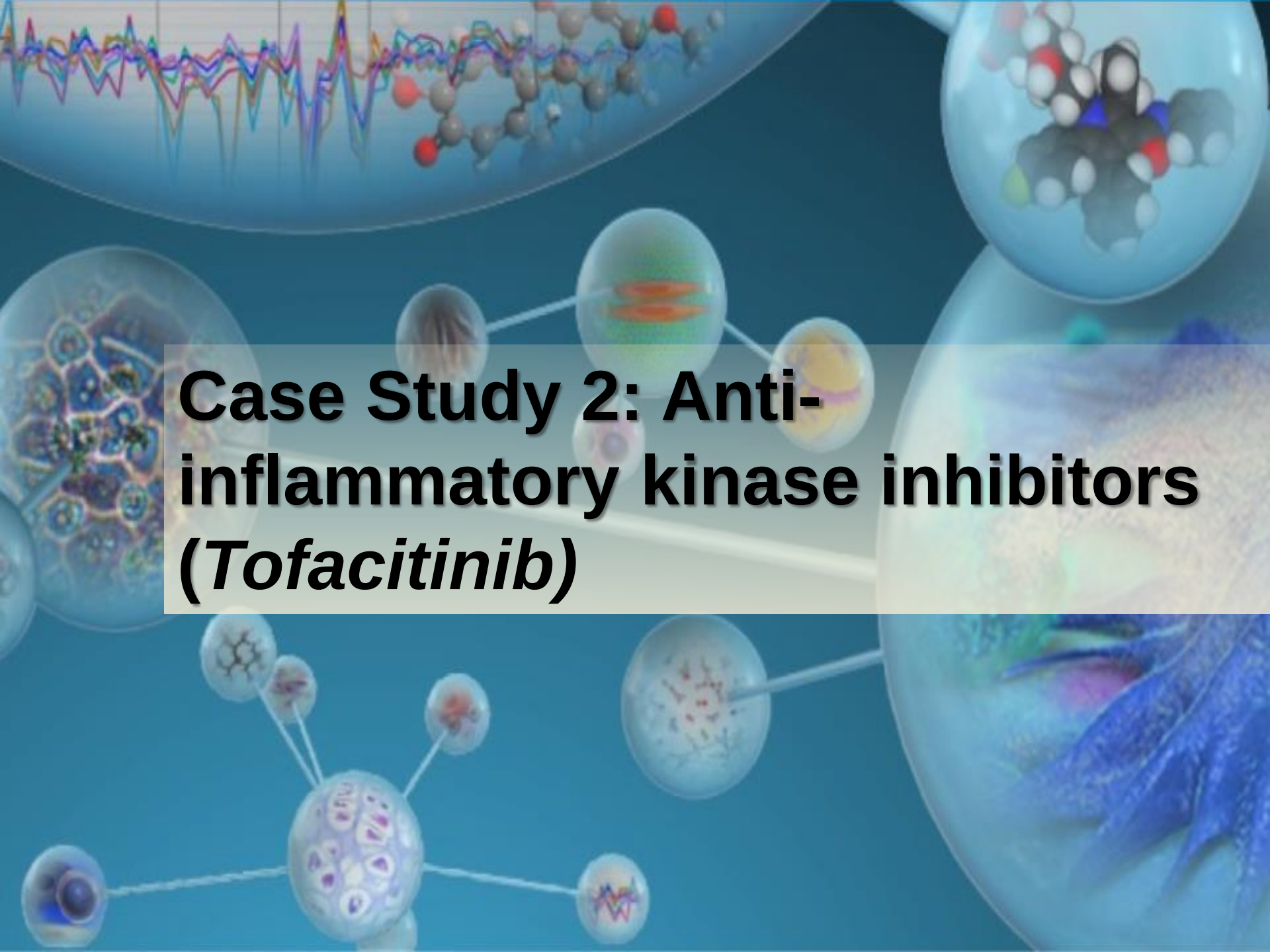
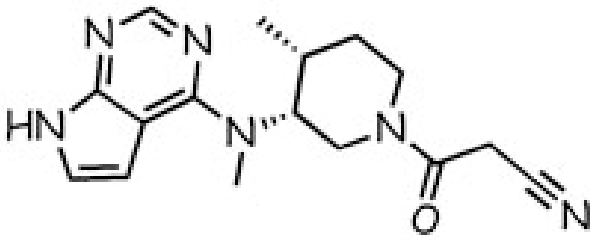
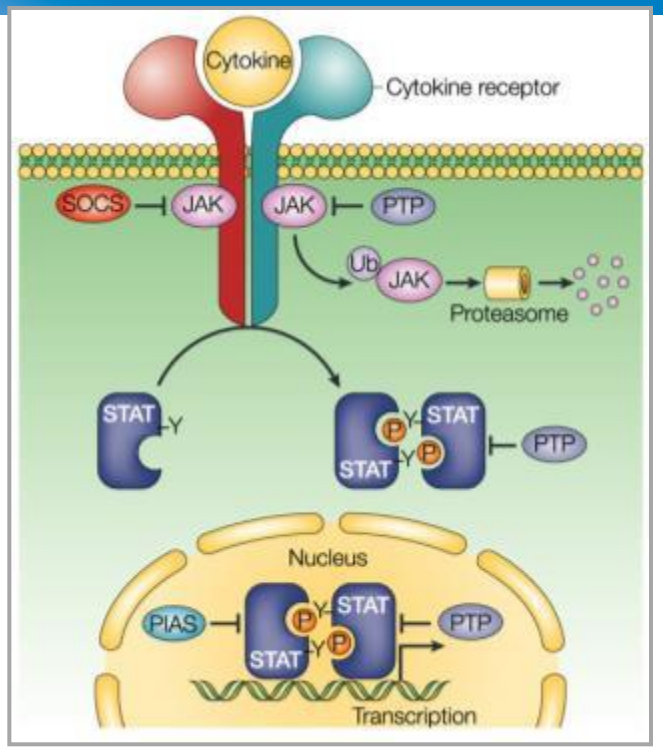


Case Study 2: Anti-inflammatory kinase inhibitors (*Tofacitinib)*

Case Study 2: Kinase Inhibitors in RA



Tofacitinib
CP-690,550



- **Jak 3 kinase selective inhibitor**
- **Jak family kinases: Jak1, Jak2, Jak3 and Tyk2**
  - Family of intracellular, nonreceptor tyrosine kinases that mediate signaling from a number of cytokine receptors (IL-2, IL-5, IL-7, IL-9, IL-15 and IL-21)
  - Act as dimers (hetero and homodimers)
- **Jak 3 is predominantly expressed in hematopoietic cells**

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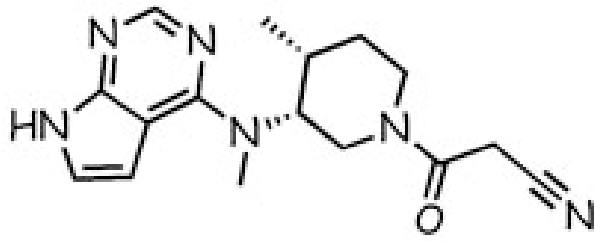
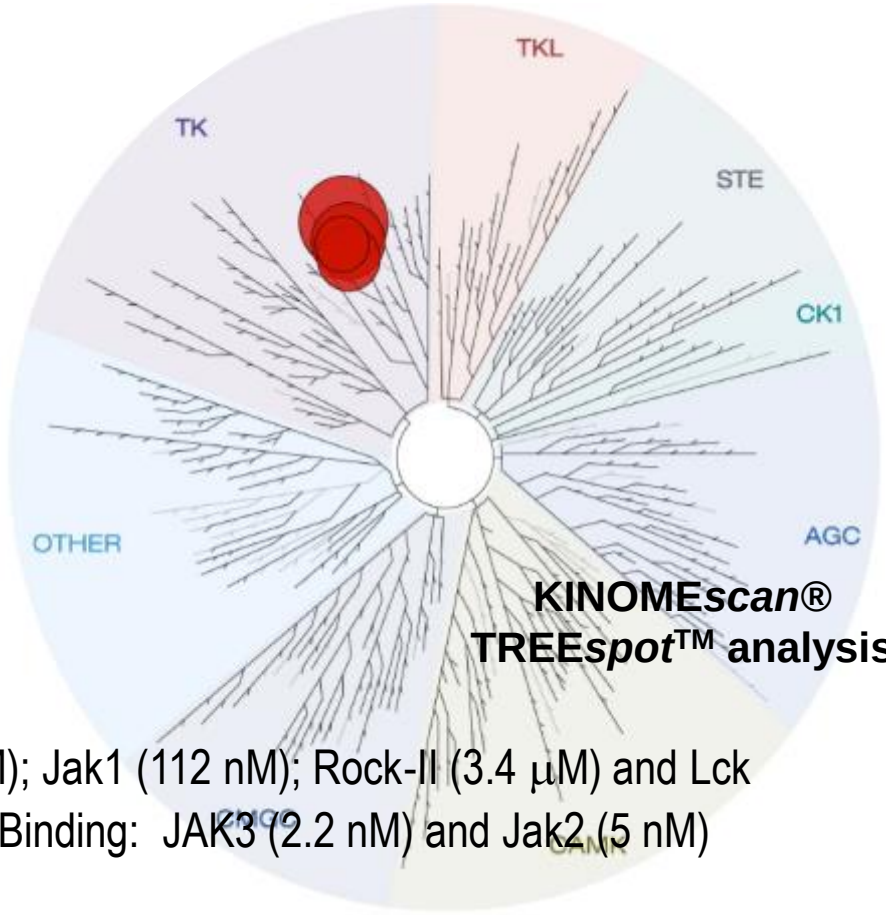
Case Study 2: Kinase Inhibitors in RA



Tofacitinib
CP-690,550



KINOME*scan*®
TREE*spot*™ analysis

- **Jak family selective inhibitor**
  - Kinase activity: EC_{50}s: Jak3 (1 nM); Jak2 (20 nM); Jak1 (112 nM); Rock-II (3.4 μM) and Lck (3.8 μM) (Changelian, Science 2003, 302:875). Binding: JAK3 (2.2 nM) and Jak2 (5 nM) (Karaman, Nat Biotech 2008, 26:127).
- **Safe and effective in rheumatoid arthritis**
  - Kremer, Arthr & Rheum 2012, 60:1895; Fleischmann, Arth & Rheum 2012, 64:619
  - Approved for rheumatoid arthritis (Pfizer)

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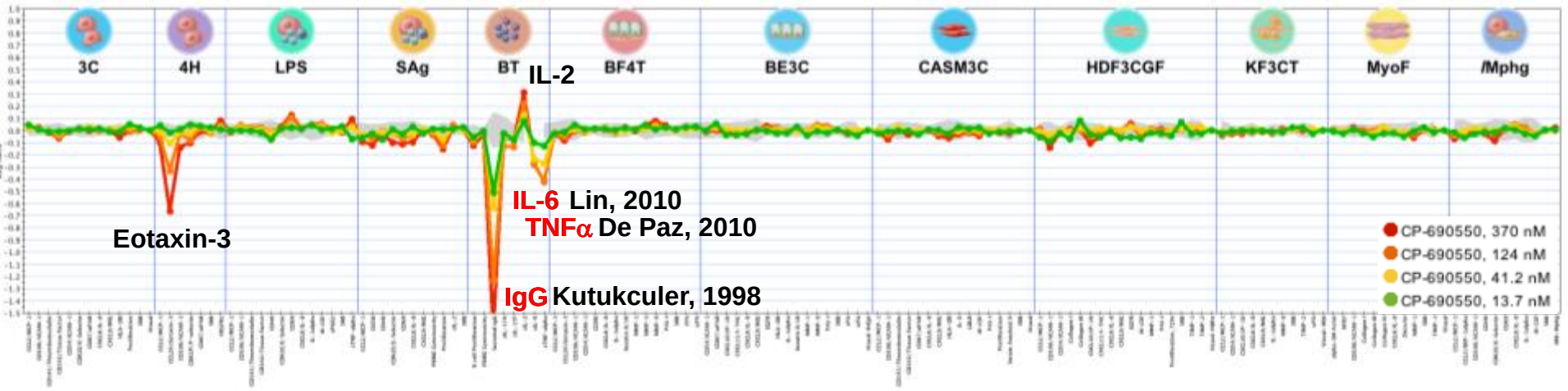
BioMAP Profile of Tofacitinib (≤ 370 nM)



- **Key activities of Tofacitinib (CP-690,550) ≤ 370 nM**
  - Selective inhibition of T-cell-dependent B cell activation (BT system)
  - Inhibition of IL-4 dependent signaling in endothelial cells (4H system)
  - Several activities consistent with clinical efficacy biomarkers (in **red**)

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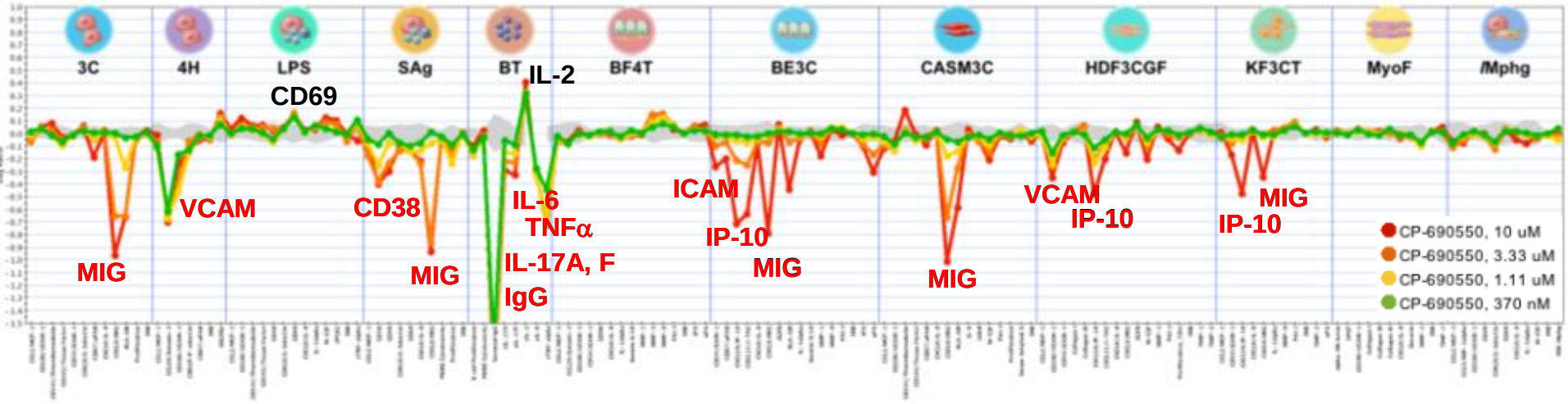
BioMAP Profile of Tofacitinib (≥ 370 nM)



- **Many additional activities at higher doses (≥ 370 nM):**
  - Most are clinical efficacy biomarkers for RA (Kuan, 2010; Klimiuk, 2002; Kutukcular, 1998; Dolhain, 1998; Metawi, 2011) (in **red**)
- **Activities are consistent with clinical effects and dosing**
  - Van Gurp, Transpl. 2009, 87:79
  - Cmax in one clinical study was ~1 microM (Cohen, BJCP 2010, 69:143)
- **Higher dose (less selective) profile is more "efficacious" in BioMAP and in the clinic**

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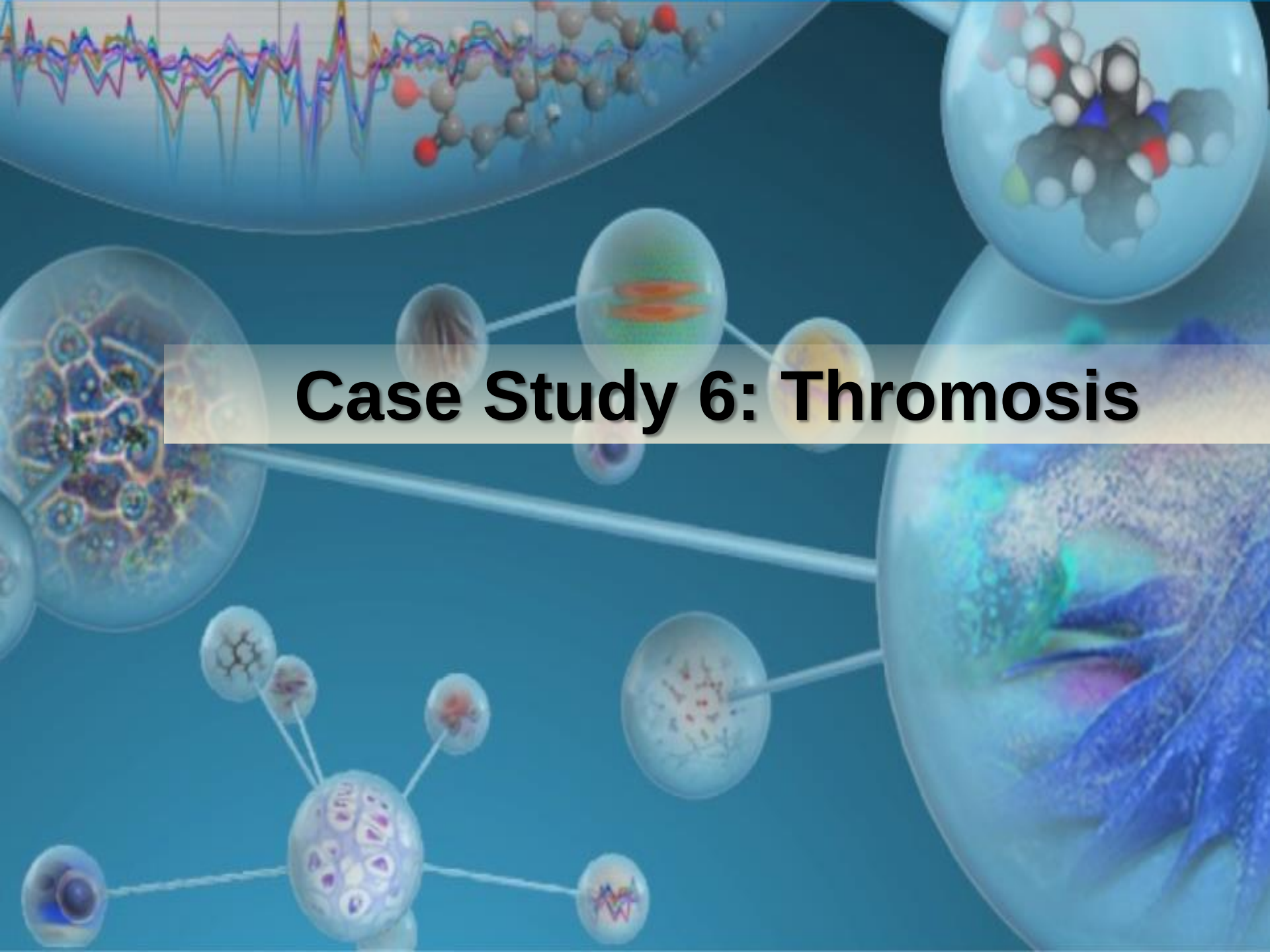
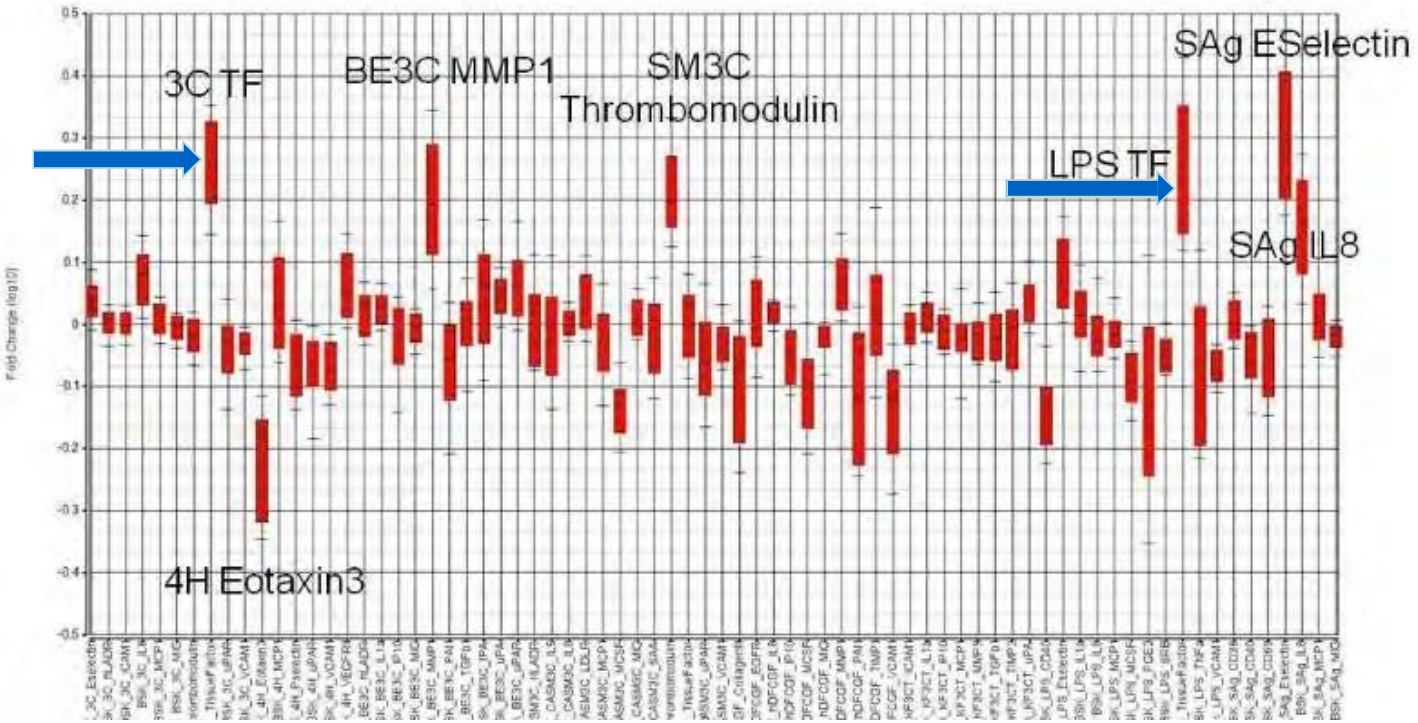

Case Study 6: Thromosis

Thrombosis

- Pathologic setting - Venous thrombosis
  - The formation of a blood clot (coagulation) within a vein
  - Deep vein thrombosis (DVT)
  - Pulmonary embolism
- Associated with:
  - Smoking (Polycyclic aromatic hydrocarbons AhR agonists)
  - Contraceptives, hormonal replacement therapy
  - Various other drugs
    - mTOR inhibitors (everolimus)
    - 2nd generation anti-psychotics
- Normal process
  - Wound healing, angiogenesis, tissue repair

Aryl Hydrocarbon Receptor Agonists



Nicole Kleinstreuer, NBT, 2014 in the press

- Cluster of chemicals defined by their BioMAP signature
  - Key feature: Increased Tissue Factor (TF) in 3C and LPS systems

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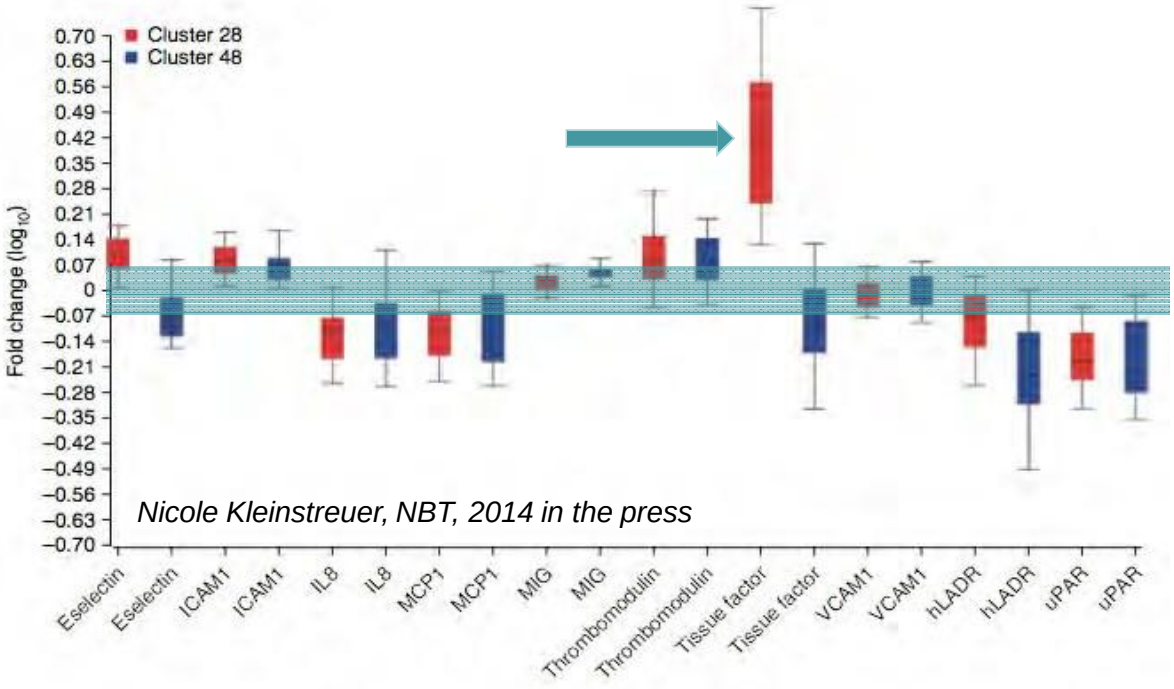
Estrogen Receptor Actives in Phase II



- Two clusters of chemicals defined by their BioMAP signatures
  - Blue = Estradiol, Estrogen Receptor Agonists
  - Red = Estrogen Receptor Antagonists, “Selective Estrogen R Modulators”
- Increased Tissue Factor by SERMs and ER antagonists

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Some Announced Partnerships

Announced Collaborations and Joint Publications or Presentations

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BioSeek

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