Advances in tools to compare potency, efficacy, safety & quality of follow-on biologics or biosimilars

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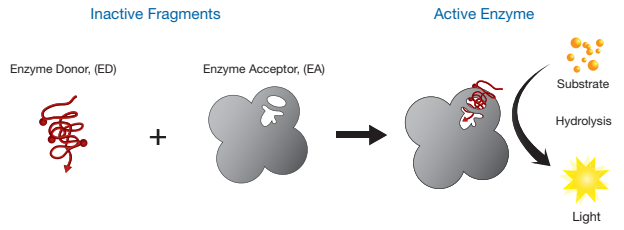
Abstract

Regulatory authorities define biosimilars as drugs that are “highly similar” to or “interchangeable” with an approved biologic. The lack of a pre-defined path to demonstrate similarity leaves the burden of proof on the biosimilar developers. Key requirements for efficacy, quality and potency testing often require a complex set of bioassays and/or cell-based assays that are also used to assess any unwanted clinical immune response to their biosimilar. This is particularly challenging to address for biosimilars.

Commercially available cell-based assays for the development of biosimilars targeting different marketed drugs can significantly shorten development time while enabling easy adoption and greater reproducibility across multiple global sites. Examples discussed here include cell-based assays for Bevacizumab, highlighting how commercial tools can enable a biosimilar developer to move more quickly and be the first to market or submission.

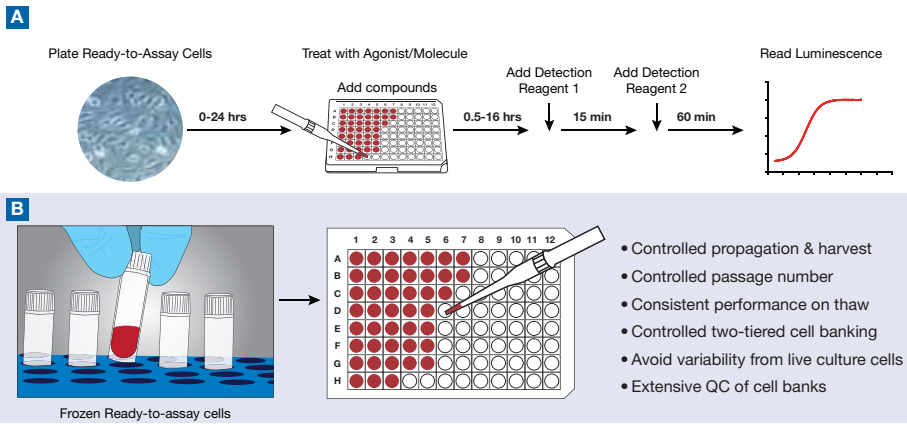
Additionally, the emergence of complex human primary cell culture and co-culture in vitro models as well as biomarker-based phenotypic assays offer high-throughput and robust solutions for assessing mechanism of action and human safety and efficacy.

PathHunter Enzyme Fragment Complementation



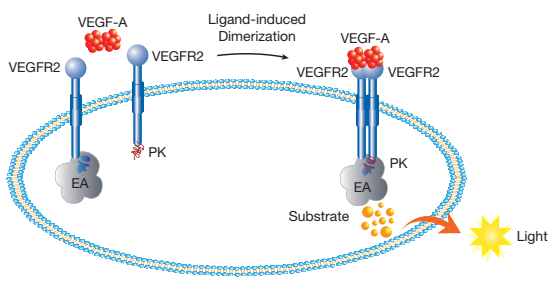
DiscoveRx’s proprietary PathHunter Enzyme Fragment Complementation (EFC) technology consists of the β-galactosidase (β-gal) enzyme, split into two inactive components, the enzyme donor peptide (ED) and enzyme acceptor (EA). When brought together in close proximity, ED complements with EA forming active β-gal. The active enzyme catalyzes the substrate generating chemiluminescent light, providing a highly amplified signal and thus an assay of high sensitivity.

A Simple Homogenous Protocol With Rapid Results



PathHunter bioassay kits use a simple homogenous protocol with rapid results. **A.** Ready-to-assay cells from DiscoveRx are plated on a 96-well plate and incubated for 0-24 hours at 37°C. The agonist/test molecule is added to the plate and incubated for 0.5-16 hours. The detection reagents are added sequentially in two addition steps and the chemiluminescent signal can be detected on any plate-reading luminometer. **B.** Cells manufactured in bioassay kits are meant for single use in ready-to-assay vials. The frozen cells are plated directly onto plates to run the assay and this format has several advantages as outlined above.

PathHunter Bevacizumab Bioassay



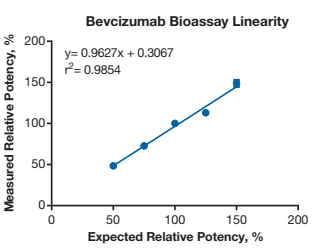
Using EFC, we have generated a simple cell-based assay for VEGF-A and the VEGFR2 receptor. The assay measures VEGFR2 receptor homodimerization by VEGF-A as the first step in its activation cascade that eventually leads to angiogenesis. The VEGFR2 receptor is tagged with both the ProLink (PK) and the Enzyme Acceptor (EA). Upon ligand induced activation, receptor dimerization occurs which forces the two b-gal components to complement. This creates an active enzyme that hydrolyzes substrate generating a chemiluminescent signal.

Excellent Accuracy and Precision for PathHunter Bevacizumab Bioassay

Assay Accuracy & Precision

	Expected Potency, %	Measured Potency, %	Mean Potency, %	SD	Recovery, %	RSD, %
Day 1	150	151.0	148.7	4.04	99.1	5.12
Day 2		144.0				
Day 3		151.0				
Day 1	125	109.0	113.0	3.46	90.4	3.07
Day 2		115.0				
Day 3		115.0				
Day 1	75	74.4	72.7	2.47	97.0	3.12
Day 2		73.9				
Day 3		69.9				
Day 1	50	48.8	48.5	2.42	96.9	4.99
Day 2		45.9				
Day 3		50.7				

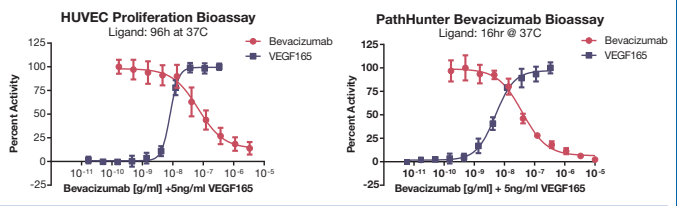
Accuracy= 95.9% **Precision= 4.1%**



VEGF-A is known to cause homodimerization of VEGFR2 (KDR), as the first step in the activation cascade of these receptors. Anti-VEGF-A antibodies such as Avastin (Bevacizumab) , Ranibizumab and Aflibercept bind to VEGF-A and prevent this dimer formation, leading to inhibition of VEGF-A dependent signaling. Here we have tested the VEGFR2 homodimer assay with VEGF-A, demonstrating a robust response and a high level of reproducibility with multiple runs.

The VEGFR dimerization assay was tested with four test samples, from 50% to 150%, compared to a reference standard (100%). The measured relative potencies were plotted against the expected relative potencies with a very high degree of accuracy and precision.

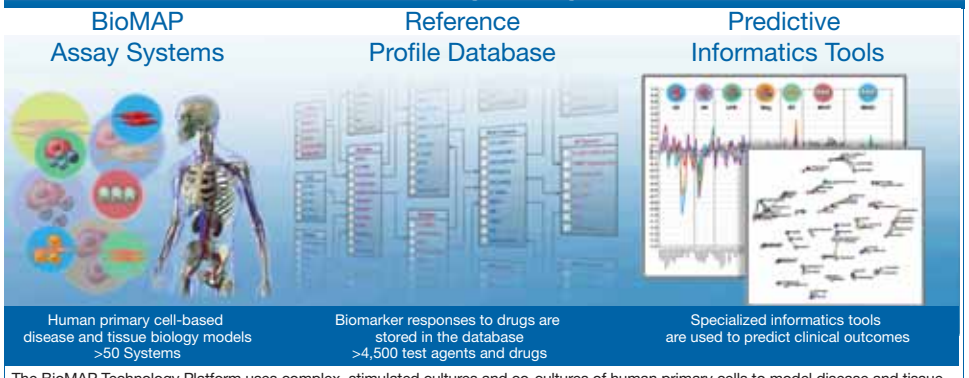
HUVEC Proliferation Assay vs. PathHunter Bevacizumab Bioassay



	HUVEC Proliferation Assay	PathHunter Bevacizumab Bioassay
EC_{50} VEGF165	8.18 ng/mL	5 ng/mL
EC_{50} Bevacizumab	67.88 ng/mL	38.96 ng/mL
S:B Ratio	2.5 fold	>4.7 fold
Assay run time	96 hours	16 hours
Specificity	Low	High
Assay Output	MTT Readout	Chemiluminescence
Cell type	Primary cells with donor variability	Clonal, frozen ready-to-assay cells
Cell Culture	Required	No cell culture necessary

The HUVEC proliferation assay is another method to test drug potency for the anti-VEGF drugs. VEGF-A (VEGF165 is an active splice variant of VEGF-A) will cause HUVEC cells to proliferate and Bevacizumab will inhibit this by inhibiting VEGF-A interaction with VEGFR2. Here we have tested the PathHunter Bevacizumab bioassay with VEGF-A and bevacizumab (top right), demonstrating the robust and precise response to both agents, and EC/IC-50’s comparable to those obtained in the HUVEC proliferation assay (top left). The table below compares the proliferation assay and the PathHunter assay, demonstrating that the latter is quicker, more robust, highly specific and generates the better data without the need for any cell culture.

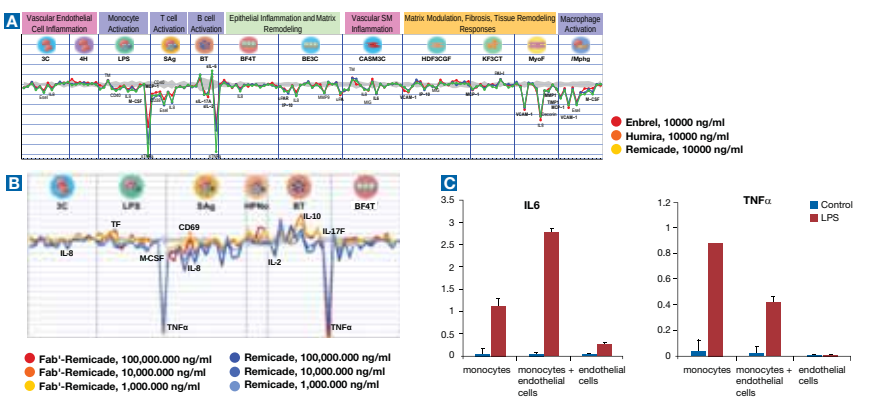
BioMAP® Platform of Human Primary Cell Systems



The BioMAP Technology Platform uses complex, stimulated cultures and co-cultures of human primary cells to model disease and tissue biology and relevant, activated signaling pathways. Combined with hundreds of translational protein biomarker assays, large reference database and proprietary informatics tools for analysis, the BioMAP Technology provides a quantitative, robust and reproducible solution for mechanism informed phenotypic screening. Characteristic signature profiles for each biologic tested can be used for comparison analysis to benchmark biosimilars to their innovator drug and can serve as an invaluable tool for helping predict clinical outcomes for efficacy and safety. Characteristic signature profiles can also be used to performed unsupervised similarity search against BioMAP Reference Database of >4,500 test agents and drugs to see if there are other biologics or compounds with similar profiles to potentially further elucidate the mechanism of action or on-target and/or off-target biology (data not shown).

BioMAP Diversity PLUS Panel Systems

System	Primary Human Cell Types	Disease /Tissue Relevance	Readouts
3C	Venular endothelial cells	Cardiovascular Disease, Chronic Inflammation	MCP-1, VCAM-1, TM, TF, ICAM-1, E-selectin, uPAR, IL-8, MIG, HLA-DR, Proliferation, SRB
4H	Venular endothelial cells	Asthma, Allergy, Oncology	MCP-1, Eotaxin-3, VCAM-1, P-selectin, uPAR, SRB, VEGFRII
LPS	Peripheral blood mononuclear cells + Venular endothelial cells	Cardiovascular Disease, Chronic Inflammation	MCP-1, VCAM-1, TM, TF, CD40, E-selectin, CD69, IL-8, IL-1α, M-CSF, sPGE2, SRB, sTNFα
SAg	Peripheral blood mononuclear cells + Venular endothelial cells	Autoimmune Disease, Chronic Inflammation	MCP-1, CD38, CD40, E-selectin, CD69, IL-8, MIG, PBMC Cytotoxicity, Proliferation, SRB
BT	B cells + Peripheral blood mononuclear cells	Asthma, Allergy, Oncology, Autoimmunity	B cell Proliferation, PBMC Cytotoxicity, Secreted IgG, sIL-17A, sIL-17F, sIL-2, sIL-6, sTNFα
BE3C	Bronchial epithelial cells	COPD, Lung Inflammation	ICAM-1, uPAR, IP-10, I-TAC, IL-8, MIG, EGFR, HLA-DR, IL-1α, Keratin 8/18, MMP-1, MMP-9, PAI-1, SRB, tPA, uPA
BF4T	Bronchial epithelial cells + Dermal fibroblasts	Asthma, Allergy, Fibrosis, Lung	MCP-1, Eotaxin-3, VCAM-1, ICAM-1, CD90, IL-8, IL-1α, Keratin 8/18, MMP-1, MMP-3, MMP-9, PAI-1, SRB, tPA, uPA
HDF3CGF	Dermal Fibroblasts	Fibrosis, Chronic Inflammation	MCP-1, VCAM-1, ICAM-1, Collagen I, Collagen III, IP-10, I-TAC, IL-8, MIG, EGFR, M-CSF, MMP-1, PAI-1, Proliferation, SRB, TIMP-1, TIMP-2
KF3CT	Keratinocytes + Dermal fibroblasts	Psoriasis, Dermatitis, Skin	MCP-1, ICAM-1, IP-10, IL-8, MIG, IL-1α, MMP-9, PAI-1, SRB, TIMP-2, uPA
CASM3C	Coronary artery smooth muscle cells	Cardiovascular Inflammation, Restenosis	MCP-1, VCAM-1, TM, TF, uPAR, IL-8, MIG, HLA-DR, IL-6, LDLR, M-CSF, PAI-1, Proliferation, SAA, SRB
MyoF	Lung fibroblasts	Fibrosis, Chronic Inflammation	α-SMA, bFGF, VCAM-1, Collagen I, Collagen III, Collagen IV, IL-8, Decorin, MMP-1, PAI-1, SRB, TIMP-1
IMphg	Venular endothelial cells + macrophages	Cardiovascular Inflammation, Restenosis, Chronic Inflammation	MCP-1, MIP-1α, VCAM-1, CD40, E-selectin, CD69, IL-8, IL-1α, M-CSF, sIL-10, SRB, SRB-Mphg



A. Overlay of BioMAP profiles for Enbrel, Humira and Remicade in the BioMAP Diversity PLUS™ panel. All three drugs show highly similar biomarker profiles, yet some differences are apparent. **B.** Bivalent Remicade and its monovalent Fab’ Remicade can be compared and differentiated through an overlay of their BioMAP profiles. **C.** In the BioMAP LPS System (Monocyte + HUVEC co-culture with LPS), IL-6 expression is enhanced and TNFα expression is suppressed relative to the non-BioMAP single cell type assay, showing that the BioMAP LPS System more closely resembles the *in vivo* human chronic inflammation and cardiovascular disease.

Summary & Conclusions

As innovator drugs come off patent and global biosimilar development activity increases, the need for specific tools to accelerate and reduce costs of biosimilar development is evident. Commercially available, ready-to-use cell-based assays will enable biosimilar developers to rapidly advance to the critical assay validation stage for potency and NAb assays. The ability to benchmark biosimilars against the innovator drug in a predictive, *in vitro* human primary cell-based system will provide a more systemic approach to predicting biosimilar efficacy and/or toxicity in human patients. Drug developers who are the quickest to demonstrate “similarity” of their molecule using available commercial tools can hope to be the first to market with their biosimilars.

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