DiscoverX

BioMAP®

Diversity PLUS Data Report

Prepared For

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Contents

Introduction of the Study

The goal of study DTM004-01-b-00001-000-00 was to characterize 1 test agent (DTM_JA-1-58; Table 1) in the BioMAP Diversity PLUS panel of 12 human primary cell-based systems (Table 2). These systems are designed to model complex human tissue and disease biology of the vasculature, skin, lung and inflammatory tissues. Quantitative measurements of biomarker activities across this broad panel, along with comparative analysis of the biological activities of known bioactive agents in the BioMAP reference database are used to predict the safety, efficacy and function of these test agents.

BioMAP Technology Platform

BioMAP panels consist of human primary cell-based systems designed to model different aspects of the human body in an *in vitro* format.[1; 2; 3; 4; 5; 6; 7] The 12 systems in the Diversity PLUS panel (Table 2) allow test agent characterization in an unbiased way across a broad set of systems modeling various human disease states. BioMAP systems are constructed with one or more primary cell types from healthy human donors, with stimuli (such as cytokines or growth factors) added to capture relevant signaling networks that naturally occur in human tissue or pathological conditions. Vascular biology is modeled in both a Th1 (**3C system**) and a Th2 (**4H system**) inflammatory environment, as well as in a Th1 inflammatory state specific to arterial smooth muscle cells (**CASM3C system**). Additional systems recapitulate aspects of the systemic immune response including monocyte-driven Th1 inflammation (**LPS system**) or T cell stimulation (**SAg system**), chronic Th1 inflammation driven by macrophage activation (**/Mphg system**) and the T cell-dependent activation of B cells that occurs in germinal centers (**BT system**). The **BE3C system** (Th1) and the **BF4T system** (Th2) represent airway inflammation of the lung, while the **MyoF system** models myofibroblast-lung tissue remodeling. Lastly, skin biology is addressed in the **KF3CT system** modeling Th1 cutaneous inflammation and the **HDF3CGF system** modeling wound healing.[1; 2] A subset of 8 of these BioMAP systems has previously been used in the U.S. Environmental Protection Agency (EPA)'s ToxCast™ program to characterize environmental chemicals, define mechanisms of toxicity and to develop predictive signatures of toxicity.[1; 8; 9]

Each test agent generates a signature BioMAP profile that is created from the changes in protein biomarker readouts within individual system environments. Biomarker readouts (7 - 17 per system) are selected for therapeutic and biological relevance, are predictive for disease outcomes or specific drug effects and are validated using agents with known mechanism of action (MoA).[1; 2; 3; 4; 5; 6; 7] Each readout is measured quantitatively by immune-based methods that detect protein (e.g., ELISA) or functional assays that measure proliferation and viability. BioMAP readouts are diverse and include cell surface receptors, cytokines, chemokines, matrix molecules and enzymes. In total, the Diversity PLUS panel contains 148 biomarker readouts that capture biological changes that occur within the physiological context of the particular BioMAP system.

Using custom-designed software containing data mining tools, a BioMAP profile can be compared against a proprietary reference database of > 4,000 BioMAP profiles of bioactive agents (biologics, approved drugs, chemicals and experimental agents) to classify and identify the most similar profiles. This robust data platform allows rapid evaluation and interpretation of BioMAP profiles by performing the unbiased mathematical identification of similar activities. Specific BioMAP activities have been correlated to *in vivo* biology[10; 11; 12], and multiparameter BioMAP profiles have been used to distinguish compounds based on MoA and target selectivity[8; 11] and can provide a predictive signature for *in vivo* toxicological outcomes (e.g., vascular toxicity, developmental toxicity, etc.) across diverse physiological systems.[8; 9; 13]

Diversity PLUS Deliverables

The Diversity PLUS project deliverables include a Profile plot which is an overlay of the BioMAP signature of four concentrations of a particular test agent. Significant biomarker readouts are annotated, and these key activities are classified and listed into biologically relevant categories. Profile plots can identify dose-dependent, cytotoxic, antiproliferative and potential off-target secondary effects. The Profile plot is followed by an overlay of one concentration of the test agent with one concentration of a selected Reference Benchmark. In this comparison, common or differentiating biomarker activities are annotated and listed by system, along with a description of the Reference Benchmark agent. Next, the test agent is mathematically compared against the BioMAP Reference Database to identify the top 3 agents with the most similar overall biomarker signature across the 12 systems. The top match is overlayed against the test agent with annotation of common biomarker activities, description of the similarity search result and the mathematical scores comparing the two profiles. If there are 3 or more test agents, a cluster analysis is performed using pairwise correlation analysis where test agents with similar profiles are graphically linked by lines. Lastly, test agents and consensus profiles for 19 predictive models that are based on profiles from multiple compounds in a known mechanism class are displayed in a Mechanism HeatMAP Analysis.[8]

Test Agents Profiled in BioMAP Diversity PLUS

	Test Agent Name	Concentration	Agent Type	Requested Benchmark
1	DTM_JA-1-58	30, 10, 3.3, 1.1 µM	Small Molecule	2-Methoxyestradiol

Table 1. Test Agents. Table containing the information for test agents profiled in the BioMAP Diversity PLUS Panel as indicated on the Compound Shipping Table (CST) supplied by Dartmouth University.

Table of Systems in BioMAP Diversity PLUS

System Name	Icon	Cell	Disease	Readouts
3C		Venular endothelial cells	Cardiovascular Disease, Chronic Inflammation	CCL2/MCP-1, CD106/VCAM-1, CD141/Thrombomodulin, CD142/Tissue Factor, CD54/ICAM-1, CD62E/E-Selectin, CD87/uPAR, CXCL8/IL-8, CXCL9/MIG, HLA-DR, Proliferation, SRB
4H		Venular endothelial cells	Autoimmunity, Allergy, Asthma	CCL26/Eotaxin-3, CCL2/MCP-1, CD106/VCAM-1, CD62P/P-Selectin, CD87/uPAR, SRB, VEGFR2
LPS		Venular endothelial cells, Peripheral blood mononuclear cells	Chronic Inflammation, Cardiovascular Disease	CCL2/MCP-1, CD106/VCAM-1, CD141/Thrombomodulin, CD142/Tissue Factor, CD40, CD62E/E-Selectin, CD69, CXCL8/IL-8, IL-1 alpha, M-CSF, sPGE2, SRB, sTNF-alpha
SAg		Venular endothelial cells, Peripheral blood mononuclear cells	Chronic Inflammation, Autoimmune Disease	CCL2/MCP-1, CD38, CD40, CD62E/E-Selectin, CD69, CXCL8/IL-8, CXCL9/MIG, PBMC Cytotoxicity, Proliferation, SRB
BT		Peripheral blood mononuclear cells, B cells	Autoimmunity, Oncology, Allergy, Asthma	B cell Proliferation, PBMC Cytotoxicity, Secreted IgG, sIL-17A, sIL-17F, sIL-2, sIL-6, sTNF-alpha
BF4T		Bronchial epithelial cells, Dermal fibroblasts	Fibrosis, Lung Inflammation, Allergy, Asthma	CCL26/Eotaxin-3, CCL2/MCP-1, CD106/VCAM-1, CD54/ICAM-1, CD90, CXCL8/IL-8, IL-1 alpha, Keratin 8/18, MMP-1, MMP-3, MMP-9, PAI-I, SRB, tPA, uPA
BE3C		Bronchial epithelial cells	COPD, Lung Inflammation	CD54/ICAM-1, CD87/uPAR, CXCL10/IP-10, CXCL11/I-TAC, CXCL8/IL-8, CXCL9/MIG, EGFR, HLA-DR, IL-1 alpha, Keratin 8/18, MMP-1, MMP-9, PAI-I, SRB, tPA, uPA
CASM3C		Coronary artery smooth muscle cells	Restenosis, Cardiovascular Inflammation	CCL2/MCP-1, CD106/VCAM-1, CD141/Thrombomodulin, CD142/Tissue Factor, CD87/uPAR, CXCL8/IL-8, CXCL9/MIG, HLA-DR, IL-6, LDLR, M-CSF, PAI-I, Proliferation, Serum Amyloid A, SRB
HDF3CGF		Dermal fibroblasts	Chronic Inflammation, Fibrosis	CCL2/MCP-1, CD106/VCAM-1, CD54/ICAM-1, Collagen I, Collagen III, CXCL10/IP-10, CXCL11/I-TAC, CXCL8/IL-8, CXCL9/MIG, EGFR, M-CSF, MMP-1, PAI-I, Proliferation_72hr, SRB, TIMP-1, TIMP-2
KF3CT		Keratinocytes, Dermal fibroblasts	Dermatitis, Psoriasis	CCL2/MCP-1, CD54/ICAM-1, CXCL10/IP-10, CXCL8/IL-8, CXCL9/MIG, IL-1 alpha, MMP-9, PAI-I, SRB, TIMP-2, uPA
MyoF		Lung fibroblasts	Wound Healing, Fibrosis, Chronic Inflammation, Matrix Remodeling	alpha-SM Actin, bFGF, CD106/VCAM-1, Collagen I, Collagen III, Collagen IV, CXCL8/IL-8, Decorin, MMP-1, PAI-I, SRB, TIMP-1
/Mphg		Macrophages, Venular endothelial cells	Restenosis, Cardiovascular Disease, Chronic Inflammation	CCL2/MCP-1, CCL3/MIP-1 alpha, CD106/VCAM-1, CD40, CD62E/E-Selectin, CD69, CXCL8/IL-8, IL-1 alpha, M-CSF, sIL-10, SRB, SRB-Mphg

Table 2. Table of the 12 systems in the BioMAP Diversity PLUS Panel, including a list of the cell types, disease context and list of biomarker readouts optimized for each system. Biomarker end point measurements are cell-surface levels unless indicated by the prefix “s” which denotes soluble levels of biomarker endpoints measured in the system supernatant. Additional information on the materials and methods, and on the biomarker readouts in Diversity PLUS can be found in Appendices A and C respectively.

Analysis of Test Agent 1: DTM_JA-1-58

BioMAP Profile

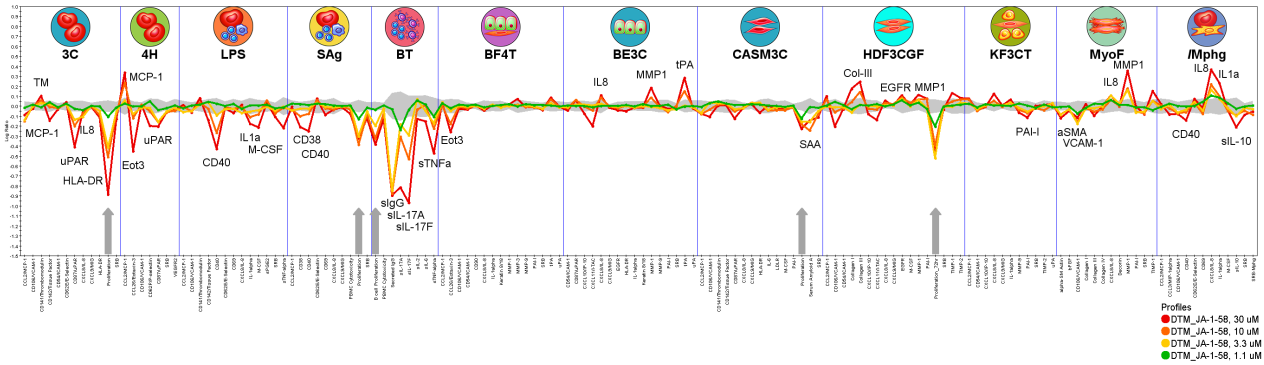


Figure 1. BioMAP profile of DTM_JA-1-58 in the Diversity PLUS Panel. The X-axis lists the quantitative protein-based biomarker readouts measured in each system. The Y-axis represents a log-transformed ratio of the biomarker readouts for the drug-treated sample (n = 1) over vehicle controls (n ≥ 6). The grey region around the Y-axis represents the 95% significance envelope generated from historical vehicle controls. Biomarker activities are annotated when 2 or more consecutive concentrations change in the same direction relative to vehicle controls, are outside of the significance envelope, and have at least one concentration with an effect size > 20% ($|\log_{10} ratio| > 0.1$). Biomarker key activities are described as modulated if these activities increase in some systems, but decrease in others. Cytotoxicity is indicated on the profile plot by a thin black arrow above the X-axis, and antiproliferative effects are indicated by a thick grey arrow. Cytotoxicity and antiproliferative arrows only require one concentration to meet the indicated threshold for profile annotation. Additional information can be found in Appendix A.

Biological and Disease Relevance Category	Decreased activity	Increased activity	Modulated activity
Inflammation-related activities	Eot3, VCAM-1, SAA, sTNFa		MCP-1, IL8, IL1a
Immunomodulatory activities	CD38, CD40, HLA-DR, M-CSF, sIgG, sIL-10, sIL-17A, sIL-17F		
Tissue remodeling activities	aSMA, uPAR, PAI-I	Col-III, EGFR, MMP1, tPA	
Hemostasis-related activities		TM	

DTM_JA-1-58 is active with 34 annotated readouts. DTM_JA-1-58 is not cytotoxic at the concentrations tested in this study. DTM_JA-1-58 is antiproliferative to human primary B cells (30 µM, 10 µM, 3.3 µM), T cells (30 µM, 10 µM, 3.3 µM, 1.1 µM), coronary artery smooth muscle cells (30 µM, 10 µM, 3.3 µM, 1.1 µM), endothelial cells (30 µM, 10 µM, 3.3 µM, 1.1 µM), and fibroblasts (30 µM, 10 µM, 3.3 µM, 1.1 µM) (grey arrows).

DTM_JA-1-58 mediated changes in key biomarker activities are listed by biological and disease classifications. DTM_JA-1-58 impacts inflammation-related activities (decreased Eotaxin 3, SAA, VCAM-1, sTNFα; modulated MCP-1, IL-8, IL-1α), immunomodulatory activities (decreased CD40, sIgG, sIL-10, M-CSF, HLA-DR, sIL-17A, CD38, sIL-17F), tissue remodeling activities (decreased PAI-1, uPAR, αSMA; increased tPA, MMP-1, Collagen III, EGFR), and hemostasis-related activities (increased TM).

Reference Benchmark Overlay

DTM_JA-1-58 and 2-Methoxyestradiol

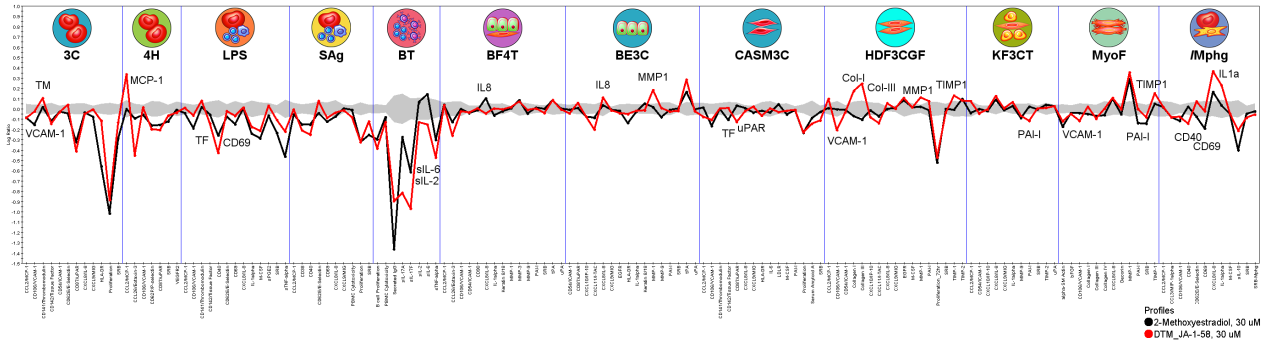


Figure 2. Reference Benchmark Overlay: DTM_JA-1-58 and Benchmark 2-Methoxyestradiol. Overlay of the test agent and a nominated compound. *Common* biomarker readouts are annotated when the readout for both profiles is outside of the significance envelope with an effect size > 20% ($|\log_{10} ratio| > 0.1$) in the same direction. Additional information can be found in Appendix A.

Overlay of DTM_JA-1-58 at 30 µM and the selected reference benchmark 2-methoxyestradiol at 30 µM. 2-Methoxyestradiol is an endogenous estradiol metabolite with potential antineoplastic activity. 2-Methoxyestradiol inhibits angiogenesis via the HIF-1α pathway and reduces endothelial cell proliferation and induces endothelial cell apoptosis. 2-Methoxyestradiol is an inhibitor of microtubule assembly and the polymerization of tubulin, leading to a block in mitosis.

There are 30 common activities that are annotated within the following systems: 3C (TF, uPAR, HLA-DR, Prolif, SRB), 4H (P-selectin, uPAR), LPS (CD40, IL-1α, M-CSF, SRB, sTNFα), SAg (CD38, CD40, Prolif, SRB), BT (Prolif, sIgG, sIL-17A, sIL-17F, sTNFα), BF4T (Eotaxin 3), BE3C (tPA), CASM3C (VCAM-1, Prolif), HDF3CGF (Prolif 72), MyoF (αSMA, MMP-1), and lMphg (IL-8, sIL-10).

There are 24 differentiating activities (not shown) within the following systems: 3C (VCAM-1, TM), 4H (MCP-1), LPS (TF, CD69), BT (sIL-2, sIL-6), BF4T (IL-8), BE3C (IL-8, MMP-1), CASM3C (TF, uPAR), HDF3CGF (VCAM-1, Collagen I, Collagen III, MMP-1, TIMP-1), KF3CT (PAI-1), MyoF (VCAM-1, PAI-1, TIMP-1), and lMphg (CD40, CD69, IL-1α). *Differentiating* biomarkers are defined when one profile has a readout outside of the significance envelope with an effect size > 20% ($|\log_{10} ratio| > 0.1$), and the readout for the other profile is either inside the envelope or in the opposite direction.

Top Database Search Result for DTM_JA-1-58

17β-estradiol (90 μM)

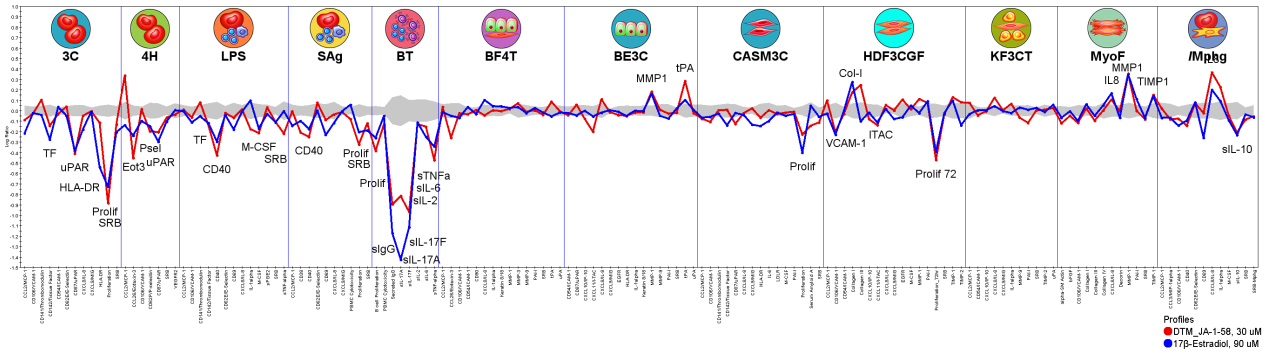


Figure 3. Top Database Search Result for DTM_JA-1-58 (30 μM) is 17β-estradiol (90 μM). Overlay of the top similarity match from an unsupervised search of the BioMAP Reference Database of > 4,000 agents with DTM_JA-1-58. *Common* biomarker readouts are annotated when the readout for both profiles is outside of the significance envelope with an effect size > 20% ($|\log_{10} ratio| > 0.1$) in the same direction. Similarity search results are filtered and ranked as described in Appendix A. Profiles are identified as having mechanistically relevant similarity if the Pearson's correlation coefficient is ≥ 0.7. Additional information can be found in Appendix A.

In our unsupervised search for mathematically similar compound profiles from the BioMAP Reference Database, DTM_JA-1-58 (30 μM) is most similar to 17β-estradiol (90 μM) (Pearson's correlation coefficient, $r = 0.830$). The Pearson's correlation coefficient between these two profiles is above our determined threshold ($r \geq 0.7$) indicating these compounds share mechanistically relevant similarity. 17β-estradiol (Estradiol) is a human sex hormone and steroid. 17β-estradiol is the primary sex hormone in females and is important in the regulation of the estrous and menstrual female reproductive cycles, the development and maintenance of female reproductive tissues and also has important effects in many other tissues including bone.

There are 34 common activities that are annotated within the following systems: 3C (TF, uPAR, HLA-DR, Prolif, SRB), 4H (Eotaxin 3, P-selectin, uPAR), LPS (TF, CD40, M-CSF, SRB), SAg (CD40, Prolif, SRB), BT (Prolif, sIgG, sIL-17A, sIL-17F, sIL-2, sIL-6, sTNFα), BE3C (MMP-1, tPA), CASM3C (Prolif), HDF3CGF (VCAM-1, Collagen I, I-TAC, Prolif 72), MyoF (IL-8, MMP-1, TIMP-1), and IMphg (IL-8, sIL-10).

Top BioMAP Reference Database Matches for DTM_JA-1-58

DTM_JA-1-58	Database Match	BioMAP Z-Standard	Pearson's Score	# of Common Readouts	Database Match (Mechanism Class)
30 µM	17β-Estradiol, 90 µM	14.308	0.830	148	ER Agonist
	Fosbretabulin Disodium, 120 nM	13.371	0.804	148	Microtubule Disruptor
	Fosbretabulin Disodium, 41 nM	13.162	0.798	148	Microtubule Disruptor
10 µM	2-Methoxyestradiol, 10 µM	14.739	0.841	148	Microtubule Disruptor
	2-Methoxyestradiol, 3.3 µM	14.485	0.835	148	Microtubule Disruptor
	2-Methoxyestradiol, 30 µM	14.425	0.833	148	Microtubule Disruptor
3.3 µM	2-Methoxyestradiol, 3.3 µM	18.486	0.911	148	Microtubule Disruptor
	Paclitaxel, 1.1 µM	17.726	0.900	148	Microtubule Stabilizer
	Paclitaxel, 370 nM	17.698	0.900	148	Microtubule Stabilizer
1.1 µM	Ro 106-9920, 1.1 µM	9.210	0.644	148	Ubiquitin Ligase Inhibitor
	Gemcitabine, 1.1 µM	9.159	0.641	148	DNA Polymerase Inhibitor
	Gemcitabine, 3.3 µM	9.150	0.641	148	DNA Polymerase Inhibitor

Figure 4. Top BioMAP Reference Database Matches for DTM_JA-1-58. A table of the top 3 similarity matches from an unsupervised search of the BioMAP Reference Database of > 4,000 agents for each concentration of test agent. The similarity between agents is determined using a combinatorial approach that accounts for the characteristics of BioMAP profiles by filtering (Tanimoto metric) and ranking (BioMAP Z-Standard) the Pearson's correlation coefficient between two profiles. Profiles are identified as having mechanistically relevant similarity if the Pearson's correlation coefficient is ≥ 0.7. Additional information can be found in Appendix A.

The Pearson's correlation coefficient between profiles that is above our determined threshold ($r \geq 0.7$) indicates these compounds share mechanistically relevant similarity. For profiles with a Pearson's correlation coefficient below our determined threshold ($r < 0.7$), the relevance of the similarity is unknown.

Mechanism HeatMAP Analysis of DTM_JA-1-58

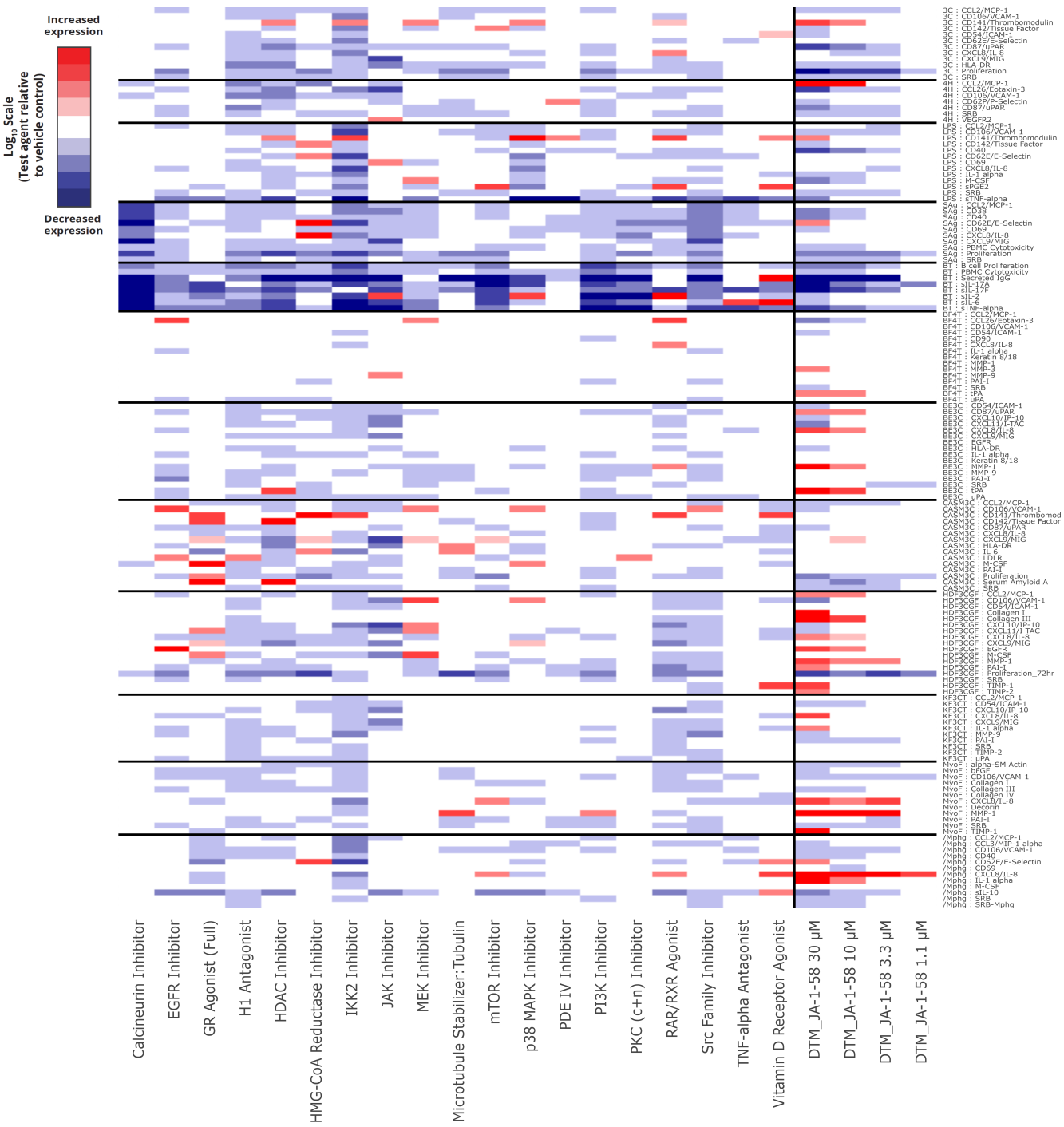


Figure 5. Mechanism HeatMAP Analysis for DTM_JA-1-58. HeatMAP analysis of the 148 biomarker readouts (rows) within the Diversity PLUS panel by DTM_JA-1-58 in comparison to 19 consensus mechanism class profiles (columns). Horizontal grey lines separate the 12 Diversity PLUS systems, while the vertical grey line separates DTM_JA-1-58 from the 19 consensus mechanism profiles. Biomarker activities outside of the significance envelope are red if protein levels are increased, blue if protein levels are decreased and white if levels are within the envelope or unchanged. Darker shades of color represent greater change in biomarker activity relative to vehicle control. These class profiles were generated from a large reference dataset as described in Appendix A. This Mechanism HeatMAP analysis informs on the regulatory mechanisms controlling increases or decreases in each of the biomarker readouts. For example, the increase in IL-8 in the /Mphg System is a feature of mTOR inhibitors, RAR/RXR agonists and VDR agonists.

Summary of Project Profiles

Test Agent	Cytotoxic System (Concentration)	Antiproliferative Effects (Concentration)	# of Annotated Biomarkers	Database Match	Database Match Mechanism Class	Pearson's r ≥ 0.7
DTM_JA-1-58	None	B cells (30 µM, 10 µM, 3.3 µM) Coronary artery smooth muscle cells (30 µM, 10 µM, 3.3 µM, 1.1 µM) Endothelial cells (30 µM, 10 µM, 3.3 µM, 1.1 µM) Fibroblasts (30 µM, 10 µM, 3.3 µM, 1.1 µM) T cells (30 µM, 10 µM, 3.3 µM, 1.1 µM)	34	17β-Estradiol	ER Agonist	Yes

Figure 6. Test Agent Summary for DTM004-01-b-00001-000-00. Table summarizing conditions with detectable cytotoxicity, the number of annotated biomarkers in the Profile plots, the Database Match for the Similarity Search of the top non-overtly cytotoxic concentration and the Mechanism Class for this Database Match. Similarity matches with a Pearson's correlation coefficient above our determined threshold ($r \geq 0.7$) share mechanistically relevant similarity. Please note, for profiles below our determined threshold ($r < 0.7$), the relevance of the similarity is unknown.

In study DTM004-01-b-00001-000-00, test agent DTM_JA-1-58 was characterized by profiling in the BioMAP Diversity PLUS panel of human primary cell based assays modeling complex tissue and disease biology of organs (vasculature, immune system, skin, lung) and general tissue biology. The Diversity PLUS panel evaluates the biological impact of test agents in conditions that preserve the complex crosstalk and feedback mechanisms that are relevant to *in vivo* outcomes.

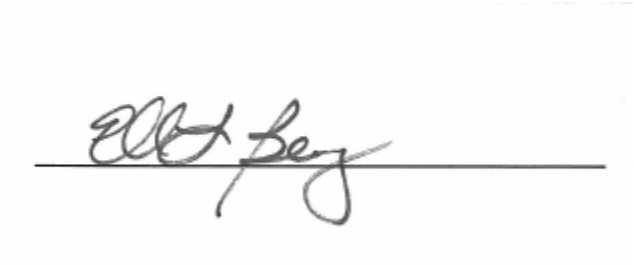
DTM_JA-1-58 was active and noncytotoxic in the BioMAP Diversity PLUS panel, with antiproliferative effects on B cells, T cells, coronary artery smooth muscle cells, endothelial cells, and fibroblasts. With 34 annotated readouts, DTM_JA-1-58 impacted inflammation-related activities (Eotaxin 3, SAA, VCAM-1, sTNFα, MCP-1, IL-8, IL-1α), immunomodulatory activities (CD40, sIgG, sIL-10, M-CSF, HLA-DR, sIL-17A, CD38, sIL-17F), tissue remodeling activities (PAI-1, uPAR, αSMA, tPA, MMP-1, Collagen III, EGFR), and hemostasis-related activities (TM). Comparative overlay of DTM_JA-1-58 and selected reference benchmark 2-methoxyestradiol revealed 30 common and 24 differentiating activities across the Diversity PLUS panel. The top database search result for DTM_JA-1-58 was 17β-estradiol, an ER agonist, with a Pearson's correlation coefficient (r=0.830) that is above the threshold for mechanistic similarity. 17β-estradiol is known to block microtubule function at high concentrations (Houck, 2009; PMID: 19773588). Other reference benchmarks with high similarity to DTM_JA-1-58 include the microtubule inhibitors fosbretabulin, 2-methoxyestradiol and paclitaxel.

Next Steps. The antiproliferative and tissue remodeling effects of DTM_JA-1-58 in multiple cell types suggest potential application of this agent in oncology. To pursue this further we recommend testing in the BioMAP CRC or NSCLC Oncology panel to evaluate their impact in the BioMAP host tissue-immune-tumor microenvironment models. These models recapitulate the immune suppressed biology associated with the intratumoral milieu targeted by agents in the immune oncology (IO) indication space.

Signature Page

Project Scientist and Study Author

I certify that this written report for BioMAP Diversity PLUS study DTM004-01-b-00001-000-00 has been reviewed and is complete.



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Appendix A

Methods for Diversity PLUS

Human primary cells in BioMAP systems are used at early passage (passage 4 or earlier) to minimize adaptation to cell culture conditions and preserve physiological signaling responses. All cells are from a pool of multiple donors (n = 2 – 6), commercially purchased and handled according to the recommendations of the manufacturers. Human blood derived $CD14^+$ monocytes are differentiated into macrophages *in vitro* before being added to the ***I*Mphg system**. Abbreviations are used as follows: Human umbilical vein endothelial cells (HUVEC), Peripheral blood mononuclear cells (PBMC), Human neonatal dermal fibroblasts (HDFn), B cell receptor (BCR), T cell receptor (TCR) and Toll-like receptor (TLR).

Cell types and stimuli used in each system are as follows: **3C system** [HUVEC + *(IL-1β, TNFα and IFNγ)*], **4H system** [HUVEC + *(IL-4 and histamine)*], **LPS system** [PBMC and HUVEC + *LPS (TLR4 ligand)*], **SAg system** [PBMC and HUVEC + *TCR ligands*], **BT system** [$CD19^+$ B cells and PBMC + *(α-IgM and TCR ligands)*], **BF4T system** [bronchial epithelial cells and HDFn + *(TNFα and IL-4)*], **BE3C system** [bronchial epithelial cells + *(IL-1β, TNFα and IFNγ)*], **CASM3C system** [coronary artery smooth muscle cells + *(IL-1β, TNFα and IFNγ)*], **HDF3CGF system** [HDFn + *(IL-1β, TNFα, IFNγ, EGF, bFGF and PDGF-BB)*], **KF3CT system** [keratinocytes and HDFn + *(IL-1β, TNFα, IFNγ and TGFβ)*], **MyoF system** [differentiated lung myofibroblasts + *(TNFα and TGFβ)*] and ***I*Mphg system** [HUVEC and M1 macrophages + *Zymosan (TLR2 ligand)*].

Systems are derived from either single cell types or co-culture systems. Adherent cell types are cultured in 96 or 384-well plates until confluence, followed by the addition of PBMC (**SAg** and **LPS systems**). The **BT system** consists of $CD19^+$ B cells co-cultured with PBMC and stimulated with a BCR activator and low levels of TCR stimulation. Test agents prepared in either DMSO (small molecules; final concentration ≤ 0.1%) or PBS (biologics) are added at the indicated concentrations 1-hr before stimulation, and remain in culture for 24-hrs or as otherwise indicated (48-hrs, MyoF system; 72-hrs, BT system (soluble readouts); 168-hrs, BT system (secreted IgG)). Each plate contains drug controls (e.g., legacy control test agent colchicine at 1.1 μM), negative controls (e.g., non-stimulated conditions) and vehicle controls (e.g., 0.1% DMSO) appropriate for each system. Direct ELISA is used to measure biomarker levels of cell-associated and cell membrane targets. Soluble factors from supernatants are quantified using either HTRF® detection, bead-based multiplex immunoassay or capture ELISA. Overt adverse effects of test agents on cell proliferation and viability (cytotoxicity) are detected by sulforhodamine B (SRB) staining, for adherent cells, and alamarBlue® reduction for cells in suspension. For proliferation assays, individual cell types are cultured at subconfluence and measured at time points optimized for each system (48-hrs: 3C and CASM3C systems; 72-hrs: BT and HDF3CGF systems; 96-hrs: SAg system). Cytotoxicity for adherent cells is measured by SRB (24-hrs: 3C, 4H, LPS, SAg, BF4T, BE3C, CASM3C, HDF3CGF, KF3CT, and *I*Mphg systems; 48-hrs: MyoF system), and by alamarBlue staining for cells in suspension (24-hrs: SAg system; 42-hrs: BT system) at the time points indicated. Additional information can be found in previous descriptions.[1; 2; 5; 6; 7; 13]

Data Analysis

Biomarker measurements in a test agent-treated sample are divided by the average of control samples (at least 6 vehicle controls from the same plate) to generate a ratio that is then $\log_{10}$ transformed. Significance prediction envelopes are calculated using historical vehicle control data at a 95% confidence interval.

Profile Analysis. Biomarker activities are annotated when 2 or more consecutive concentrations change in the same direction relative to vehicle controls, are outside of the significance envelope and have at least one concentration with an effect size > 20% ($|\log_{10}$ *ratio*$|$ > 0.1). Biomarker key activities are described as modulated if these activities increase in some systems, but decrease in others. Cytotoxic conditions are noted when total protein levels decrease by more than 50% ($\log_{10}$ *ratio* of SRB or alamarBlue levels < -0.3) and are indicated by a thin black arrow above the X-axis. A compound is considered to have broad cytotoxicity when cytotoxicity is detected in 3 or more systems. Concentrations of test agents with detectable broad cytotoxicity are excluded from biomarker activity annotation and downstream benchmarking, similarity search and cluster analysis. Antiproliferative effects are defined by an SRB or alamarBlue $\log_{10}$ *ratio* value < -0.1 from cells plated at a lower density and are indicated by grey arrows above the X-axis. Cytotoxicity and antiproliferative arrows only require one concentration to meet the indicated threshold for profile annotation.

Benchmark Analysis. *Common* biomarker readouts are annotated when the readout for both profiles is outside of the significance envelope with an effect size > 20% in the same direction. *Differentiating* biomarkers are annotated when one profile has a readout outside of the significance envelope with an effect size > 20%, and the readout for the other profile is either inside the envelope or in the opposite direction. Unless specified, the top non-cytotoxic concentration of both the test agent and benchmark agent are included in the benchmark overlay analysis.

Similarity Analysis. *Common* biomarker readouts are annotated when the readout for both profiles is outside of the significance envelope with an effect size > 20% in the same direction. Concentrations of test agents that have 3 or more detectable systems with cytotoxicity are excluded from similarity analysis. Concentrations of test agents that have 1 – 2 systems with detectable cytotoxicity will be included

in the similarity search analysis, along with an overlay of the database match with the top concentration of the test agent. This will be followed by an additional overlay of the next highest concentration of the test agent containing no systems with detectable cytotoxicity and the respective database match. To determine the extent of similarity between BioMAP profiles of compounds run in the Diversity PLUS panel, we have developed a custom similarity metric (BioMAP Z-Standard) that is a combinatorial approach that has improved performance in mechanism classification of reference agents compared to other measures tested (including Pearson's and Spearman's correlation coefficients). This approach more effectively accounts for variations in the number of data points, systems, active biomarker readouts and the amplitude of biomarker readout changes that are characteristic features of BioMAP profiles. A Pearson's correlation coefficient (*r*) is first generated to measure the linear association between two profiles that is based on the similarity in the direction and magnitude of the relationship. Since the Pearson's correlation can be influenced by the magnitude of any biomarker activity, a per-system weighted average Tanimoto metric is used as a filter to account for underrepresentation of less robust systems. The Tanimoto metric does not consider the amplitude of biomarker activity, but addresses whether the identity and number of readouts are in common on a weighted, per system basis. A real-value Tanimoto metric is calculated first by normalizing each profile to the unit vector (e.g., $A = \frac{A}{||A||}$) and then applying the following formula: $\frac{A \cdot B}{||A|| + ||B|| - A \cdot B}$, where A and B are the 2 profile vectors. Then, it is incorporated into a system weighted-averaged real-value Tanimoto metric in this calculation: $= \frac{\sum W_i \cdot T_i}{\sum W_i}$. The calculation uses the real-value Tanimoto score for each *i*th system (T_i) and the weight of each *i*th system (W_i). W_i is calculated for each system in the following formula: $\frac{1}{1+e^{-}100*(lr-0.09)}$, where *lr* is the largest absolute value of the ratios from the 2 profiles being compared. Based on the optimal performance of reference compounds, profiles are identified as having mechanistically relevant similarity if the Pearson's correlation coefficient (*r*) ≥ 0.7.[4] Finally, a Fisher *r*-to-z-transformation is used to calculate a z-score to convert a short tail distribution into a normal distribution as follows: $z = 0.5 \log_{10} \frac{1+r}{1-r}$. Then the BioMAP Z-Standard, which adjusts for the number of common readouts (CR), is generated according to the following formula: Z-Standard = $z \cdot \sqrt{CR - 3}$. A larger BioMAP Z-Standard value corresponds to a higher confidence level, and this is the metric used to rank similarity results.

Cluster Analysis. Cluster analysis (function similarity map) uses the results of pairwise correlation analysis to project the "proximity" of agent profiles from multi-dimensional space into two dimensions. Functional clustering of the agent profiles generated during this analysis uses Pearson correlation values for pairwise comparisons of the profiles for each agent at each concentration, and then subjects the pairwise correlation data to multidimensional scaling. Profiles that are similar with a Pearson's correlation coefficient (*r*) ≥ 0.7 are connected by lines. Agents that do not cluster with one another are interpreted as mechanistically distinct.[13] This analysis is performed for projects with 3 or more agents tested. Cytotoxic concentrations are excluded from cluster analysis.

Mechanism HeatMAP Analysis. Mechanism HeatMAP analysis provides a visualization of the test compound and 19 consensus mechanisms allowing comparison of biomarker activities across all compound concentrations and consensus mechanisms. The synthetic consensus profiles used in the Mechanism HeatMAP analysis are representative BioMAP profiles of the average of multiple compounds from structurally distinct chemical classes. Profiles were calculated by averaging the values for each biomarker endpoint for all profiles selected (multiple agents at different concentrations) to build the consensus mechanism profile.[8] Biomarker activities are colored in the heatmap for consensus mechanisms and compounds when they have expression relative to vehicle controls outside of the significance envelope. Red represents increased protein expression, blue represents decreased expression and white indicates levels that were unchanged or within filtering conditions. Darker shades of color represent greater change in biomarker activity relative to vehicle control. The Mechanism HeatMAP was prepared using R[14] and the *gplots* package[15] for R.

Assay Acceptance Criteria

A BioMAP assay includes the multi-parameter data sets generated by the BioMAP platform for agents tested in the systems that make up the Diversity PLUS panel. Assays contain drug controls (e.g., legacy control test agent colchicine), negative controls (e.g., non-stimulated conditions), and vehicle controls (e.g., DMSO) appropriate for each system. BioMAP assays are plate-based, and data acceptance criteria depend on both plate performance (% CV of vehicle control wells) and system performance across historical controls for that system. The QA/QC Pearson Test is performed by first establishing the 1% false negative Pearson cutoff from the reference dataset of historical positive controls. The process iterates through every profile of system biomarker readouts in the positive control reference dataset, calculating Pearson values between each profile and the mean of the remaining profiles in the dataset. The overall number of Pearson values used to determine the 1% false negative cutoff is the total number of profiles present in the reference dataset. The Pearson value at the one percentile of all values calculated is the 1% false negative Pearson cutoff. A system will pass if the Pearson value between the experimental plate's negative control or drug control profile and the mean of the historical control profiles in the reference dataset exceeds this 1% false negative Pearson cutoff. Overall assays are accepted when each individual system passes the Pearson test and 95% of all project plates have % CV <20%.

Quality Assurance

DiscoverX® ensures the quality of all internal testing, operations and data release using a comprehensive Quality Management System (QMS). The QMS is implemented through detailed Standard Operating Procedures (SOPs) within the Documentation Management System and controlled and maintained by a Quality Assurance Unit (QAU).

References

[1] E. J. Kunkel, M. Dea, A. Ebens, E. Hytopoulos, J. Melrose, D. Nguyen, et al. "An integrative biology approach for analysis of drug action in models of human vascular inflammation". In: *The FASEB Journal* 18.11 (2004), pp. 1279-1281.

[2] E. J. Kunkel, I. Plavec, D. Nguyen, J. Melrose, E. S. Rosler, L. T. Kao, et al. "Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models". In: *Assay Drug Development Technologies* 2.4 (2004), pp. 431-442.

[3] E. L. Berg, E. J. Kunkel, E. Hytopoulos and I. Plavec. "Characterization of compound mechanisms and secondary activities by BioMAP analysis". In: *Journal of Pharmacological and Toxicological Methods* 53.1 (2006), pp. 67-74.

[4] K. A. Houck, D. J. Dix, R. S. Judson, R. J. Kavlock, J. Yang and E. L. Berg. "Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems". In: *Journal of Biomolecular Screening* (2009).

[5] D. Xu, Y. Kim, J. Postelnek, M. D. Vu, D. Hu, C. Liao, et al. "RN486, a selective Bruton's tyrosine kinase inhibitor, abrogates immune hypersensitivity responses and arthritis in rodents". In: *Journal of Pharmacology and Experimental Therapeutics* 341.1 (2012), pp. 90-103.

[6] G. Bergamini, K. Bell, S. Shimamura, T. Werner, A. Cansfield, K. Müller, et al. "A selective inhibitor reveals PI3Kγ dependence of T(H)17 cell differentiation". In: *Nature Chemical Biology* 8.6 (2012), pp. 576-582.

[7] A. C. Melton, J. Melrose, L. Alajoki, S. Privat, H. Cho, N. Brown, et al. "Regulation of IL-17A production is distinct from IL-17F in a primary human cell co-culture model of T cell-mediated B cell activation". In: *PLOS ONE* 8.3 (2013), p. e58966.

[8] E. L. Berg, J. Yang and M. A. Polokoff. "Building predictive models for mechanism-of-action classification from phenotypic assay data sets". In: *Journal of Biomolecular Screening* (2013), pp. 1260-9. DOI: 1087057113505324.

[9] N. C. Kleinstreuer, J. Yang, E. L. Berg, T. B. Knudsen, A. M. Richard, M. T. Martin, et al. "Phenotypic screening of the ToxCast chemical library to classify toxic and therapeutic mechanisms". In: *Nature Biotechnology* 32.6 (2014), pp. 583-591.

[10] E. L. Berg, M. A. Polokoff, A. O'Mahony, D. Nguyen and X. Li. "Elucidating Mechanisms of Toxicity Using Phenotypic Data from Primary Human Cell Systems-A Chemical Biology Approach for Thrombosis-Related Side Effects". In: *International Journal of Molecular Sciences* 16.1 (2015), pp. 1008-1029.

[11] E. L. Berg and A. O'Mahony. "Complex Primary Human Cell Systems for Drug Discovery". In: *Human-based Systems for Translational Research*. Ed. by R. Coleman. RSC Drug Discovery. The Royal Society of Chemistry, 2014, pp. 88-109. ISBN: 978-1-84973-825-5. DOI: 10.1039/9781782620136.

[12] E. L. Berg, Y. Hsu and J. A. Lee. "Consideration of the cellular microenvironment: physiologically relevant co-culture systems in drug discovery". In: *Advanced Drug Delivery Reviews* 69 (2014), pp. 190-204.

[13] E. L. Berg, J. Yang, J. Melrose, D. Nguyen, S. Privat, E. Rosler, et al. "Chemical target and pathway toxicity mechanisms defined in primary human cell systems". In: *Journal of Pharmacological and Toxicological Methods* 61.1 (2010), pp. 3-15.

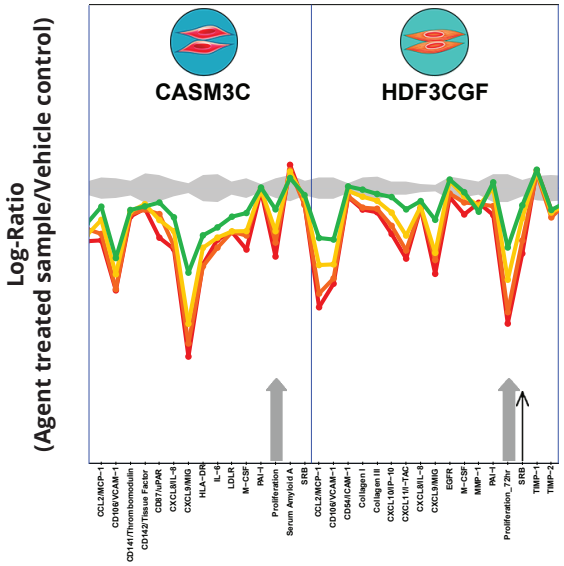
[14] R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. Vienna, Austria, 2013.

[15] G. R. Warnes, B. Bolker, L. Bonebakker, R. Gentleman, W. H. A. Liaw, T. Lumley, et al. *gplots: Various R Programming Tools for Plotting Data*. R package version 2.17.0. 2015.

Appendix B

Explanation of BioMAP Terms

BioMAP Profile - A graphical representation of the signature biomarker readout changes quantitated within BioMAP systems in response to test agent treatment.



System Icon: Pictoral depiction of the cells and stimuli within the system.

CASM3C

System Name: Abbreviated description of the cells and stimuli within the system.

Significance Envelope: Symmetrical upper and lower bound values of historical vehicle controls at a 95% confidence interval (CI).

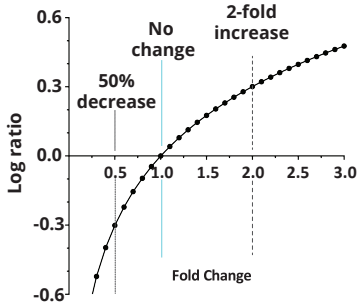
Cytotoxicity: Indicated by a thin black arrow above the X-axis and determined by measuring changes in total protein content.

Antiproliferation: Indicated by grey arrow above the X-axis and determined by changes in total protein content of samples from cells plated at a lower density.

Biomarker Readouts (X axis): Therapeutically relevant cell surface or secreted proteins that have been validated with well-characterized agents or mechanism of action compounds.

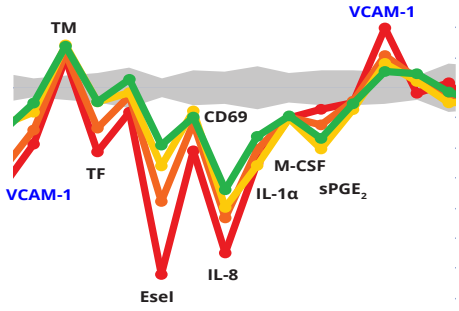
Log-Ratio Values (Y axis): Biomaker values in a test sample are divided by the average of vehicle controls to generate a ratio that is Log_{10} transformed.

Log_{10} scale *vs.* Fold change



Scaling of Biomarker Expression: Comparing the normalized biomarker readout expression of a Log_{10} scale to fold change demonstrates that a Log_{10} of -0.3 is about a 50% decrease in fold change, while a Log_{10} of 0.3 is about a 2-fold increase.

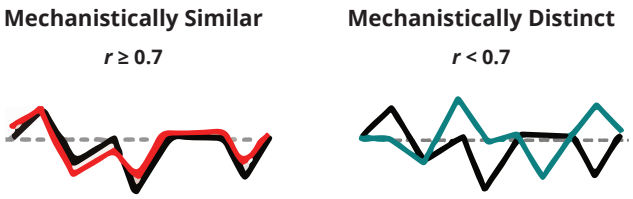
Annotation of Key Activities or Hits (biomarkers in black in figure below):



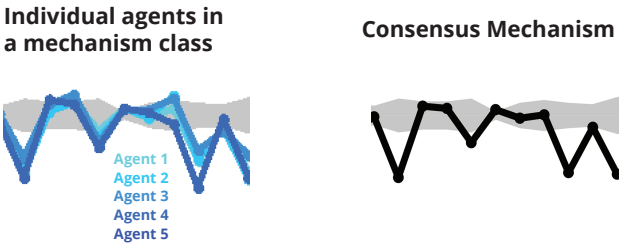
Biomarker readouts are designated hits if:
(1) Two or more consecutive concentrations are changed in the same direction relative to vehicle controls
(2) values are outside the significance envelope
(3) and at least one concentration has an effect size > 20% *vs.* vehicle controls.

Modulated: Biomarkers that are increased in some systems, but decreased in others **(biomarkers in blue)**.

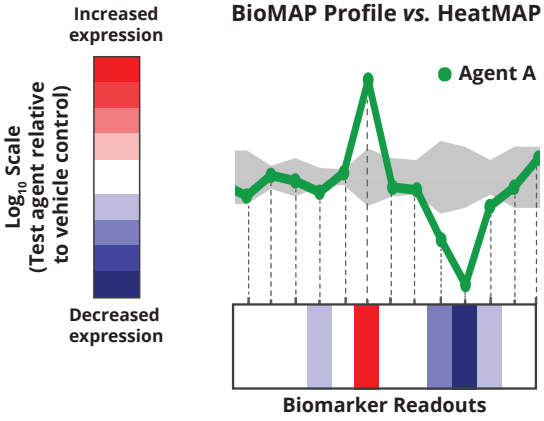
Similarity Analysis: Similarity between agents is evaluated using a combinatorial approach that ranks the Pearson's Correlation Coefficient (*r*) between two agents using a custom BioMAP Z-Standard. Profiles are identified as being mechanistically similar if $r \geq 0.7$.



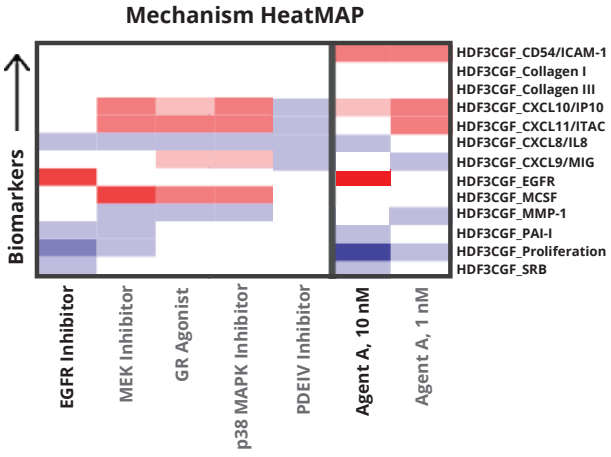
Consensus Mechanism: A consensus mechanism represents the average profile of multiple agents in a known mechanism class. This average has been filtered to exclude profiles that were toxic, had weak activity or low similarity correlation ($r < 0.7$).



Mechanism HeatMAP: Biomarker activites outside of the significance envelope are red if protein levels are increased, blue if protein levels are decreased and white if levels are within the envelope or unchanged. Darker shades of color represent greater change in biomarker activity relative to vehicle control.



HeatMAP Interpretation: In the HDF3CGF system, Agent A at 10 µM shares a similar increase in EGFR expression with the EGFR Inhibitor consensus mechanism, but has increased expression of ICAM-1 that is not shared by any of the other consensus mechanisms shown.



Explanation of Diversity PLUS Deliverables

Plot	Description	Analysis Criteria
Profile Analysis	Profile Plot of individual test agents at 4 concentrations with biomarker readout annotation and interpretation of key activities, including relevance to biological pathways and *in vivo* correlations / predictions.	Biomarker readouts are designated as "key activities" and annotated if: i. 2 or more consecutive concentrations are changed in the same direction relative to vehicle controls ii. biomarker readouts are outside of the significance envelope iii. at least 1 of the concentrations has an effect size > 20% ($\|\log_{10} ratio\|$ > 0.1) compared to vehicle controls Cytotoxicity (thin black arrow) is annotated when total protein levels decrease by more than 50% in at least one concentration ($\log_{10} ratio$ of SRB or alamarBlue levels < -0.3). Antiproliferative effects (grey arrows) are defined by an SRB or alamarBlue $\log_{10} ratio$ value < -0.1 in at least one concentration (adherent cells plated at a lower density).
Benchmark Analysis	Overlay of one concentration of a test agent with one concentration of a specified reference benchmark.	*Common* biomarker readouts are annotated when the readout for both profiles is outside of the significance envelope with an effect size > 20% in the same direction. *Differentiating* biomarkers are annotated when one profile has a readout outside of the significance envelope with an effect size > 20%, and the readout for the other profile is either inside the envelope or in the opposite direction.
Similarity Analysis	Results from unsupervised search for biologically similar agents from the BioMAP reference database of > 4,000 test agents including biologics, approved drugs and experimental chemical compounds.	*Common* biomarker readouts are annotated when the readout for both profiles is outside of the significance envelope with an effect size > 20% in the same direction. Profiles are identified as having mechanistically relevant similarity if the Pearson's correlation coefficient (r) ≥ 0.7.
Cluster Analysis	Overall comparison of agents using pairwise correlation analysis and clustering of most similar profiles.	Available with > 3 test agents. Cytotoxic concentrations will be excluded from the analysis. Profiles that are similar with a Pearson's correlation coefficient (r) ≥ 0.7 are connected by lines. Agents that do not cluster with one another are interpreted as mechanistically distinct.
Mechanism HeatMAP Analysis	Each agent is compared in a heatmap format to the BioMAP profile of 19 consensus mechanism class profiles of well-characterized drugs (columns) by biomarker readout (rows).	Biomarker activity is compared to vehicle control and filtered for biomarker activity outside of the significance envelope. Red represents increased protein expression, blue represents decreased expression and white indicates levels that were unchanged or within filtering conditions. Darker shades of color represent greater change in biomarker activity relative to vehicle controls.

Explanation of Diversity PLUS Excel Data Table

Worksheet	Description
Profile Data	Biomarker readouts in a drug-treated sample are divided by the average of controls (> 6 vehicle controls from the same plate) to generate a ratio that is $\log_{10}$ transformed. Values in blue are outside the significance envelope. Values in red are outside the significance envelope for SRB and Alamar Blue assays.
Cytotoxicity	1 indicates detection of overt cytotoxicity within a system, which is defined as more than 50% reduction in total protein levels. The 1 will match the values in red in the Profile Data worksheet.
Envelope	Symmetrical upper bound values and negative lower bound values of $\log_{10}$ transformed historical vehicle controls at a 95% confidence interval (CI).
Biomarker Hits	1 denotes biomarker readouts outside of the significance envelope. This 1 will match the values in blue in the Profile Data worksheet.

Appendix C

Description of Biomarkers in BioMAP Diversity PLUS Panel

System	Biomarker	Description
3C	CCL2/MCP-1	Monocyte chemoattractant protein-1 (MCP-1/CCL2) is a chemoattractant cytokine (chemokine) that mediates recruitment of monocytes and T cells into sites of inflammation. MCP-1 is categorized as an inflammation-related activity in the 3C system modeling Th1 vascular inflammation. MCP-1 in the 3C system is regulated by the following pathways: HDAC, Histamine H1R, IKK2.
	CD106/VCAM-1	Vascular Cell Adhesion Molecule 1 (VCAM-1/CD106) is a cell adhesion molecule that mediates adhesion of monocytes and T cells to endothelial cells. VCAM-1 is categorized as an inflammation-related activity in the 3C system modeling Th1 vascular inflammation. VCAM-1 in the 3C system is regulated by the following pathways: HDAC, Histamine H1R, IKK2.
	CD141 / Thrombomodulin	Thrombomodulin/CD141 is a cell surface receptor for complement factor 3b with anti-coagulant, anti-inflammatory and cytoprotective activities during the process of fibrinolysis, coagulation and thrombosis. Thrombomodulin is categorized as a hemostasis-related activity in the 3C system modeling Th1 vascular inflammation.
	CD142/Tissue Factor	Tissue Factor/CD142 is a cell surface receptor for coagulation factor VII that promotes the formation of thrombin during the process of vascular thrombosis and coagulation. Tissue Factor is categorized as a hemostasis-related activity in the 3C system modeling Th1 vascular inflammation. TF in the 3C system is regulated by the following pathways: HDAC, IKK2.
	CD54/ICAM-1	Intercellular Adhesion Molecule 1 (ICAM-1/CD54) is a cell adhesion molecule that mediates leukocyte-endothelial cell adhesion and leukocyte recruitment. ICAM-1 is categorized as an inflammation-related activity in the 3C system modeling Th1 vascular inflammation. ICAM-1 in the 3C system is regulated by the following pathways: IKK2.
	CD62E/E-Selectin	E-Selectin/CD62E is a cell adhesion molecule expressed only on endothelial cells that mediates leukocyte-endothelial cell interactions. E-Selectin is categorized as an inflammation-related activity in the 3C system modeling Th1 vascular inflammation. E-selectin in the 3C system is regulated by the following pathways: IKK2.
	CD87/uPAR	Urokinase plasminogen activator receptor (uPAR/CD87) is a cell surface receptor for urokinase plasminogen activator (uPA) involved in the regulation of pericellular proteolysis, cell migration, cancer cell invasion, and angiogenesis. uPAR is categorized as a tissue remodeling-related activity in the 3C system modeling Th1 vascular inflammation. uPAR in the 3C system is regulated by the following pathways: HDAC, HMG-CoA Reductase, Histamine H1R, Microtubule, PI3K, mTOR.
	CXCL8/IL-8	Interleukin-8 (IL-8/CXCL8) is a chemokine that mediates neutrophil recruitment into acute inflammatory sites. IL-8 is categorized as an inflammation-related activity in the 3C system modeling Th1 vascular inflammation. IL-8 in the 3C system is regulated by the following pathways: IKK2, p38 MAPK.
	CXCL9/MIG	Monokine induced by gamma interferon (MIG/CXCL9) is a chemokine that mediates T cell recruitment. MIG is categorized as an inflammation-related activity in the 3C system modeling Th1 vascular inflammation. MIG in the 3C system is regulated by the following pathways: HDAC, Histamine H1R, IKK2, JAK.
	HLA-DR	HLA-DR is a cell surface heterodimer involved in antigen presentation. HLA-DR binds peptides and presents them to the T cell receptor and is involved in T cell activation and immune responses. HLA-DR is categorized as an immunomodulatory-related activity in the 3C system modeling Th1 vascular inflammation. HLA-DR in the 3C system is regulated by the following pathways: HDAC, Histamine H1R, IKK2, JAK, PI3K, RAR/RXR, p38 MAPK.
	Proliferation	Proliferation in the 3C system is a measure of endothelial cell proliferation which is important to the process of wound healing and angiogenesis. Prolif in the 3C system is regulated by the following pathways: EGFR, HDAC, HMG-CoA Reductase, Histamine H1R, IKK2, MEK, Microtubule, PI3K, Src, mTOR.
	SRB	SRB in the 3C system is a measure of the total protein content of venular endothelial cells. Cell viability of adherent cells is measured by Sulforhodamine B (SRB) staining, a method that determines cell density by measuring total protein content of test wells. SRB in the 3C system is regulated by the following pathways: HMG-CoA Reductase, Histamine H1R, Microtubule, PI3K.
4H	CCL2/MCP-1	Monocyte chemoattractant protein-1 (MCP-1/CCL2) is a chemoattractant cytokine (chemokine) that regulates the recruitment of monocytes and T cells into sites of inflammation. MCP-1 is categorized as an inflammation-related activity in the 4H system modeling Th2 vascular inflammation. MCP-1 in the 4H system is regulated by the following pathways: HMG-CoA Reductase, Histamine H1R.
	CCL26/Eotaxin-3	Eotaxin-3/CCL26 is a chemokine that mediates recruitment of eosinophils and basophils into sites of tissue inflammation. Eotaxin-3 is categorized as an inflammation-related activity in the 4H system modeling Th2 vascular inflammation. Eotaxin 3 in the 4H system is regulated by the following pathways: HDAC, Histamine H1R, IKK2, JAK, RAR/RXR.
	CD106/VCAM-1	Vascular Cell Adhesion Molecule 1 (VCAM-1/CD106) is a cell adhesion molecule that mediates adhesion of monocytes and T cells to endothelial cells. VCAM-1 is categorized as an inflammation-related activity in the 4H system modeling Th2 vascular inflammation. VCAM-1 in the 4H system is regulated by the following pathways: Calcineurin, HDAC, Histamine H1R, IKK2, JAK, RAR/RXR.
	CD62P/P-Selectin	P-Selectin/CD62P is a cell adhesion molecule that mediates platelet-endothelial cell and leukocyte-endothelial cell interactions. P-Selectin is categorized as an inflammation-related activity in the 4H system modeling Th2 vascular inflammation. P-selectin in the 4H system is regulated by the following pathways: JAK, PI3K.
	CD87/uPAR	Urokinase plasminogen activator receptor (uPAR/CD87) is a cell surface receptor for urokinase plasminogen activator (uPA) involved in the regulation of pericellular proteolysis, cell migration, cancer cell invasion, and angiogenesis. uPAR is categorized as a tissue remodeling-related activity in the 4H system modeling Th2 vascular inflammation. uPAR in the 4H system is regulated by the following pathways: EGFR, HDAC, Histamine H1R, MEK, Microtubule.
	SRB	SRB in the 4H system is a measure of the total protein content of venular endothelial cells. Cell viability of adherent cells is measured by Sulforhodamine B (SRB) staining, a method that determines cell density by measuring total protein content of test wells. SRB in the 4H system is regulated by the following pathways: Histamine H1R.
	VEGFR2	Vascular endothelial growth factor receptor 2 (VEGFR2) in the 4H system modeling Th2 vascular inflammation is a cell surface tyrosine kinase receptor for VEGF-A and is involved in angiogenesis, endothelial cell proliferation and vascular permeability.
LPS	CCL2/MCP-1	Monocyte chemoattractant protein-1 (MCP-1/CCL2) is a chemoattractant cytokine (chemokine) that regulates the recruitment of monocytes and T cells into sites of inflammation. MCP-1 is categorized as an inflammation-related activity in the LPS system modeling monocyte-driven Th1 vascular inflammation. MCP-1 in the LPS system is regulated by the following pathways: HDAC, Histamine H1R, IKK2, p38 MAPK.
	CD106/VCAM-1	Vascular Cell Adhesion Molecule 1 (VCAM-1/CD106) is a cell adhesion molecule that mediates adhesion of monocytes and T cells to endothelial cells. VCAM-1 is categorized as an inflammation-related activity in the LPS system modeling monocyte-driven Th1 vascular inflammation. VCAM-1 in the LPS system is regulated by the following pathways: HDAC, IKK2.
	CD141 / Thrombomodulin	Thrombomodulin/CD141 is a cell surface receptor for complement factor 3b with anti-coagulant, anti-inflammatory and cytoprotective activities during the process of fibrinolysis, coagulation and thrombosis. Thrombomodulin is categorized as a hemostasis-related activity in the LPS system modeling monocyte-driven Th1 vascular inflammation. TM in the LPS system is regulated by the following pathways: IKK2, RAR/RXR, p38 MAPK.

System	Biomarker	Description
LPS	CD142/Tissue Factor	Tissue Factor/CD142 is a cell surface receptor for coagulation factor VII that promotes the formation of thrombin during the process of thrombosis and coagulation. Tissue Factor is categorized as a hemostasis-related activity in the LPS system modeling monocyte-driven Th1 vascular inflammation. TF in the LPS system is regulated by the following pathways: IKK2, p38 MAPK.
	CD40	CD40 is a cell surface adhesion receptor and costimulatory receptor for T cell activation that is expressed on antigen presenting cells, endothelial cells, smooth muscle cells, fibroblasts and epithelial cells. CD40 is categorized as an immunomodulatory-related activity in the LPS system modeling monocyte-driven Th1 vascular inflammation. CD40 in the LPS system is regulated by the following pathways: Histamine H1R, IKK2, PI3K, RAR/RXR, Src, mTOR.
	CD62E/E-Selectin	E-Selectin/CD62E is a cell adhesion molecule expressed only on endothelial cells that mediates leukocyte-endothelial cell interactions. E-Selectin is categorized as an inflammation-related activity in the LPS system modeling monocyte-driven Th1 vascular inflammation. E-selectin in the LPS system is regulated by the following pathways: HDAC, IKK2, p38 MAPK.
	CD69	CD69 is a cell surface activation antigen. CD69 is categorized as an immunomodulatory-related activity in the LPS system modeling monocyte-driven Th1 vascular inflammation. CD69 in the LPS system is regulated by the following pathways: Histamine H1R, IKK2.
	CXCL8/IL-8	Interleukin-8 (IL-8/CXCL8) is a chemokine that mediates neutrophil recruitment into acute inflammatory sites. IL-8 is categorized as an inflammation-related activity in the LPS system modeling monocyte-driven Th1 vascular inflammation. IL-8 in the LPS system is regulated by the following pathways: IKK2, p38 MAPK.
	IL-1 alpha	Interleukin-1 alpha (IL-1α) is a secreted proinflammatory cytokine involved in endothelial cell activation and neutrophil recruitment. IL-1α is categorized as an inflammation-related activity in the LPS system modeling monocyte-driven Th1 vascular inflammation. IL-1a in the LPS system is regulated by the following pathways: HDAC, HMG-CoA Reductase, IKK2, p38 MAPK.
	M-CSF	Macrophage colony-stimulating factor (M-CSF) is a secreted and cell surface cytokine that mediates macrophage differentiation. M-CSF is categorized as a tissue remodeling-related activity in the LPS system modeling monocyte-driven Th1 vascular inflammation. M-CSF in the LPS system is regulated by the following pathways: HDAC, IKK2, RAR/RXR, p38 MAPK.
	sPGE2	Prostaglandin E2 (PGE2) is an immunomodulatory lipid mediator involved in muscle contractility, inflammatory pain and kidney function. Secreted PGE2 (sPGE2) is categorized as an inflammation-related activity in the LPS system modeling monocyte-driven Th1 vascular inflammation. sPGE2 in the LPS system is regulated by the following pathways: IKK2, MEK, PKC (c+n), RAR/RXR, Vitamin D R, mTOR, p38 MAPK.
	SRB	SRB in the LPS system is a measure of the total protein content of venular endothelial cells and PBMC. Cell viability of adherent cells is measured by Sulforhodamine B (SRB) staining, a method that determines cell density by measuring total protein content of test wells. SRB in the LPS system is regulated by the following pathways: HMG-CoA Reductase, Histamine H1R, IKK2.
	sTNF-alpha	Tumor necrosis factor alpha (TNFα) is a secreted proinflammatory cytokine involved in Th1 vascular inflammation. Secreted TNFα (sTNFα) is categorized as an inflammation-related activity in the LPS system modeling monocyte-driven Th1 vascular inflammation. sTNFa in the LPS system is regulated by the following pathways: EGFR, Glucocorticoid R, HDAC, Histamine H1R, IKK2, MEK, PDE4, PI3K, PKC (c+n), RAR/RXR, Src, TNF-alpha, Vitamin D R, p38 MAPK.
SAg	CCL2/MCP-1	Monocyte chemoattractant protein-1 (MCP-1/CCL2) is a chemoattractant cytokine (chemokine) that regulates the recruitment of monocytes and T cells into sites of inflammation. MCP-1 is categorized as an inflammation-related activity in the SAg system modeling T cell-driven Th1 vascular inflammation. MCP-1 in the SAg system is regulated by the following pathways: Calcineurin, HDAC, IKK2, JAK, MEK, PI3K, PKC (c+n), Src.
	CD38	CD38 is a cell surface enzyme and marker of cell activation that is involved in T cell activation/co-stimulation and chemotaxis. CD38 is categorized as an immunomodulatory-related activity in the SAg system modeling T cell-driven Th1 vascular inflammation. CD38 in the SAg system is regulated by the following pathways: Calcineurin, HDAC, IKK2, JAK, MEK, PI3K, PKC (c+n), RAR/RXR, Src.
	CD40	CD40 is a cell surface adhesion receptor and costimulatory receptor for T cell activation that is expressed on antigen presenting cells, endothelial cells, smooth muscle cells, fibroblasts and epithelial cells. CD40 is categorized as an immunomodulatory-related activity in the SAg system modeling T cell-driven Th1 vascular inflammation. CD40 in the SAg system is regulated by the following pathways: Calcineurin, HMG-CoA Reductase, Histamine H1R, IKK2, JAK, PI3K, RAR/RXR, Src, mTOR.
	CD62E/E-Selectin	E-Selectin/CD62E is a cell adhesion molecule expressed only on endothelial cells that mediates leukocyte-endothelial cell interactions. E-Selectin is categorized as an inflammation-related activity in the SAg system modeling T cell-driven Th1 vascular inflammation. E-selectin in the SAg system is regulated by the following pathways: Calcineurin, Glucocorticoid R, HMG-CoA Reductase, Histamine H1R, IKK2, JAK, MEK, PDE4, PI3K, PKC (c+n), RAR/RXR, Src, TNF-alpha.
	CD69	CD69 is a cell surface activation antigen that is induced early during immune activation and is involved in lymphocyte proliferation and activation. CD69 is categorized as an immunomodulatory-related activity in the SAg system modeling T cell-driven Th1 vascular inflammation. CD69 in the SAg system is regulated by the following pathways: Calcineurin, EGFR, HMG-CoA Reductase, Histamine H1R, IKK2, JAK, MEK, PKC (c+n), Src.
	CXCL8/IL-8	Interleukin-8 (IL-8/CXCL8) is a chemokine that mediates neutrophil recruitment into acute inflammatory sites. IL-8 is categorized as an inflammation-related activity in the SAg system modeling T cell-driven Th1 vascular inflammation. IL-8 in the SAg system is regulated by the following pathways: Calcineurin, HMG-CoA Reductase, IKK2, JAK, MEK, PKC (c+n), Src, TNF-alpha, p38 MAPK.
	CXCL9/MIG	Monokine induced by gamma interferon (MIG/CXCL9) is a chemokine that mediates T cell recruitment. MIG is categorized as an inflammation-related activity in the SAg system modeling T cell-driven Th1 vascular inflammation. MIG in the SAg system is regulated by the following pathways: Calcineurin, HDAC, IKK2, JAK, RAR/RXR, Src.
	PBMC Cytotoxicity	PBMC Cytotoxicity in the SAg system is a measure of the cell death of PBMC. Cell viability of non-adherent cells is measured by alamarBlueÂ® staining, a method based on a cell permeable compound that emitts fluorescence after entering cells. The number of living cells is proportional to the amount of fluorescence produced. Pcyto in the SAg system is regulated by the following pathways: Histamine H1R, IKK2.
	Proliferation	Proliferation in the SAg system is a measure of T cell proliferation which is the critical event driving both adaptive immunity as well as many auto-immune diseases (RA, PsA, MS, IBD etc). Prolif in the SAg system is regulated by the following pathways: Calcineurin, EGFR, HDAC, HMG-CoA Reductase, Histamine H1R, IKK2, JAK, MEK, Microtubule, PI3K, PKC (c+n), RAR/RXR, Src, mTOR.
	SRB	SRB in the SAg system is a measure of the total protein content of venular endothelial cells. Cell viability of adherent cells is measured by Sulforhodamine B (SRB) staining, a method that determines cell density by measuring total protein content of test wells. SRB in the SAg system is regulated by the following pathways: HMG-CoA Reductase, Histamine H1R.
BT	B cell Proliferation	B cell proliferation is a critical event driving both adaptive immunity (antibody production) as well as auto-immune diseases where B cells are key disease players (Lupus, MS, RA etc). Inhibition of B cell proliferation is considered an immune suppressive effect. Prolif in the BT system is regulated by the following pathways: Calcineurin, EGFR, HDAC, HMG-CoA Reductase, Histamine H1R, IKK2, JAK, Microtubule, PI3K, PKC (c+n), Src, mTOR.
	PBMC Cytotoxicity	PBMC Cytotoxicity in the BT system is a measure of the cell death of PBMC. Cell viability of non-adherent cells is measured by alamarBlueÂ® staining, a method based on a cell permeable compound that emits fluorescence after entering cells. The number of living cells is proportional to the amount of fluorescence produced. Pcyto in the BT system is regulated by the following pathways: Calcineurin, Glucocorticoid R, HDAC, Histamine H1R, IKK2, PI3K.
	Secreted IgG	Secreted IgG (sIgG) is produced by B cells and is the main type of antibody found in blood and extracellular fluid that mediates the immune response against pathogens. sIgG is categorized as an immunomodulatory-related activity in the BT system modeling T cell dependent B cell activation. sIgG in the BT system is regulated by the following pathways: Calcineurin, EGFR, HDAC, HMG-CoA Reductase, Histamine H1R, IKK2, JAK, Microtubule, PDE4, PI3K, PKC (c+n), Src, Vitamin D R, mTOR, p38 MAPK.
	sIL-17A	Interleukin-17A (IL-17A) is a proinflammatory cytokine produced by T cells that induces cytokine production and mediates monocyte and neutrophil recruitment to sites of inflammation. Secreted IL-17A (sIL-17A) is categorized as an immunomodulatory-related activity in the BT system modeling T cell dependent B cell activation. sIL-17A in the BT system is regulated by the following pathways: Calcineurin, EGFR, Glucocorticoid R, HDAC, HMG-CoA Reductase, Histamine H1R, IKK2, JAK, MEK, Microtubule, PI3K, PKC (c+n), Src, Vitamin D R, mTOR, p38 MAPK.

System	Biomarker	Description
BT	sIL-17F	Interleukin-17F (IL-17F) is a proinflammatory cytokine produced by T cells that induces cytokine, chemokine and adhesion molecule production and mediates neutrophil recruitment to sites of inflammation. Secreted IL-17F (sIL-17F) is categorized as an immunomodulatory-related activity in the BT system modeling T cell dependent B cell activation. sIL-17F in the BT system is regulated by the following pathways: Calcineurin, EGFR, Glucocorticoid R, HDAC, HMG-CoA Reductase, Histamine H1R, JAK, MEK, Microtubule, PKC (c+n), RAR/RXR, Src, TNF-alpha, Vitamin D R, mTOR, p38 MAPK.
	sIL-2	Interleukin-2 (IL-2) is a secreted proinflammatory cytokine produced by T cells that regulates lymphocyte proliferation and promotes T cell differentiation. Secreted IL-2 (IL-2) is categorized as an immunomodulatory-related activity in the BT system modeling T cell dependent B cell activation. sIL-2 in the BT system is regulated by the following pathways: Calcineurin, EGFR, Glucocorticoid R, HDAC, Histamine H1R, IKK2, JAK, MEK, PI3K, PKC (c+n), RAR/RXR, Src, Vitamin D R, mTOR, p38 MAPK.
	sIL-6	Interleukin-6 (IL-6) is a secreted proinflammatory cytokine and acute phase reactant. Secreted IL-6 (sIL-6) is categorized as an immunomodulatory-related activity in the BT system modeling T cell dependent B cell activation. sIL-6 in the BT system is regulated by the following pathways: Calcineurin, EGFR, Glucocorticoid R, HDAC, Histamine H1R, IKK2, JAK, MEK, PI3K, PKC (c+n), Src, TNF-alpha, Vitamin D R, mTOR, p38 MAPK.
	sTNF-alpha	Tumor necrosis factor alpha (TNFα) is a secreted proinflammatory cytokine involved in Th1 inflammation. Secreted TNFα (sTNFα) is categorized as an inflammation-related activity in the BT system modeling T cell dependent B cell activation. sTNFa in the BT system is regulated by the following pathways: Calcineurin, EGFR, Glucocorticoid R, HDAC, HMG-CoA Reductase, Histamine H1R, IKK2, JAK, MEK, PI3K, PKC (c+n), RAR/RXR, Src, TNF-alpha, Vitamin D R, mTOR.
BF4T	CCL2/MCP-1	Monocyte chemoattractant protein-1 (MCP-1/CCL2) is a chemoattractant cytokine (chemokine) that regulates the recruitment of monocytes and T cells into sites of inflammation. MCP-1 is categorized as an inflammation-related activity in the BF4T system modeling Th2 airway inflammation.
	CCL26/Eotaxin-3	Eotaxin-3/CCL26 is a chemokine that mediates recruitment of eosinophils and basophils into tissue sites. Eotaxin-3 is categorized as an inflammation-related activity in the BF4T system modeling Th2 airway inflammation. Eotaxin 3 in the BF4T system is regulated by the following pathways: EGFR, RAR/RXR.
	CD106/VCAM-1	Vascular Cell Adhesion Molecule 1 (VCAM-1/CD106) is a cell adhesion molecule that mediates adhesion of monocytes and T cells to endothelial cells. VCAM-1 is categorized as an inflammation-related activity in the BF4T system modeling Th2 airway inflammation.
	CD54/ICAM-1	Intercellular Adhesion Molecule 1 (ICAM-1/CD54) is a cell adhesion molecule that mediates leukocyte-endothelial cell adhesion and leukocyte recruitment. ICAM-1 is categorized as an inflammation-related activity in the BF4T system modeling Th2 airway inflammation.
	CD90	CD90 is a cell surface glycoprotein that mediates cell-cell and cell-matrix interactions. CD90 is categorized as a tissue remodeling-related activity in the BF4T system modeling Th2 airway inflammation.
	CXCL8/IL-8	Interleukin-8 (IL-8/CXCL8) is a chemokine that mediates neutrophil recruitment into acute inflammatory sites. IL-8 is categorized as an inflammation-related activity in the BF4T system modeling Th2 airway inflammation. IL-8 in the BF4T system is regulated by the following pathways: IKK2.
	IL-1 alpha	Interleukin-1 alpha (IL-1α) is a secreted proinflammatory cytokine involved in endothelial cell activation and neutrophil recruitment. Secreted IL-1α (sIL-1α) is categorized as an inflammation-related activity in the BF4T system modeling Th2 airway inflammation.
	Keratin 8/18	Keratin 8/18 is an intermediate filament heterodimer of fibrous structural proteins involved in Epithelial cell death, EMT, COPD, Lung Inflammation. Keratin 8/18 is categorized as a tissue remodeling-related activity in the BF4T system modeling Th2 airway inflammation.
	MMP-1	Matrix metalloproteinase-1 (MMP-1) is an interstitial collagenase that degrades collagens I, II and III and is involved in the process of tissue remodeling. MMP-1 is categorized as a tissue remodeling-related activity in the BF4T system modeling Th2 airway inflammation.
	MMP-3	Matrix metalloproteinase-3 (MMP-3) is an enzyme involved in tissue remodeling that can activate other MMPs (MMP-1, MMP-7 and MMP-9) and degrade collagens (II, III, IV, IX and X), proteoglycans, fibronectin, laminin and elastin. MMP-3 is categorized as a tissue remodeling-related activity in the BF4T system modeling Th2 airway inflammation.
	MMP-9	Matrix metalloproteinase-9 (MMP-9) is a gelatinase B that degrades collagen IV and gelatin and is involved in airway matrix remodeling. MMP-9 is categorized as a tissue remodeling-related activity in the BF4T system modeling Th2 airway inflammation.
	PAI-I	Plasminogen activator inhibitor-1 (PAI-I) is a serine proteinase inhibitor and inhibitor of tissue plasminogen activator (tPA) and urokinase (uPA) and is involved in tissue remodeling and fibrinolysis. PAI-I is categorized as a tissue remodeling-related activity in the BF4T system modeling Th2 airway inflammation.
	SRB	SRB in the BF4T system is a measure of the total protein content of bronchial epithelial cells and dermal fibroblasts. Cell viability of adherent cells is measured by Sulforhodamine B (SRB) staining, a method that determines cell density by measuring total protein content of test wells.
	tPA	Tissue plasminogen activator (tPA) is a serine protease that catalyzes the cleavage of plasminogen to plasmin and regulates clot degradation. tPA is involved in fibrinolyis, cell migration and tissue remodeling. tPA is categorized as a tissue remodeling-related activity in the BF4T system modeling Th2 airway inflammation.
	uPA	Urokinase plasminogen activator (uPA) is a serine protease with thrombolytic activity. Triggers fibrinolysis and extracellular matrix degradation. uPA is categorized as a tissue remodeling-related activity in the BF4T system modeling Th2 airway inflammation.
BE3C	CD54/ICAM-1	Intercellular Adhesion Molecule 1 (ICAM-1/CD54) is a cell adhesion molecule that mediates leukocyte-endothelial cell adhesion and leukocyte recruitment. ICAM-1 is categorized as an inflammation-related activity in the BE3C system modeling Th1 lung inflammation.
	CD87/uPAR	Urokinase plasminogen activator receptor (uPAR/CD87) is a cell surface receptor for urokinase plasminogen activator (uPA) involved in the regulation of pericellular proteolysis, cell migration, cancer cell invasion, and angiogenesis. uPAR is categorized as a tissue remodeling-related activity in the BE3C system modeling Th1 lung inflammation. uPAR in the BE3C system is regulated by the following pathways: EGFR, HDAC, IKK2, JAK, Src.
	CXCL10/IP-10	Interferon-gamma-inducible protein 10 (IP-10/CXCL10) is a chemokine that mediates T cell, monocyte and dendritic cell chemotaxis. IP-10 is categorized as an inflammation-related activity in the BE3C system modeling Th1 lung inflammation. IP-10 in the BE3C system is regulated by the following pathways: IKK2, JAK, RAR/RXR.
	CXCL11/I-TAC	Interferon-inducible T Cell Alpha Chemoattractant (I-TAC/CXCL11) is a chemokine that mediates T cell and monocyte chemotaxis. I-TAC is categorized as an inflammation-related activity in the BE3C system modeling Th1 lung inflammation. I-TAC in the BE3C system is regulated by the following pathways: HDAC, IKK2, JAK.
	CXCL8/IL-8	Interleukin-8 (IL-8/CXCL8) is a chemokine that mediates neutrophil recruitment into acute inflammatory sites. IL-8 is categorized as an inflammation-related activity in the BE3C system modeling Th1 lung inflammation. IL-8 in the BE3C system is regulated by the following pathways: IKK2.
	CXCL9/MIG	Monokine induced by gamma interferon (MIG/CXCL9) is a chemokine that mediates T cell recruitment. MIG is categorized as an inflammation-related activity in the BE3C system modeling Th1 lung inflammation. MIG in the BE3C system is regulated by the following pathways: JAK.
	EGFR	Epidermal growth factor receptor (EGFR) is a cell surface receptor for epidermal growth factor involved in cell proliferation, cell differentiation, tissue remodeling and tumor growth. EGFR is categorized as a tissue remodeling-related activity in the BE3C system modeling Th1 lung inflammation.
	HLA-DR	HLA-DR is a cell surface heterodimer involved in antigen presentation. HLA-DR binds and presents peptides to T cell receptors and is involved in T cell activation and immune responses. HLA-DR is categorized as an immunomodulatory-related activity in the BE3C system modeling Th1 lung inflammation. HLA-DR in the BE3C system is regulated by the following pathways: JAK.
	IL-1 alpha	Interleukin-1 alpha (IL-1α) is a secreted proinflammatory cytokine involved in endothelial cell activation and neutrophil recruitment. Secreted IL-1α (sIL-1α) is categorized as an inflammation-related activity in the BE3C system modeling Th1 lung inflammation. IL-1a in the BE3C system is regulated by the following pathways: EGFR, Src.

System	Biomarker	Description
BE3C	Keratin 8/18	Keratin 8/18 is an intermediate filament heterodimer of fibrous structural proteins involved in Epithelial cell death, EMT, COPD, Lung Inflammation. Keratin 8/18 is categorized as a tissue remodeling-related activity in the BE3C system modeling Th1 lung inflammation.
	MMP-1	Matrix metalloproteinase-1 (MMP-1) is an interstitial collagenase that degrades collagens I, II and III and is involved in the process of tissue remodeling. MMP-1 is categorized as a tissue remodeling-related activity in the BE3C system modeling Th1 lung inflammation. MMP-1 in the BE3C system is regulated by the following pathways: EGFR, MEK, Src.
	MMP-9	Matrix metalloproteinase-9 (MMP-9) is a gelatinase B that degrades collagen IV and gelatin and is involved in airway matrix remodeling. MMP-9 is categorized as a tissue remodeling-related activity in the BE3C system modeling Th1 lung inflammation. MMP-9 in the BE3C system is regulated by the following pathways: EGFR, IKK2, Microtubule, PI3K.
	PAI-I	Plasminogen activator inhibitor-1 (PAI-I) is a serine proteinase inhibitor and inhibitor of tissue plasminogen activator (tPA) and urokinase (uPA) and is involved in tissue remodeling and fibrinolysis. PAI-I is categorized as a tissue remodeling-related activity in the BE3C system modeling Th1 lung inflammation. PAI-1 in the BE3C system is regulated by the following pathways: EGFR, MEK, Src.
	SRB	SRB in the BE3C system is a measure of the total protein content of bronchial epithelial cells. Cell viability of adherent cells is measured by Sulforhodamine B (SRB) staining, a method that determines cell density by measuring total protein content of test wells.
	tPA	Tissue plasminogen activator (tPA) is a serine proteases that catalyzes the cleavage of plasminogen to plasmin and regulates clot degradation. tPA is involved in cell migration, tissue remodeling and fibrinolysis. tPA is categorized as a tissue remodeling-related activity in the BE3C system modeling Th1 lung inflammation. tPA in the BE3C system is regulated by the following pathways: EGFR, HDAC, HMG-CoA Reductase.
	uPA	Urokinase plasminogen activator (uPA) is a serine protease with thrombolytic activity. Triggers fibrinolysis and extracellular matrix degradation. uPA is categorized as a tissue remodeling-related activity in the BE3C system modeling Th1 lung inflammation. uPA in the BE3C system is regulated by the following pathways: EGFR, HDAC, IKK2, JAK, Src.
CASM3C	CCL2/MCP-1	Monocyte chemoattractant protein-1 (MCP-1/CCL2) is a chemoattractant cytokine (chemokine) that regulates the recruitment of monocytes and T cells into sites of inflammation. MCP-1 is categorized as an inflammation-related activity in the CASM3C system modeling Th1 vascular smooth muscle inflammation. MCP-1 in the CASM3C system is regulated by the following pathways: Glucocorticoid R, HDAC, IKK2.
	CD106/VCAM-1	Vascular Cell Adhesion Molecule 1 (VCAM-1/CD106) is a cell adhesion molecule that mediates adhesion of monocytes and T cells to endothelial cells. VCAM-1 is categorized as an inflammation-related activity in the CASM3C system modeling Th1 vascular smooth muscle inflammation. VCAM-1 in the CASM3C system is regulated by the following pathways: EGFR, HDAC, JAK.
	CD141 / Thrombomodulin	Thrombomodulin/CD141 is a cell surface receptor for complement factor 3b with anti-coagulant, anti-inflammatory and cytoprotective activities during the process of fibrinolysis, coagulation and thrombosis. Thrombomodulin is categorized as a hemostasis-related activity in the CASM3C system modeling Th1 vascular smooth muscle inflammation. TM in the CASM3C system is regulated by the following pathways: Glucocorticoid R, HMG-CoA Reductase, IKK2, RAR/RXR, Vitamin D R.
	CD142/Tissue Factor	Tissue Factor/CD142 is a cell surface receptor for coagulation factor VII that promotes the formation of thrombin during the process of thrombosis and coagulation in the vascular smooth muscle environment. Tissue Factor is categorized as a hemostasis-related activity in the CASM3C system modeling Th1 vascular smooth muscle inflammation. TF in the CASM3C system is regulated by the following pathways: Glucocorticoid R, HDAC.
	CD87/uPAR	Urokinase plasminogen activator receptor (uPAR/CD87) is a cell surface receptor for urokinse plasminogen activator (uPA) involved in the regulation of pericellular proteolysis, cell migration, cancer cell invasion, and angiogenesis. uPAR is categorized as a tissue remodeling-related activity in the CASM3C system modeling Th1 vascular smooth muscle inflammation. uPAR in the CASM3C system is regulated by the following pathways: EGFR, Glucocorticoid R, HDAC, Histamine H1R, JAK.
	CXCL8/IL-8	Interleukin-8 (IL-8/CXCL8) is a chemokine that mediates neutrophil recruitment into acute inflammatory sites. IL-8 is categorized as an inflammation-related activity in the CASM3C system modeling Th1 vascular smooth muscle inflammation. IL-8 in the CASM3C system is regulated by the following pathways: Glucocorticoid R, IKK2.
	CXCL9/MIG	Monokine induced by gamma interferon (MIG/CXCL9) is a chemokine that mediates T cell recruitment. MIG is categorized as an inflammation-related activity in the CASM3C system modeling Th1 vascular smooth muscle inflammation. MIG in the CASM3C system is regulated by the following pathways: HDAC, IKK2, JAK.
	HLA-DR	HLA-DR is a cell surface heterodimer involved in antigen presentation and is involved in T cell activation and immune responses. HLA-DR is categorized as an immunomodulatory-related activity in the CASM3C system modeling Th1 vascular smooth muscle inflammation. HLA-DR in the CASM3C system is regulated by the following pathways: HDAC, JAK, p38 MAPK.
	IL-6	Interleukin-6 (IL-6) is a secreted proinflammatory cytokine and acute phase reactant. Secreted IL-6 (sIL-6) is categorized as an inflammation-related activity in the CASM3C system modeling Th1 vascular smooth muscle inflammation. IL-6 in the CASM3C system is regulated by the following pathways: Glucocorticoid R, HDAC, IKK2, Vitamin D R.
	LDLR	Low density lipoprotein receptor (LDLR) in the CASM3C system modeling Th1 vascular smooth muscle inflammation is a cell surface receptor involved in cholesterol regulation that mediates endocytosis of low density lipoprotein (LDL).
	M-CSF	Macrophage colony-stimulating factor (M-CSF) is a secreted and cell surface cytokine that mediates macrophage differentiation. M-CSF is categorized as an immunomodulatory-related activity in the CASM3C system modeling Th1 vascular smooth muscle inflammation. M-CSF in the CASM3C system is regulated by the following pathways: Glucocorticoid R, JAK.
	PAI-I	Plasminogen activator inhibitor-1 (PAI-I) is a serine proteinase inhibitor and inhibitor of tissue plasminogen activator (tPA) and urokinase (uPA) and is involved in tissue remodeling and fibrinolysis. PAI-I is categorized as a tissue remodeling-related activity in the CASM3C system modeling Th1 vascular smooth muscle inflammation.
	Proliferation	Proliferation in the CASM3C system is a measure of coronary artery smooth muscle cell proliferation which is important to the process of vascular biology and restenosis. Prolif in the CASM3C system is regulated by the following pathways: EGFR, HDAC, HMG-CoA Reductase, Histamine H1R, IKK2, Microtubule, PI3K, RAR/RXR, mTOR.
	Serum Amyloid A	Serum Amyloid A (SAA) is a member of the apolipoprotein family that is an acute phase reactant. SAA is categorized as an inflammation-related activity in the CASM3C system modeling Th1 vascular smooth muscle inflammation. SAA in the CASM3C system is regulated by the following pathways: Glucocorticoid R, HDAC.
	SRB	SRB in the CASM3C system is a measure of the total protein content of coronary artery smooth muscle cells. Cell viability of adherent cells is measured by Sulforhodamine B (SRB) staining, a method that determines cell density by measuring total protein content of test wells. SRB in the CASM3C system is regulated by the following pathways: Histamine H1R.
HDF3CGF	CCL2/MCP-1	Monocyte chemoattractant protein-1 (MCP-1/CCL2) is a chemoattractant cytokine (chemokine) that regulates the recruitment of monocytes and T cells into sites of inflammation. MCP-1 is categorized as an inflammation-related activity in the HDF3CGF system modeling Th1 inflammation involved in wound healing and matrix remodeling of the skin. MCP-1 in the HDF3CGF system is regulated by the following pathways: IKK2, PI3K, RAR/RXR, Src.
	CD106/VCAM-1	Vascular Cell Adhesion Molecule 1 (VCAM-1/CD106) is a cell adhesion molecule that mediates adhesion of monocytes and T cells to endothelial cells. VCAM-1 is categorized as an inflammation-related activity in the HDF3CGF system modeling Th1 inflammation involved in wound healing and matrix remodeling of the skin. VCAM-1 in the HDF3CGF system is regulated by the following pathways: IKK2, JAK, MEK, RAR/RXR, Src, Vitamin D R.
	CD54/ICAM-1	Intercellular Adhesion Molecule 1 (ICAM-1/CD54) is a cell adhesion molecule that mediates leukocyte-endothelial cell adhesion and leukocyte recruitment. ICAM-1 is categorized as an inflammation-related activity in the HDF3CGF system modeling Th1 inflammation involved in wound healing and matrix remodeling of the skin. ICAM-1 in the HDF3CGF system is regulated by the following pathways: RAR/RXR.

System	Biomarker	Description
HDF3CGF	Collagen I	Collagen I is involved in tissue remodeling and fibrosis, and is the most common fibrillar collagen that is found in skin, bone, tendons and other connective tissues. Collagen I is categorized as a tissue remodeling-related activity in the HDF3CGF system modeling Th1 inflammation involved in wound healing and matrix remodeling of the skin.
	Collagen III	Collagen III is an extracellular matrix protein and fibrillar collagen found in extensible connective tissues (skin, lung and vascular system) and is involved in cell adhesion, cell migration, tissue remodeling. Collagen III is categorized as a tissue remodeling-related activity in the HDF3CGF system modeling Th1 inflammation involved in wound healing and matrix remodeling of the skin. Collagen III in the HDF3CGF system is regulated by the following pathways: RAR/RXR.
	CXCL10/IP-10	Interferon-gamma-inducible protein 10 (IP-10/CXCL10) is a chemokine that mediates T cell, monocyte and dendritic cell chemotaxis. IP-10 is categorized as an inflammation-related activity in the HDF3CGF system modeling Th1 inflammation involved in wound healing and matrix remodeling of the skin. IP-10 in the HDF3CGF system is regulated by the following pathways: HDAC, Histamine H1R, IKK2, JAK, RAR/RXR, Src.
	CXCL11/I-TAC	Interferon-inducible T Cell Alpha Chemoattractant (I-TAC/CXCL11) is a chemokine that mediates T cell and monocyte chemotaxis. I-TAC is categorized as an inflammation-related activity in the HDF3CGF system modeling Th1 inflammation involved in wound healing and matrix remodeling of the skin. I-TAC in the HDF3CGF system is regulated by the following pathways: HDAC, JAK, RAR/RXR.
	CXCL8/IL-8	Interleukin-8 (IL-8/CXCL8) is a chemokine that mediates neutrophil recruitment into acute inflammatory sites. IL-8 is categorized as an inflammation-related activity in the HDF3CGF system modeling Th1 inflammation involved in wound healing and matrix remodeling of the skin. IL-8 in the HDF3CGF system is regulated by the following pathways: IKK2.
	CXCL9/MIG	Monokine induced by gamma interferon (MIG/CXCL9) is a chemokine that mediates T cell recruitment. MIG is categorized as an inflammation-related activity in the HDF3CGF system modeling Th1 inflammation involved in wound healing and matrix remodeling of the skin. MIG in the HDF3CGF system is regulated by the following pathways: HDAC, JAK, RAR/RXR, Src.
	EGFR	Epidermal growth factor receptor (EGFR) is a cell surface receptor for epidermal growth factor involved in cell proliferation, cell differentiation, tissue remodeling and tumor growth. EGFR is categorized as a tissue remodeling-related activity in the HDF3CGF system modeling Th1 inflammation involved in wound healing and matrix remodeling of the skin. EGFR in the HDF3CGF system is regulated by the following pathways: EGFR.
	M-CSF	Macrophage colony-stimulating factor (M-CSF) is a secreted and cell surface cytokine that mediates macrophage differentiation. M-CSF is categorized as a tissue remodeling-related activity in the HDF3CGF system modeling Th1 inflammation involved in wound healing and matrix remodeling of the skin. M-CSF in the HDF3CGF system is regulated by the following pathways: IKK2, JAK, MEK, RAR/RXR, Src.
	MMP-1	Matrix metalloproteinase-1 (MMP-1) is an interstitial collagenase that degrades collagens I, II and III and is involved in the process of tissue remodeling. MMP-1 is categorized as a tissue remodeling-related activity in the HDF3CGF system modeling Th1 inflammation involved in wound healing and matrix remodeling of the skin. MMP-1 in the HDF3CGF system is regulated by the following pathways: MEK, RAR/RXR, Src.
	PAI-I	Plasminogen activator inhibitor-1 (PAI-I) is a serine proteinase inhibitor and inhibitor of tissue plasminogen activator (tPA) and urokinase (uPA) and is involved in tissue remodeling and fibrinolysis. PAI-I is categorized as a tissue remodeling-related activity in the HDF3CGF system modeling Th1 inflammation involved in wound healing and matrix remodeling of the skin. PAI-1 in the HDF3CGF system is regulated by the following pathways: EGFR, Histamine H1R, MEK, PDE4, PI3K, RAR/RXR, Src, mTOR.
	Proliferation_72hr	Proliferation_72hr in the HDF3CGF system is a measure of dermal fibroblast proliferation which is important to the process of wound healing and fibrosis. Prolif 72 in the HDF3CGF system is regulated by the following pathways: EGFR, HDAC, HMG-CoA Reductase, Histamine H1R, IKK2, Microtubule, PI3K, RAR/RXR, Src, mTOR.
	SRB	SRB in the HDF3CGF system is a measure of the total protein content of dermal fibroblasts. Cell viability of adherent cells is measured by Sulforhodamine B (SRB) staining, a method that determines cell density by measuring total protein content of test wells. SRB in the HDF3CGF system is regulated by the following pathways: IKK2, RAR/RXR.
	TIMP-1	TIMP-1 is a tissue inhibitor of matrix metalloprotease-7 (MMP-7) and other MMPs, and is involved in tissue remodeling, angiogenesis and fibrosis. TIMP-1 is categorized as a tissue remodeling-related activity in the HDF3CGF system modeling Th1 inflammation involved in wound healing and matrix remodeling of the skin. TIMP-1 in the HDF3CGF system is regulated by the following pathways: Vitamin D R.
	TIMP-2	TIMP-2 is a tissue inhibitor of matrix metalloproteases and is involved in tissue remodeling, angiogenesis and fibrosis. TIMP-2 is categorized as a tissue remodeling-related activity in the HDF3CGF system modeling Th1 inflammation involved in wound healing and matrix remodeling of the skin.
KF3CT	CCL2/MCP-1	Monocyte chemoattractant protein-1 (MCP-1/CCL2) is a chemoattractant cytokine (chemokine) that regulates the recruitment of monocytes and T cells into sites of inflammation. MCP-1 is categorized as an inflammation-related activity in the KF3CT system modeling Th1 cutaneous inflammation. MCP-1 in the KF3CT system is regulated by the following pathways: IKK2.
	CD54/ICAM-1	Intercellular Adhesion Molecule 1 (ICAM-1/CD54) is a cell adhesion molecule that mediates leukocyte-endothelial cell adhesion and leukocyte recruitment. ICAM-1 is categorized as an inflammation-related activity in the KF3CT system modeling Th1 cutaneous inflammation. ICAM-1 in the KF3CT system is regulated by the following pathways: JAK.
	CXCL10/IP-10	Interferon-gamma-inducible protein 10 (IP-10/CXCL10) is a chemokine that mediates T cell, monocyte and dendritic cell chemotaxis. IP-10 is categorized as an inflammation-related activity in the KF3CT system modeling Th1 cutaneous inflammation. IP-10 in the KF3CT system is regulated by the following pathways: JAK, RAR/RXR, Src.
	CXCL8/IL-8	Interleukin-8 (IL-8/CXCL8) is a chemokine that mediates neutrophil recruitment into acute inflammatory sites. IL-8 is categorized as an inflammation-related activity in the KF3CT system modeling Th1 cutaneous inflammation. IL-8 in the KF3CT system is regulated by the following pathways: IKK2.
	CXCL9/MIG	Monokine induced by gamma interferon (MIG/CXCL9) is a chemokine that mediates T cell recruitment. MIG is categorized as an inflammation-related activity in the KF3CT system modeling Th1 cutaneous inflammation. MIG in the KF3CT system is regulated by the following pathways: JAK, Src.
	IL-1 alpha	Interleukin-1 alpha (IL-1α) is a secreted proinflammatory cytokine involved in endothelial cell activation and neutrophil recruitment. Secreted IL-1α (sIL-1α) is categorized as an inflammation-related activity in the KF3CT system modeling Th1 cutaneous inflammation. IL-1a in the KF3CT system is regulated by the following pathways: IKK2, Src.
	MMP-9	Matrix metalloproteinase-9 (MMP-9) is a gelatinase B that degrades collagen IV and gelatin and is involved in cutaneous remodeling. MMP-9 is categorized as a tissue remodeling-related activity in the KF3CT system modeling Th1 cutaneous inflammation. MMP-9 in the KF3CT system is regulated by the following pathways: HDAC, Histamine H1R, IKK2, Src.
	PAI-I	Plasminogen activator inhibitor-1 (PAI-I) is a serine proteinase inhibitor and inhibitor of tissue plasminogen activator (tPA) and urokinase (uPA) and is involved in tissue remodeling and fibrinolysis. PAI-I is categorized as a tissue remodeling-related activity in the KF3CT system modeling Th1 cutaneous inflammation. PAI-1 in the KF3CT system is regulated by the following pathways: PI3K, RAR/RXR.
	SRB	SRB in the KF3CT system is a measure of the total protein content of keratinocytes and dermal fibroblasts. Cell viability of adherent cells is measured by Sulforhodamine B (SRB) staining, a method that determines cell density by measuring total protein content of test wells.
	TIMP-2	TIMP-2 is a tissue inhibitor of matrix metalloproteases and is involved in tissue remodeling, angiogenesis and fibrosis. TIMP-2 is categorized as a tissue remodeling-related activity in the KF3CT system modeling Th1 cutaneous inflammation. TIMP-2 in the KF3CT system is regulated by the following pathways: IKK2, Src.
	uPA	Urokinase plasminogen activator (uPA) is a serine protease with thrombolytic activity. Triggers fibrinolysis and extracellular matrix degradation. uPA is categorized as a tissue remodeling-related activity in the KF3CT system modeling Th1 cutaneous inflammation. uPA in the KF3CT system is regulated by the following pathways: HDAC, IKK2, Src.

System	Biomarker	Description
MyoF	alpha-SM Actin	Alpha-smooth muscle actin (α-SMA) is a protein involved in muscle contraction, cell motility, structure and integrity and is a marker for activated myofibroblast phenotype. α-SMA is categorized as a tissue remodeling-related activity in the MyoF system modeling pulmonary myofibroblast development. aSMA in the MyoF system is regulated by the following pathways: RAR/RXR, Src.
	bFGF	Basic fibroblast growth factor (bFGF) is a pro-fibrotic growth factor that drives fibroblast proliferation, migration and fibronectin synthesis. bFGF is categorized as a tissue remodeling-related activity in the MyoF system modeling pulmonary myofibroblast development. bFGF in the MyoF system is regulated by the following pathways: HDAC.
	CD106/VCAM-1	Vascular Cell Adhesion Molecule 1 (VCAM-1/CD106) is a cell adhesion molecule that mediates adhesion of monocytes and T cells to endothelial cells. VCAM-1 is categorized as an inflammation-related activity in the MyoF system modeling pulmonary myofibroblast development. VCAM-1 in the MyoF system is regulated by the following pathways: Histamine H1R, IKK2, PI3K, RAR/RXR, TNF-alpha.
	Collagen I	Collagen I is involved in tissue remodeling and fibrosis, and is the most common fibrillar collagen that is found in skin, bone, tendons and other connective tissues. Collagen I is categorized a tissue remodeling-related activity in the MyoF system modeling pulmonary myofibroblast development. Collagen I in the MyoF system is regulated by the following pathways: RAR/RXR.
	Collagen III	Collagen III is an extracellular matrix protein and fibrillar collagen found in extensible connective tissues (skin, lung and vascular system) and is involved in cell adhesion, cell migration, tissue remodeling. Collagen III is categorized a tissue remodeling-related activity in the MyoF system modeling pulmonary myofibroblast development. Collagen III in the MyoF system is regulated by the following pathways: HDAC, Histamine H1R, IKK2, RAR/RXR.
	Collagen IV	Collagen IV is the major structural component of the basal lamina. Collagen IV is categorized a tissue remodeling-related activity in the MyoF system modeling pulmonary myofibroblast development.
	CXCL8/IL-8	Interleukin-8 (IL-8/CXCL8) is a chemokine that mediates neutrophil recruitment into acute inflammatory sites. IL-8 is categorized as an inflammation-related activity in the MyoF system modeling pulmonary myofibroblast development. IL-8 in the MyoF system is regulated by the following pathways: Glucocorticoid R, IKK2, TNF-alpha, Vitamin D R, p38 MAPK.
	Decorin	Decorin is a proteoglycan that is a component of connective tissue and is involved in collagen and matrix assembly. Decorin is categorized as a tissue remodeling-related activity in the MyoF system modeling pulmonary myofibroblast development.
	MMP-1	Matrix metalloproteinase-1 (MMP-1) is an interstitial collagenase that degrades collagens I, II and III and is involved in the process of tissue remodeling. MMP-1 is categorized as a tissue remodeling-related activity in the MyoF system modeling pulmonary myofibroblast development. MMP-1 in the MyoF system is regulated by the following pathways: IKK2, Microtubule.
	PAI-I	Plasminogen activator inhibitor-1 (PAI-I) is a serine proteinase inhibitor and inhibitor of tissue plasminogen activator (tPA) and urokinase (uPA) and is involved in tissue remodeling and fibrinolysis. PAI-I is categorized as a tissue remodeling-related activity in the MyoF system modeling pulmonary myofibroblast development. PAI-1 in the MyoF system is regulated by the following pathways: PI3K, Src.
	SRB	SRB in the MyoF system is a measure of the total protein content of lung fibroblasts. Cell viability of adherent cells is measured by Sulforhodamine B (SRB) staining, a method that determines cell density by measuring total protein content of test wells. SRB in the MyoF system is regulated by the following pathways: HDAC, Histamine H1R, IKK2, RAR/RXR.
	TIMP-1	TIMP-1 is a tissue inhibitor of matrix metalloprotease-7 (MMP-7) and other MMPs, and is involved in tissue remodeling, angiogenesis and fibrosis. TIMP-1 is categorized as a tissue remodeling-related activity in the MyoF system modeling pulmonary myofibroblast development. TIMP-1 in the MyoF system is regulated by the following pathways: Glucocorticoid R.
/Mphg	CCL2/MCP-1	Monocyte chemoattractant protein-1 (MCP-1/CCL2) is a chemoattractant cytokine (chemokine) that regulates the recruitment of monocytes and T cells into sites of inflammation. MCP-1 is categorized as an inflammation-related activity in the /Mphg system modeling macrophage-driven Th1 vascular inflammation. MCP-1 in the lMphg system is regulated by the following pathways: Glucocorticoid R, IKK2.
	CCL3/MIP-1 alpha	Macrophage inflammatory protein 1 alpha (MIP-1α/CCL3) is a pro-inflammatory chemokine that mediates leukocyte recruitment to sites of inflammation. MIP-1α is categorized as an inflammation-related activity in the /Mphg system modeling macrophage-driven Th1 vascular inflammation. MIP-1a in the lMphg system is regulated by the following pathways: Glucocorticoid R, IKK2.
	CD106/VCAM-1	Vascular Cell Adhesion Molecule 1 (VCAM-1/CD106) is a cell adhesion molecule that mediates adhesion of monocytes and T cells to endothelial cells. VCAM-1 is categorized as an inflammation-related activity in the /Mphg system modeling macrophage-driven Th1 vascular inflammation. VCAM-1 in the lMphg system is regulated by the following pathways: IKK2, TNF-alpha.
	CD40	CD40 is a cell surface adhesion receptor and costimulatory receptor for T cell activation that is expressed on antigen presenting cells, endothelial cells, smooth muscle cells, fibroblasts and epithelial cells. CD40 is categorized as an immunomodulatory-related activity in the /Mphg system modeling macrophage-driven Th1 vascular inflammation. CD40 in the lMphg system is regulated by the following pathways: IKK2.
	CD62E/E-Selectin	E-Selectin/CD62E is a cell adhesion molecule expressed only on endothelial cells that mediates leukocyte-endothelial cell interactions. E-Selectin is categorized as an inflammation-related activity in the /Mphg system modeling macrophage-driven Th1 vascular inflammation. E-selectin in the lMphg system is regulated by the following pathways: Glucocorticoid R, HMG-CoA Reductase, IKK2, TNF-alpha, p38 MAPK.
	CD69	CD69 is a cell surface activation antigen that is induced early during immune activation and is involved in macrophage activation. CD69 is categorized as an immunomodulatory-related activity in the /Mphg system modeling macrophage-driven Th1 vascular inflammation.
	CXCL8/IL-8	Interleukin-8 (IL-8/CXCL8) is a chemokine that mediates neutrophil recruitment into acute inflammatory sites. IL-8 is categorized as an inflammation-related activity in the /Mphg system modeling macrophage-driven Th1 vascular inflammation. IL-8 in the lMphg system is regulated by the following pathways: Glucocorticoid R, IKK2, Vitamin D R, p38 MAPK.
	IL-1 alpha	Interleukin-1 alpha (IL-1α) is a secreted proinflammatory cytokine involved in endothelial cell activation and neutrophil recruitment. Secreted IL-1α (sIL-1α) is categorized as an inflammation-related activity in the /Mphg system modeling macrophage-driven Th1 vascular inflammation. IL-1a in the lMphg system is regulated by the following pathways: Glucocorticoid R, IKK2.
	M-CSF	Macrophage colony-stimulating factor (M-CSF) is a secreted and cell surface cytokine that mediates macrophage differentiation. M-CSF is categorized as an immunomodulatory-related activity in the /Mphg system modeling macrophage-driven Th1 vascular inflammation.
	sIL-10	Interleukin-10 (IL-10) is a secreted anti-inflammatory cytokine. Secreted IL-10 (sIL-10) is categorized as an immunomodulatory-related activity in the /Mphg system modeling macrophage-driven Th1 vascular inflammation. sIL-10 in the lMphg system is regulated by the following pathways: EGFR, Glucocorticoid R, HDAC, Histamine H1R, JAK, MEK, PDE4, RAR/RXR, Src, TNF-alpha, mTOR, p38 MAPK.
	SRB	SRB in the /Mphg system is a measure of the total protein content of venular endothelial cells and macrophages. Cell viability of adherent cells is measured by Sulforhodamine B (SRB) staining, a method that determines cell density by measuring total protein content of test wells.
	SRB-Mphg	SRB-Mphg in the /Mphg system is a measure of the total protein content of macrophages alone. Cell viability of adherent cells is measured by Sulforhodamine B (SRB) staining, a method that determines cell density by measuring total protein content of test wells.

BioMAP Service Offerings

Service Product	Description	Data Report Deliverables
Diversity PLUS Panel	• 12 Systems, 148 readouts • 4 conc. / test agent / singlicate	• Profile plots and data tables, biomarker activity annotation • Benchmarking – comparison of test agent and selected reference compound • Unsupervised similarity search of BioMAP Database, table of top matches and overlay plot of test agent and top match • Cluster analysis (for ≥ 3 test agents) • Mechanism HeatMAP Anlaysis – biomarker activity of each test agent is compared to 19 consensus mechanism class profiles of well-characterized drugs
Predictive Tox Analysis	• 28 mechanism models • 10 Tox Associations • 4 conc. / test agent / singlicate	• Overlay plot of test agent and result of SVM mechanism model prediction analysis in 8 Systems • Identification and description of the physiological relevance of potential alerts
T Cell Autoimmune Panel	• 4 Systems, 51 readouts • 4 conc. / test agent / triplicate	• Profile plots and data tables, biomarker activity annotation • Benchmarking – overlay plots of test agent and 6 standard-of-care (SOC) reference compounds • Cluster analysis of test agents and 6 SOC compounds
Oncology Panels	• Select from 2 Oncology Panels CRC Systems: StroHT29, VascHT29 NSCLC Systems: StroNSCLC, VascNSCLC • All readouts in selected Systems • 4 conc. / test agent / triplicate	• Profile plots and data tables, biomarker activity annotation • Benchmarking – comparison of test agent and selected reference compound
Fibrosis Panel	• 3 Systems, 54 readouts • SAEMyoF, MyoF, REMyoF • 4 conc. / test agent / triplicate	• Profile plots and data tables, biomarker activity annotation • Benchmarking – comparison of test agent and selected reference compound

Combo ELECT	• Select any of >50 validated Systems
	• All readouts in selected System(s)
	• Individual agent analysis
	  • 4 conc. / triplicate
	• Comparative checkerboard analysis
	  • 4x4 matrix of 16 combinations
	  • 4 conc. / triplicate
		• Profile plots and data tables, biomarker activity annotation
		• Overlay plots of single test agents with corresponding combination
		• Combination analysis – identification of significantly different biomarker activities compared to single test agents
		• Checkerboard summary of differential activities for all combinations tested

Overlay ELECT	• Select any Panel or System(s) • Overlay 2 compounds within the selected Panel or System(s)	• Benchmarking – comparison of test agent and selected reference compound
Data Package	• Select any available compound in the Diversity PLUS Panel, Oncology Panel or Fibrosis Panel	• Inquire about available reference compounds: Profile plots and data tables, biomarker activity annotation

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