BIOMED 21

A HUMAN PATHWAY-BASED APPROACH TO DISEASE AND MEDICINE

# Primary Cell-Based Phenotypic Profiling for Building Human Outcome Pathways

BioMed21, Bethesda, MD

27 June 2017

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DiscoverX

# Human Pathway-Based Approaches to Disease & Medicine

*Overall Goals*

- Support better decisions in drug discovery & chemical safety
  - Improve failure rates, protect patients
- Reduce the costs of new drug/product development
  - Leverage new technologies & innovations
    - *In vitro* assays & *in silico* methods; pathway approaches
- Build our understanding of human disease & toxicity mechanisms

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A HUMAN PATHWAY-BASED APPROACH TO DISEASE AND MEDICINE

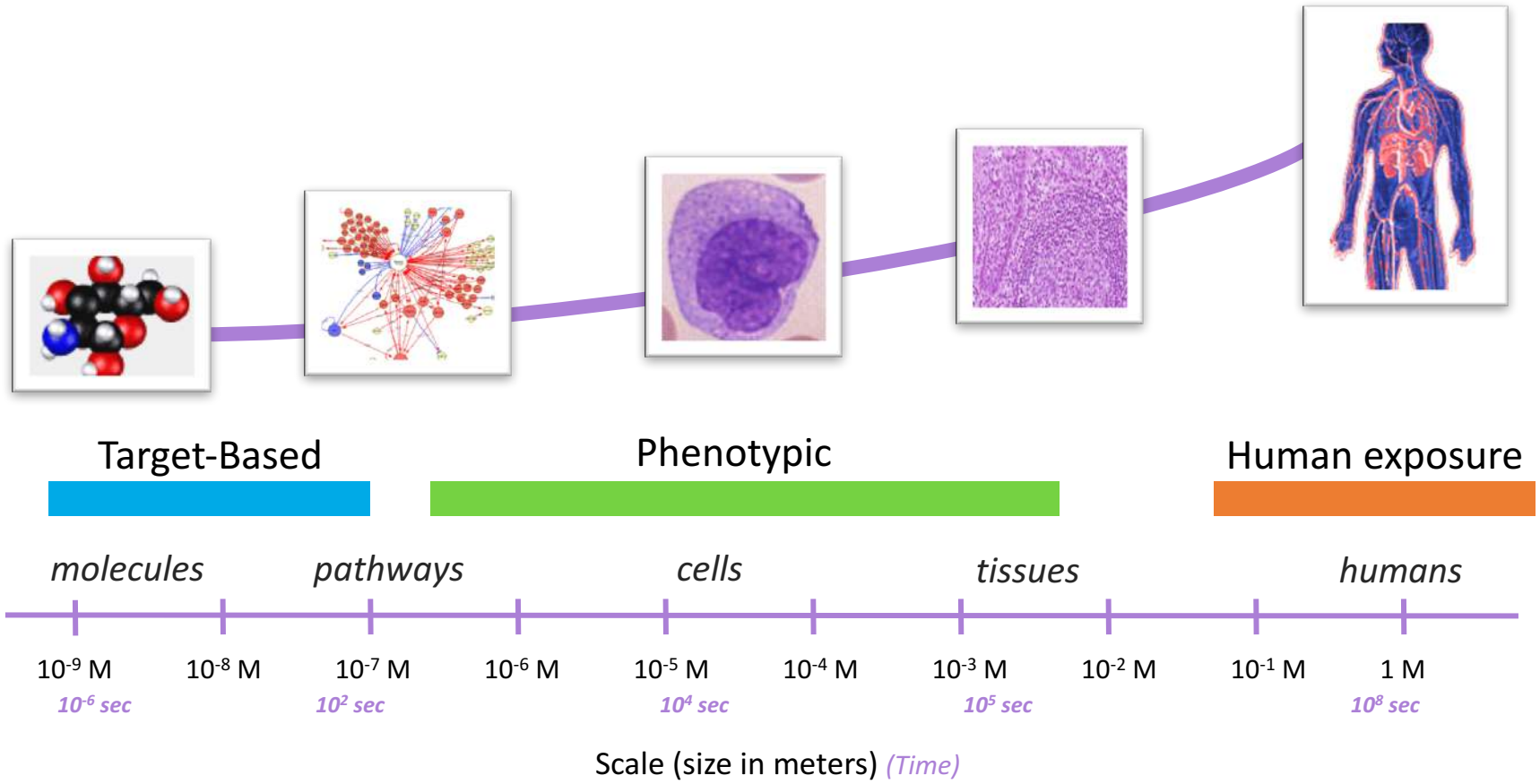
# Human Pathway-Based Approaches to Disease & Medicine

*A Role for In Vitro Human-Based Phenotypic Profiling*

- Phenotypic versus Target-Based Assays
- Human primary cell-based systems (BioMAP / BioSeek assays)
  - Key features
  - Data-driven knowledge discoveries
- Human Outcome Pathways Knowledgebase

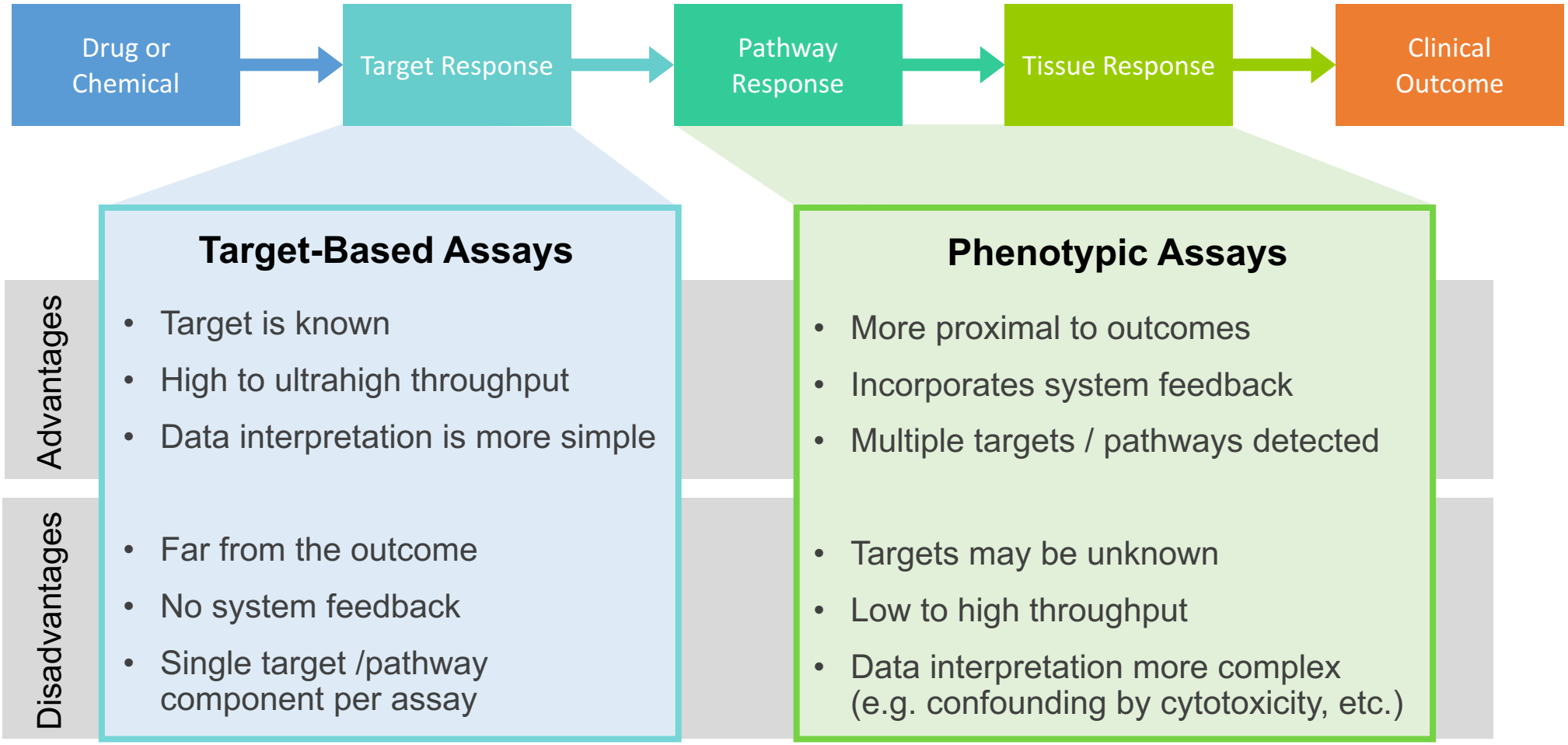
# Phenotypic Assays Are Closer to Outcomes

*Biology is Complex, Hierarchical & Modular*



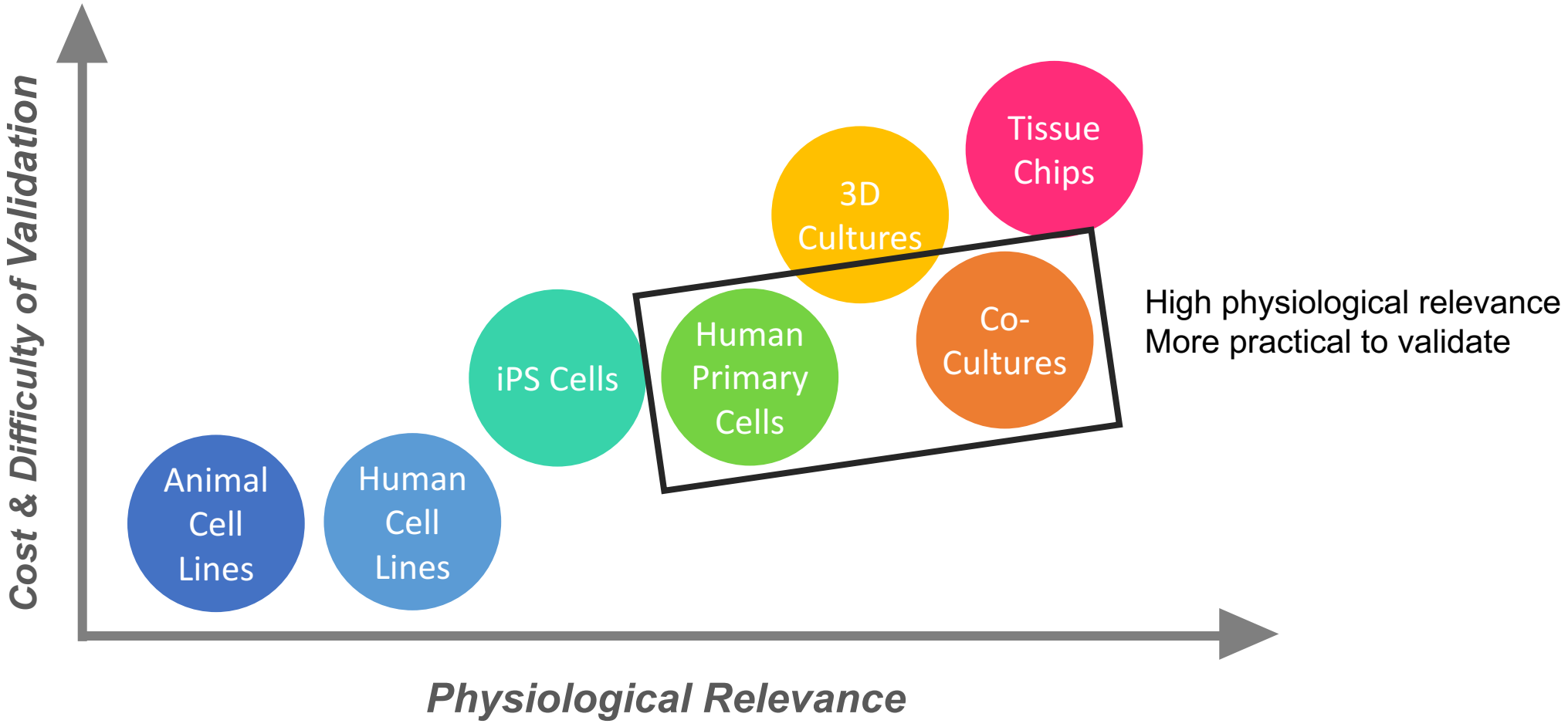
# Phenotypic Versus Target-Based Assays

*Advantages & Disadvantages*



# Phenotypic Assay Options

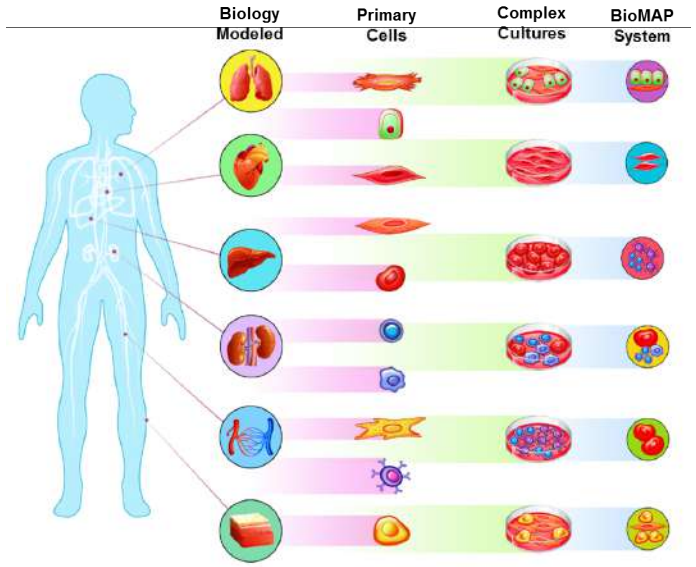
*Tradeoff: high physiological relevance versus cost & difficulty of validation*



# Human Primary Cell-Based Systems

# BioMAP® Human Phenotypic Profiling Platform

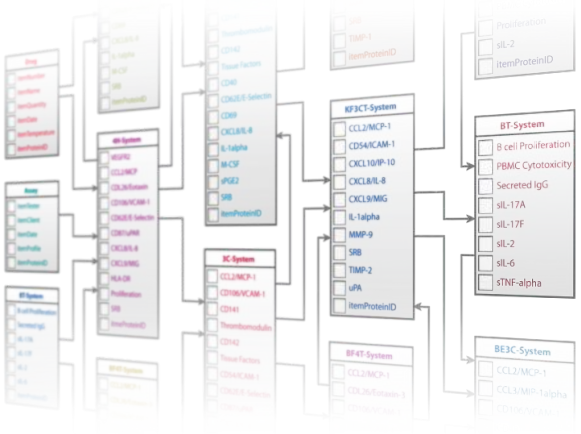
## BioMAP Systems




### High Physiological Relevance

- **Human primary cells** & co-cultures
- **Broad coverage** of tissue & disease biology
- **Clinical biomarker** (protein) endpoints

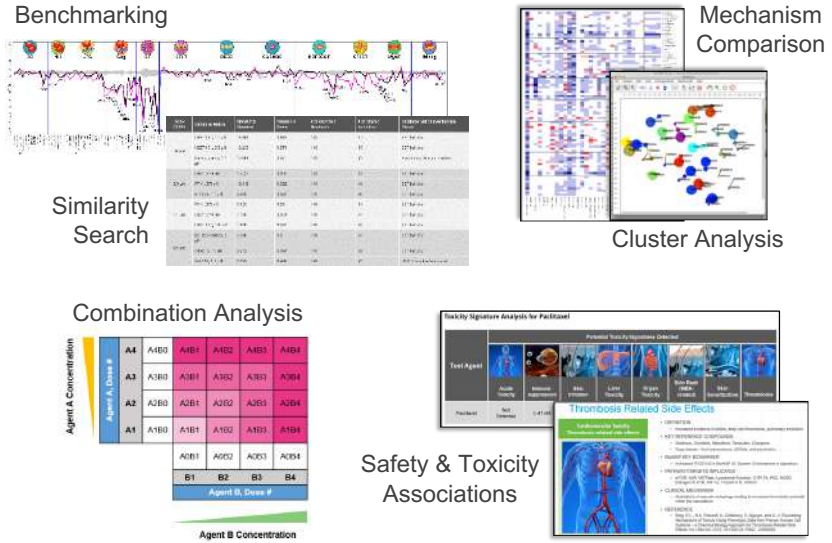
## Reference Profile Database



### Well Validated - Large database (> 4000)

- **Drugs** – Clinical stage, approved, failed
- **Experimental Compounds** - Research tools, mixtures
- **Biologics** – Antibodies, proteins, peptides

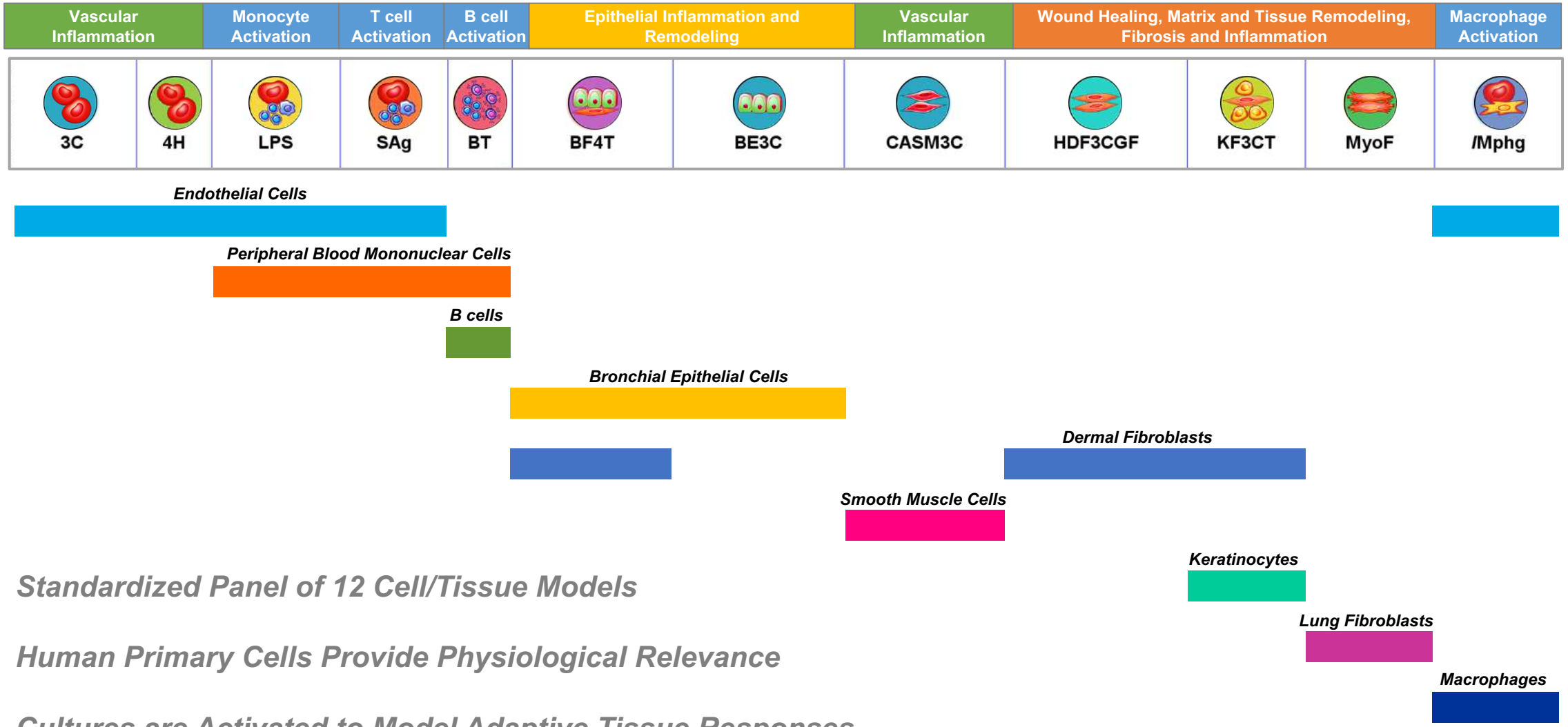
## Predictive Analytics Tools




### Analytics for Knowledge Discovery

- Mechanism of action
- Toxicity signatures, mechanism classifiers
- Benchmarking to standards-of-care

# Diversity PLUS Panel of 12 BioMAP® Systems




*Standardized Panel of 12 Cell/Tissue Models*

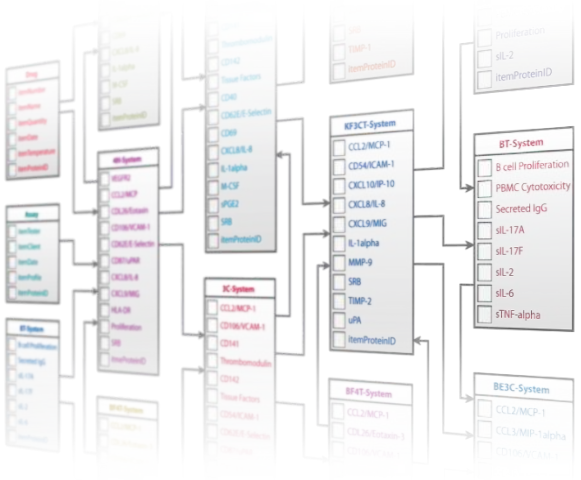
*Human Primary Cells Provide Physiological Relevance*

*Cultures are Activated to Model Adaptive Tissue Responses*

# Large Drug Reference Database

*10+ Years to Build*

**Reference Profile Database**



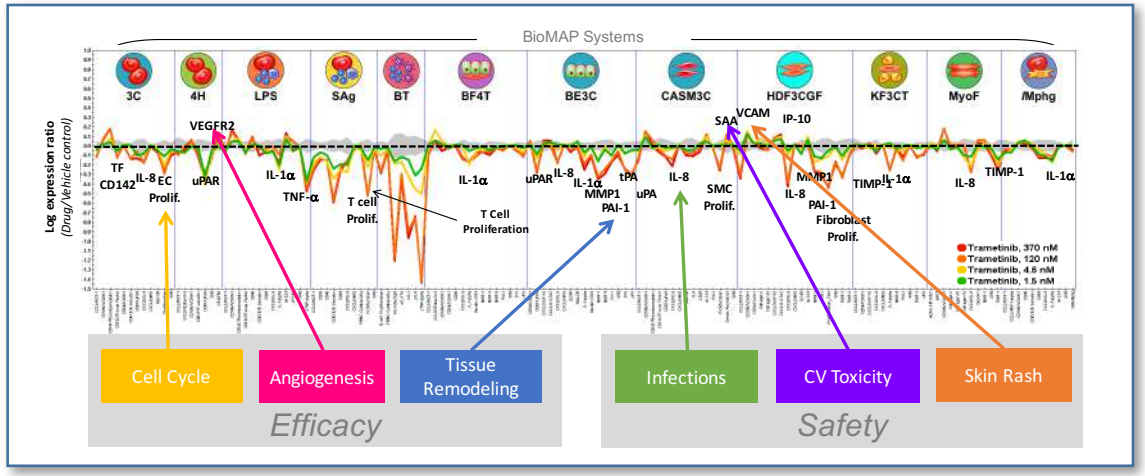
Biomarker responses stored as profiles in BioMAP database
>4000 compounds

- **More than 4000 agents**
  - **Drugs** – Clinical stage, approved, and failed
  - **Experimental Chemicals** - Research tool compounds, environmental chemicals, nanomaterials
  - **Biologics** – Antibodies, cytokines, factors, peptides, soluble receptors, extracts
- **Validation**
  - **Predictive classifiers** for mechanism classes
    - Berg, JBS 2013, 18:1260
  - **Toxicity signatures** for Adverse Effects (AEs)
    - Berg, IJTM 2015, 16:1008; Shah, Cell Chem Biol 2017, in press
  - **ToxCast** program
    - Houck, JBS 2009, 14:1054; Kleinstreuer Nat Biotech 2014, 32:538

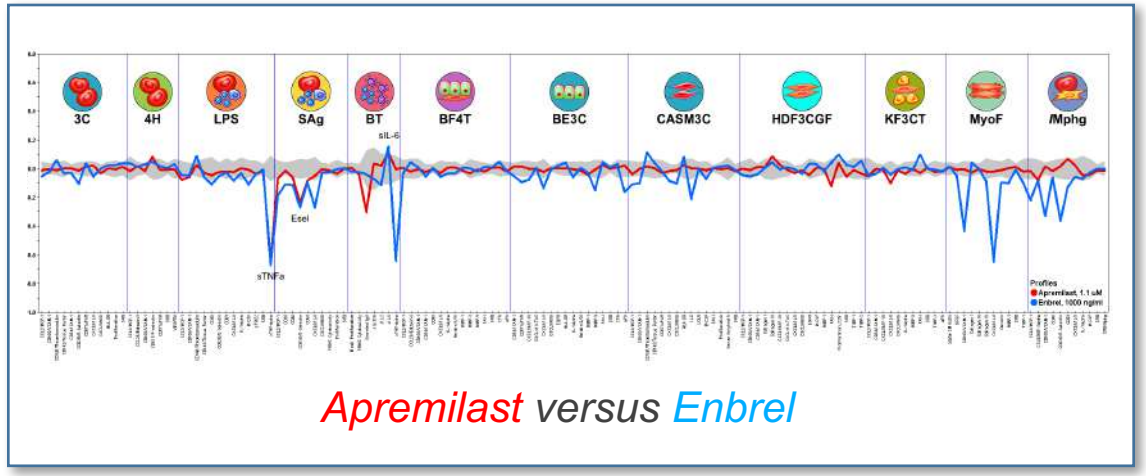
# Knowledge Discovery from Phenotypic Profiling

*Translational Biomarkers and Assessing Clinical Indication Potential*

## Identify Translational Biomarkers




## Benchmark to Clinical Standards of Care




*Apremilast* versus *Enbrel*

*Correlate biomarkers to clinical effects*

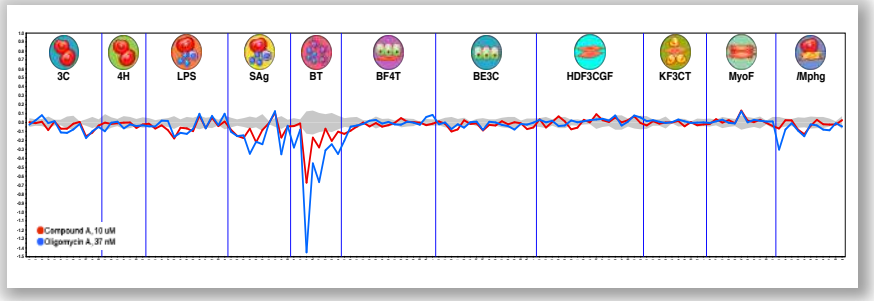
*Define signatures for clinical indications*

# Knowledge Discovery from Phenotypic Profiling

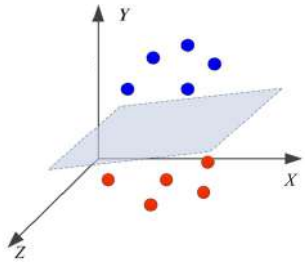
*Identifying mechanism of action*

## Similarity Search

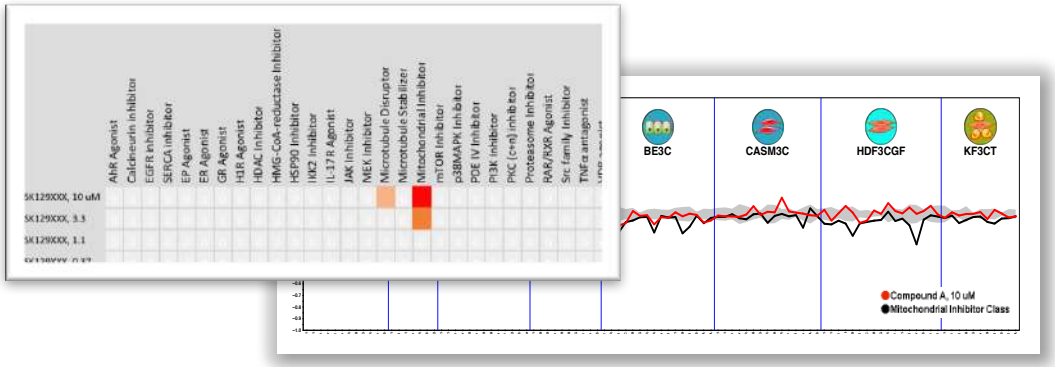
- Search for the most similar compound in the reference database
- Generate mechanistic hypothesis based on the top match




## Mechanism Classification




- 28 mechanism classifiers constructed with data on known compounds using **machine learning**
- Test compounds for class membership

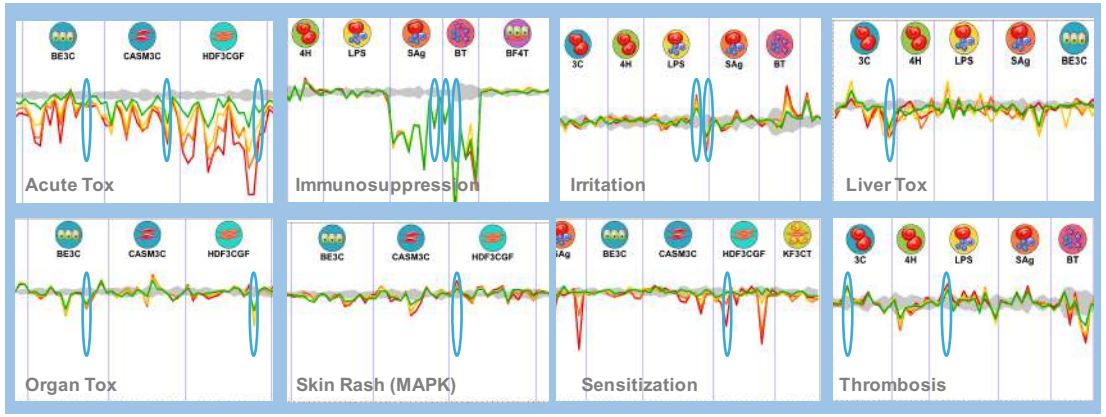



# Knowledge Discovery from Phenotypic Profiling

*Identifying Toxicity Signatures*

Adverse Effects


Toxicity Signatures

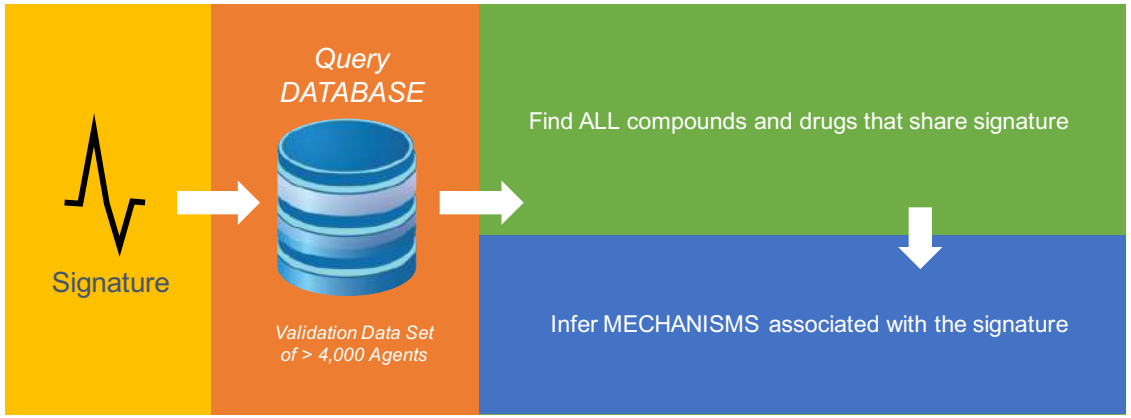



- Groups of compounds (or drugs) sharing specific toxicities or adverse effects were identified
- Common activities shared by these compounds were developed as “Toxicity Signatures”

# Knowledge Discovery from Phenotypic Profiling

*Identifying Toxicity Signatures*

## Using Signatures to Derive Pathway Mechanisms




- Data mining of reference database uncovers mechanisms associated with each signature

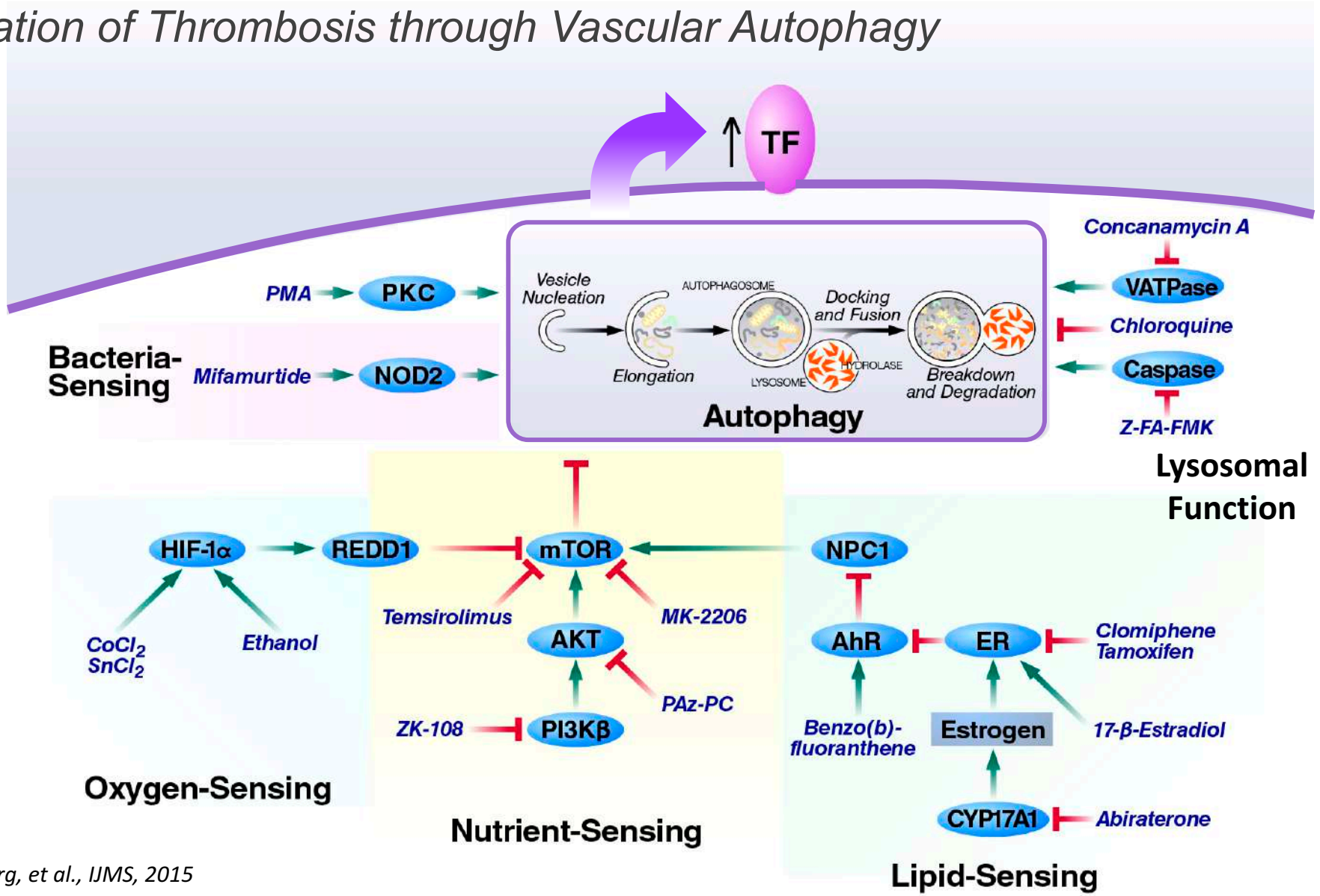
## Mechanism-based Toxicity Signatures

| | | | |
|---|---|---|---|
| **Reference Drugs** Cycloheximide, actinomycin D, digoxin, bortezomib, valinomycin<br>**Pathway/targets** Protein synthesis inhibition, RNA synthesis inhibition, $Na^+/K^+$ ATPase, proteasome inhibition<br>**Acute Tox** | **Reference Drugs** Sirolimus, Remicade, Cyclosporine, Tacrilomus, Mycophenolate, Azathioprine<br>**Pathway/targets** mTOR, calcineurin, Jak, hsp90, NFAT, DNA proliferation<br>**Immunosuppression** | **Reference Drugs** Retinoic Acid, Retinol, Vitamin D, Ritonavir, Imatinib, 2-chloroethyl ethyl sulfide, Calcitriol<br>**Pathway/targets** RAR/RXR, AhR, VDR, PKC<br>**Irritation** | **Reference Drugs** Amiodarone, Tamoxifen, Astemizole, Ketoconazole, Haloperidol, Aplaviroc<br>**Pathway/targets** V-ATPase, PIKfyve, Smoothened<br>**Liver Tox** |
| **Reference Drugs** 5-fluorouracil, vincristine, cisplatin<br>**Pathway/targets** DNA replication, microtubule function<br>**Organ Tox** | **Reference Drugs** Trametinib, AZD6244, p38 MAPK Inhibitors, Betaseron, Anakinra<br>**Pathway/targets** MEK, p38 MAPK, IL-1R, IL-4R, TweakR, IFNα/β<br>**Skin Rash (MAPK)** | **Reference Drugs** NSAIDS, Phthalates<br>**Pathway/targets** RAR/RXR, PKC, mitochondria, JNK, Prostaglandin R<br>**Sensitization** | **Reference Drugs** Sirolimus, Crizotinib, Raloxifene, Tamoxifen, Clozapine, Cigarette smoke<br>**Pathway/targets** AhR, mTOR, VATPase, Lysosomal Function, CYP17A, PKC, NOD2, Estrogen R, H1R, HIF-1α, Thyroid H R, OSM R<br>**Thrombosis** |

- **RESULT**: Toxicity Signatures are mechanism-based

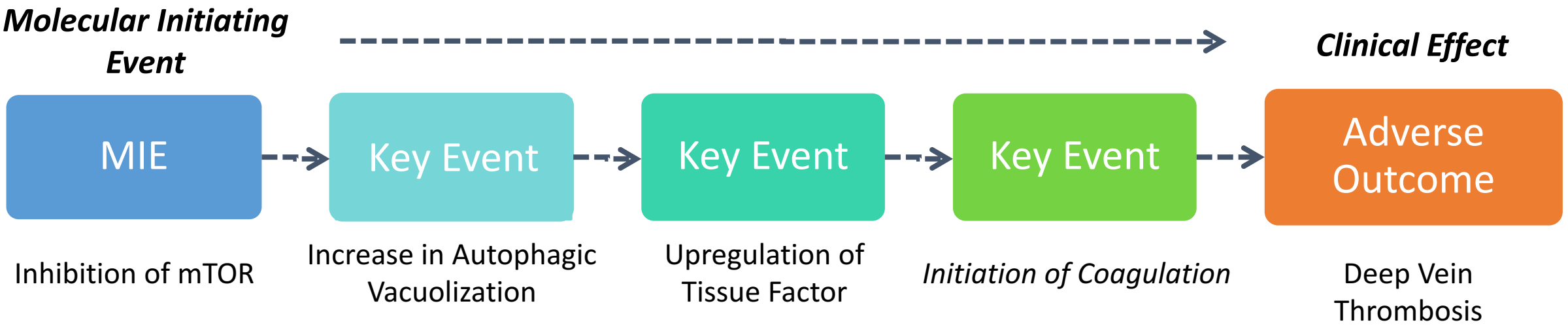
# Knowledge Discovery from Phenotypic Profiling

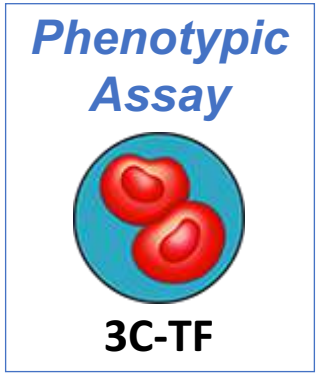
*Regulation of Thrombosis through Vascular Autophagy*




*Berg, et al., IJMS, 2015*

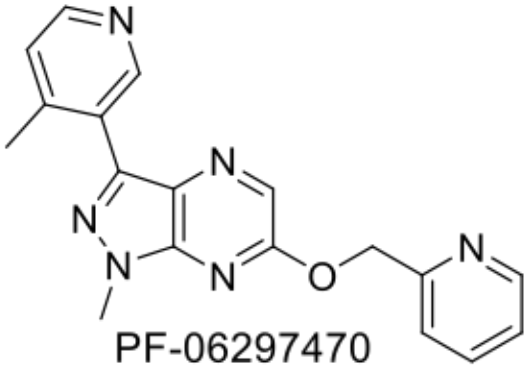
# An AOP Framework for Thrombosis

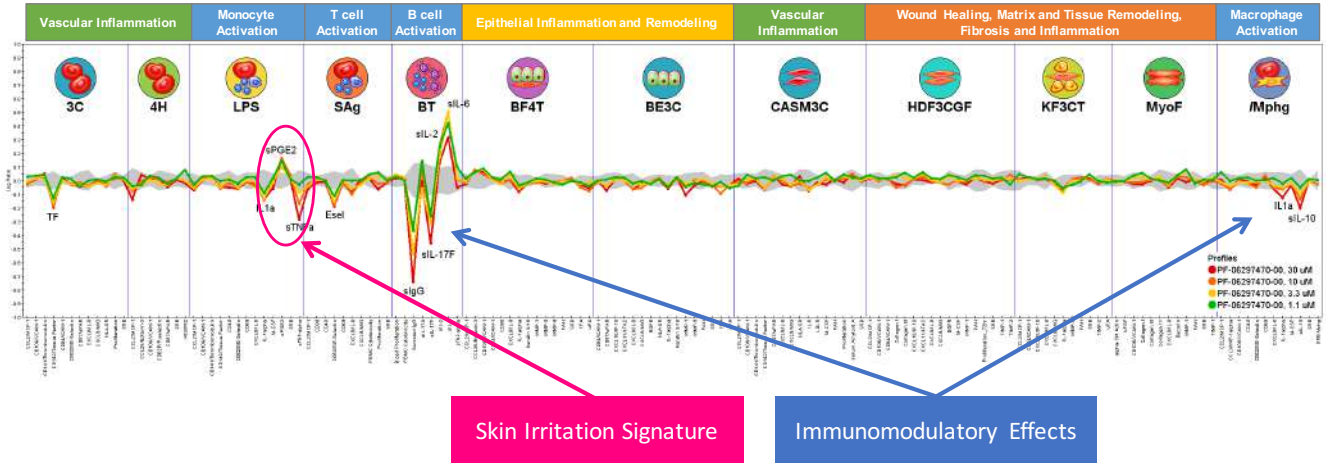


# Case Study – NHP Hypersensitivity

*Mechanism of Toxicity?*




- Pfizer mGluR5 NAM compound failed in preclinical due to skin lesions in a 90 day non-human primate (NHP) study
- Type IV hypersensitivity

- Unexpectedly active in human primary cell systems
- Toxicity Signature:  Skin irritation
- Novel immunomodulatory activities

# Case Study – NHP Hypersensitivity

*Skin Irritation Toxicity Signature*

## Adverse Effects


## Mechanism-based Toxicity Signatures

| | | | |
|---|---|---|---|
| **Reference Drugs** Cycloheximide, actinomycin D, digoxin, bortezomib, valinomycin<br>**Pathway/targets** Protein synthesis inhibition, RNA synthesis inhibition, $Na^+/K^+$ ATPase, proteasome inhibition<br>**Acute Tox** | **Reference Drugs** Sirolimus, Remicade, Cyclosporine, Tacrilomus, Mycophenolate, Azathioprine<br>**Pathway/targets** mTOR, calcineurin, Jak, hsp90, NFAT, DNA proliferation<br>**Immunosuppression** | **Reference Drugs** Retinoic Acid, Retinol, Vitamin D, Ritonavir, Imatinib, 2-chloroethyl ethyl sulfide, Calcitriol<br>**Pathway/targets** RAR/RXR, AhR, VDR, PKC<br>**Irritation** | **Reference Drugs** Amiodarone, Tamoxifen, Astemizole, Ketoconazole, Haloperidol, Aplaviroc<br>**Pathway/targets** V-ATPase, PIKfyve, Smoothened<br>**Liver Tox** |
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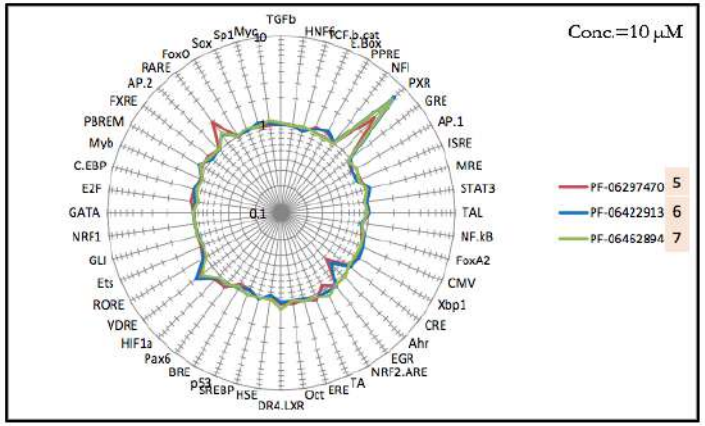
- Skin irritation is one of 8 toxicity signatures developed
- Pathway / targets associated with the signature for skin irritation
  - RAR/RXR, AhR, VDR, PKC

# Case Study – NHP Hypersensitivity

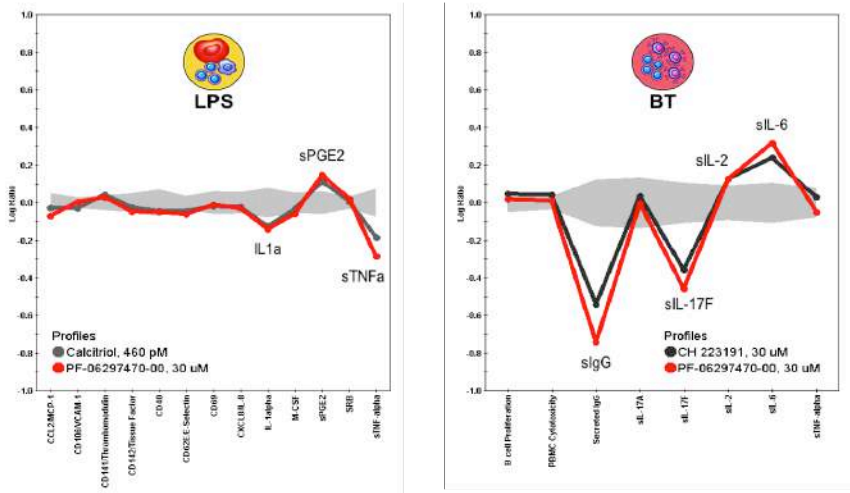
*Mechanism Confirmation*

## Transcription Factor Profiling




**5 & 6** showed downregulation of AhRE (>5-fold)
**5, 6, 7** showed up-regulation of RARE & VDRE (1.5-2 fold).

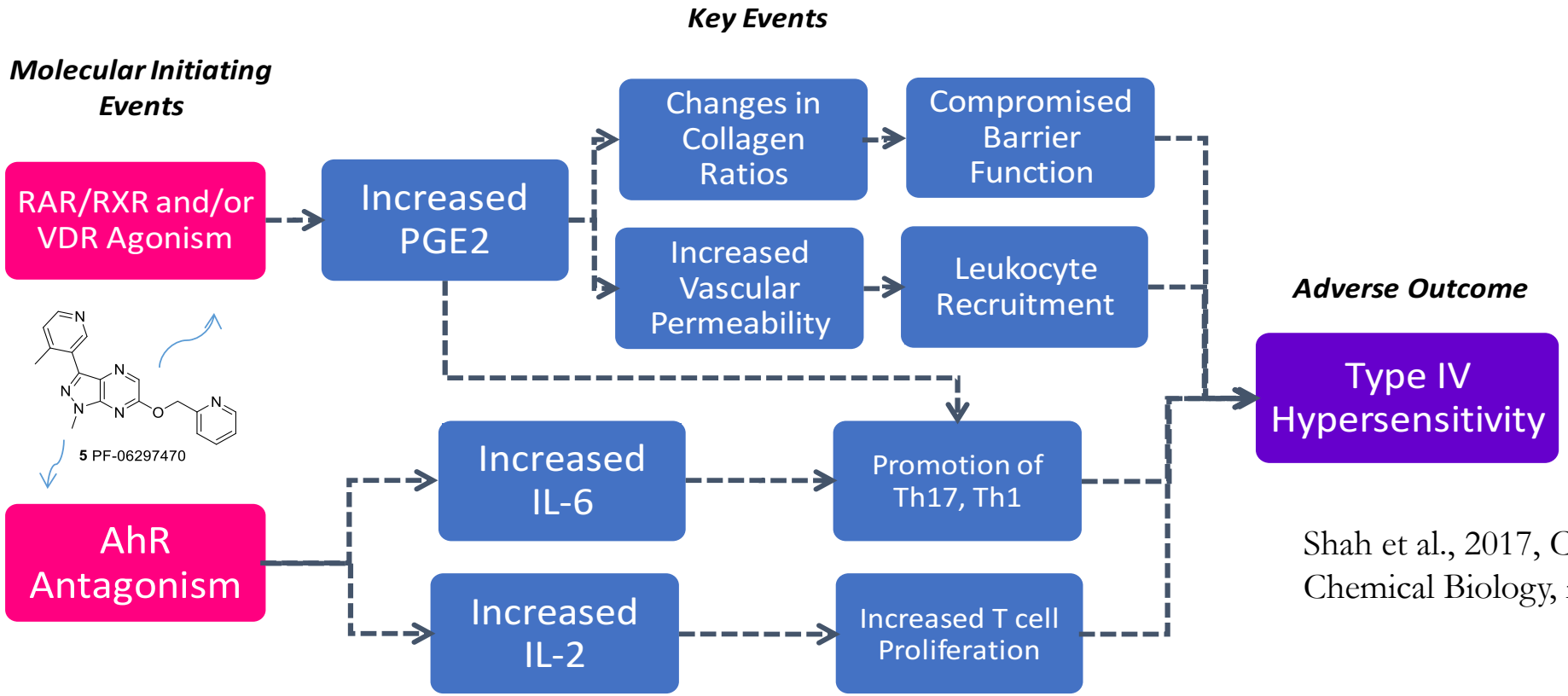
## Similarity Analysis




- Transcription Factor Profiling
  - Downregulation of AhRE (5-fold)
  - Upregulation of RARE & VDRE (1.5-2-fold)
- Custom BioMAP Similarity Analysis
  - LPS system: Calcitriol, VDR agonist
  - BT system: CH 223191, AhR antagonist

# Proposed AOP for Hypersensitivity

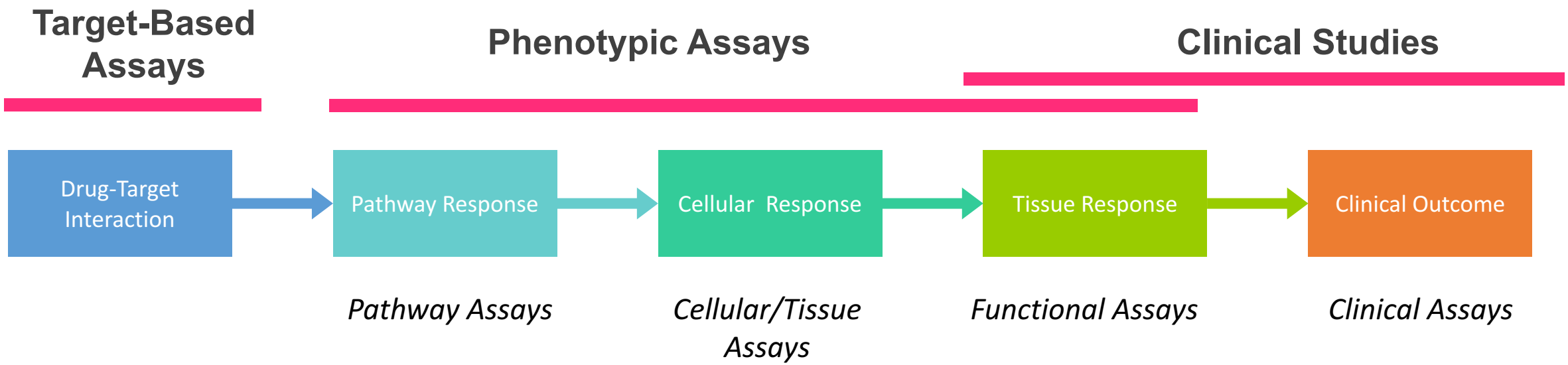
*Supports the emerging role of AhR in controlling immune responses*




Shah et al., 2017, Cell Chemical Biology, in press.

There is an emerging appreciation of the role of AhR in controlling immune responses, in particular for regulatory T cells that control allergic responses (Tousa, 2017, PMID: 28320933).

# Generalized Framework for Human Outcome Pathways




Map Assays & Data into Knowledge Framework

Use Data to Build Predictive Models

Use Predictive Models to Support Program Assessments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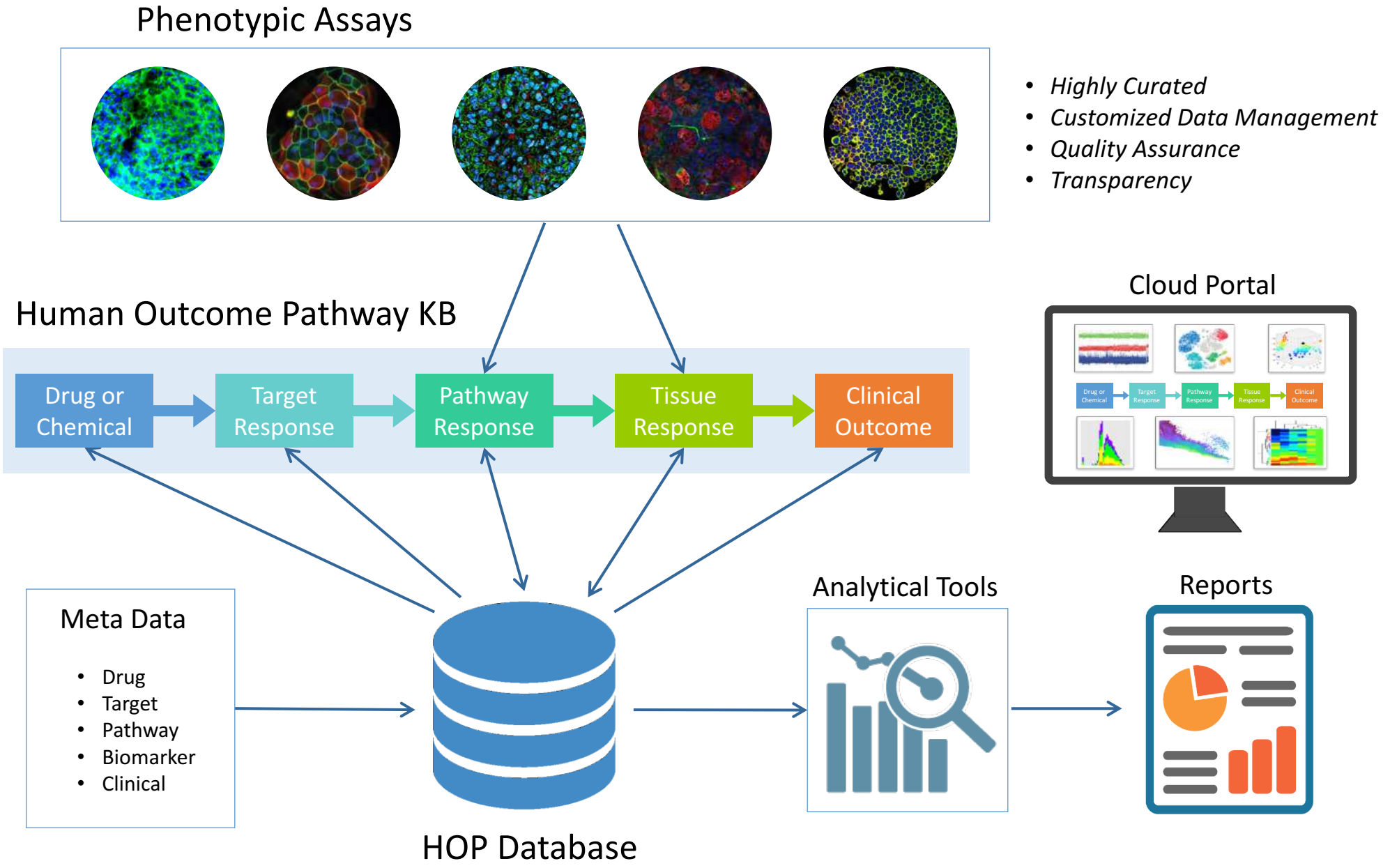
# Conceptual Framework



# Summary

- Human-based phenotypic profiling provides an opportunity for preclinical innovation
  - Use of phenotypic data can drive advancement of disease understanding, drug mechanisms
- What is needed?
  - Open science knowledge framework of human outcome pathways
  - Support for validation
    - Public/private effort
  - Clinical data
    - Data to support *in vitro* assay validation
      - Pharmacokinetics, exposure, biomarker responses
    - Data to support knowledge advances
      - Results from failed clinical trials

# American Society for Cellular and Computational Toxicology

## 6th Annual Meeting

## September 21-22, 2017

**Institute for In Vitro Science Conference Center**
**Gaithersburg, MD**

## Themed Sessions on:

- TSCA Reform
- Research Policy and Advances in Acute Toxicology

**Visit http://bit.ly/ASCCT-2017 for more information**

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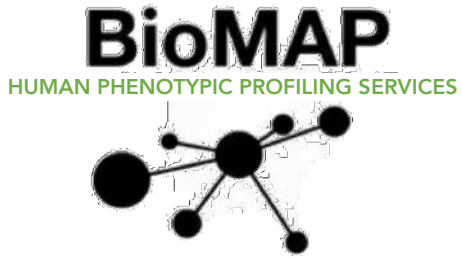


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