eurofins | DISCOVERY

ADVANCE EMERGING THERAPEUTICS FOR UNDRUGGABLE TARGETS

Targeted Protein Degradation Drug Discovery Solutions

Eurofins Discovery.
Deep resources for success.

TARGETED PROTEIN DEGRADATION SOLUTIONS

For drug discovery

The Eurofins Discovery Protein Degradation and PROTAC Discovery Portfolio includes a comprehensive suite of target-based, cellular, and phenotypic approaches for a powerful drug discovery resource in the targeted protein degradation space.

AVAILABLE PLATFORMS

- E3*scan*™ assays – Identify and characterize new, potent, and selective ligands that bind and reprogram E3 ligase substrate specificity with the new E3*scan* ligand binding assay.
- KINOME*scan*®, BROMO*scan*®, and BCL2*scan* – Develop, characterize, and validate the warhead end of novel PROTACs.
- SPRINTer™ Protein Turnover Biosensor Assays – Enable sensitive quantitation of PROTAC-mediated degradation of targets with our EFC-based cellular biosensor cell lines which combine EFC detection technology with CRISPR genome editing.
- BioMAP® Phenotypic Platform – Evaluate the impact of target inhibitors and degraders on efficacy and safety-related translational biomarkers with our BioMAP phenotypic platform.
- Proteomic mass spectrometry – Characterize PROTAC selectivity across the proteome and provide a global readout on changes in protein synthesis and degradation using Proteomic mass spectrometry-based methodologies.
- Chemistry – Apply collective expertise in synthetic and medicinal chemistry to design oral drug-like properties into larger molecules in the 'beyond rule of 5' space.

FIVE PILLARS OF EUROFINS DISCOVERY'S TARGETED PROTEIN DEGRADATION PORTFOLIO

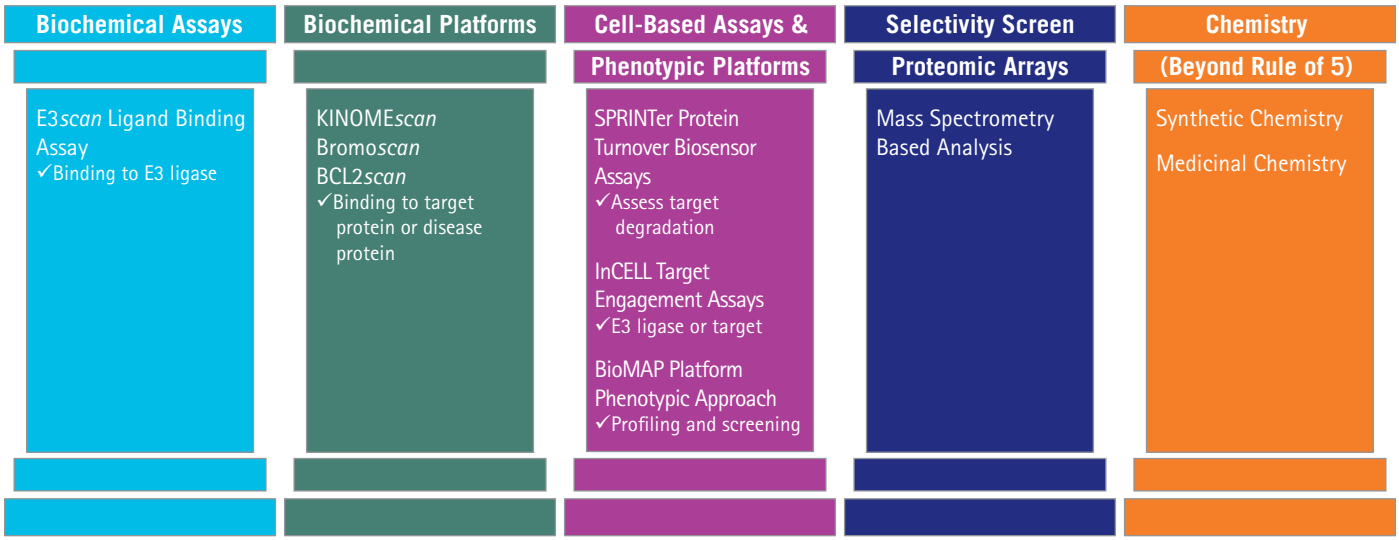

Figure 1. Eurofins Discovery's Five Pillars for Targeted Protein Degradation Includes Solutions to Progress Knowledge of Ligand–Target Interactions and Cellular Impact. Challenges to Lipinski's Rule of 5 and the design of orally available large-molecule therapies such as PROTACs are met by Eurofins Discovery chemists with expertise designing in the 'beyond rule of 5' space.

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E3*scan*™ LIGAND BINDING ASSAY TECHNOLOGY FOR TARGETED PROTEIN DEGRADATION

Eurofins Discovery has developed a first-in-class E3 ligase ligand binding assay based on proprietary KINOME*scan*® technology. KINOME*scan* technology utilizes DNA-tagged target proteins and a high sensitivity qPCR readout to enable sensitive (accurate ligand KDs measured down to low picomolar range) and quantitative competition binding assays (Figure 2).

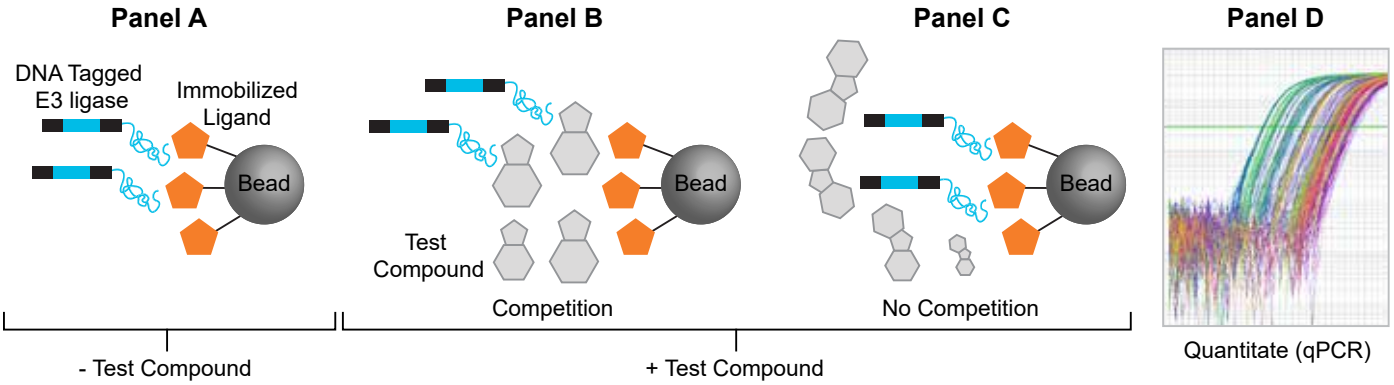

Figure 2. E3*scan* Ligand Binding Assay principle using KINOME*scan* technology.

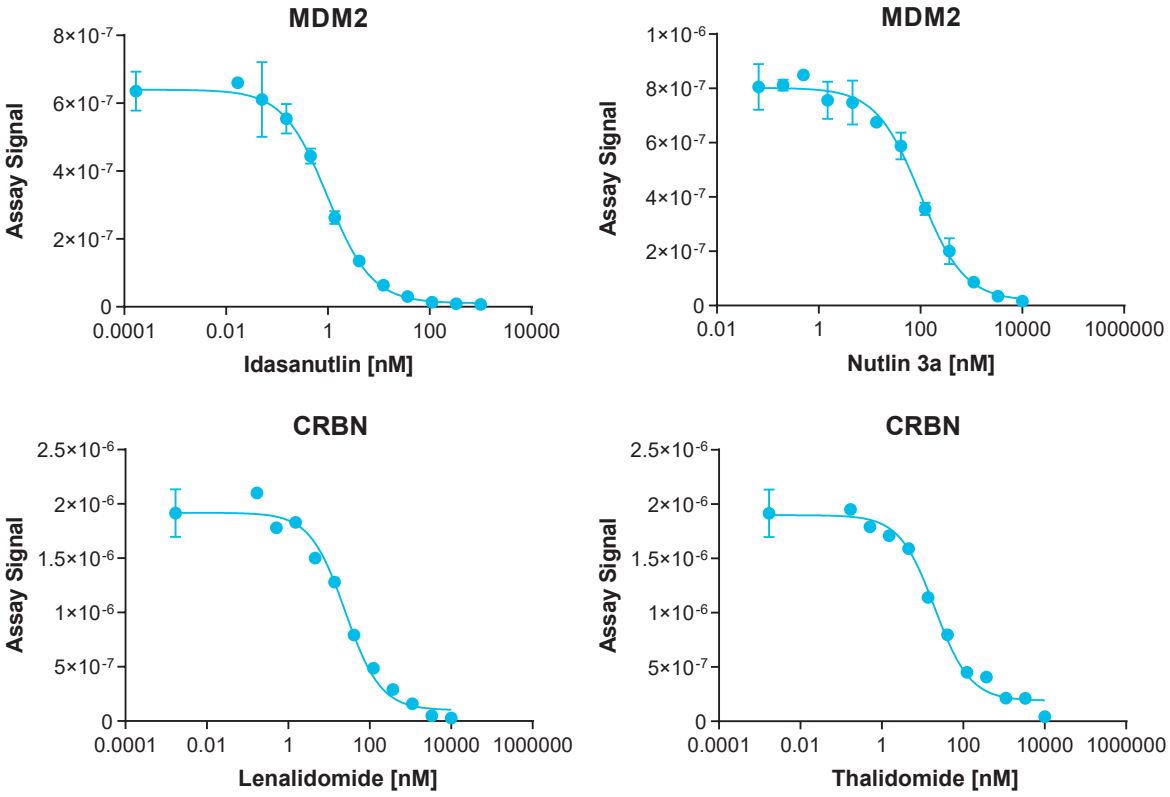

Figure 3. Representative data of MDM2 validation with two MDM2 Inhibitors Idasanutlin (Kd 1.3 nM) and Nutlin 3a (Kd 75.8 nM) and CRBN with CRBN inhibitors Thalidomide (Kd 23 nM) and Lenalidomide (Kd 28 nM).

E3*scan* Ligand Binding Assays

Assays available	VHL, MDM2, MDM4, CRBN, cIAP1, cIAP2 and XIAP (all full length)

>525 Assays available in KINOME*scan*, BROMO*scan*, and BCL2*scan*

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SPRINTer BIOSENSOR CELL LINE AND ASSAY DEVELOPMENT FOR TARGETED PROTEIN DEGRADATION

Sensitive quantitation of PROTAC-mediated endogenous protein degradation

Together with current excitement surrounding the field of targeted protein degradation, there remains an unmet need for better cell-based assays to evaluate the effects of targeted drugs such as PROTACs on endogenous protein levels. Commonly used technologies rely on the availability of specific antibodies for protein detection via immunoassay or the use of phenotypic endpoints such as proliferation. By combining CRISPR gene editing techniques with the well-established Enzyme Fragment Complementation (EFC) technology, Eurofins DiscoverX, the Eurofins Discovery Products Company, has engineered SPRINTer™ Protein Turnover Biosensor Assays that allow for sensitive quantitation of PROTAC-mediated degradation of a target of interest in physiologically relevant cell models using a homogeneous assay format.

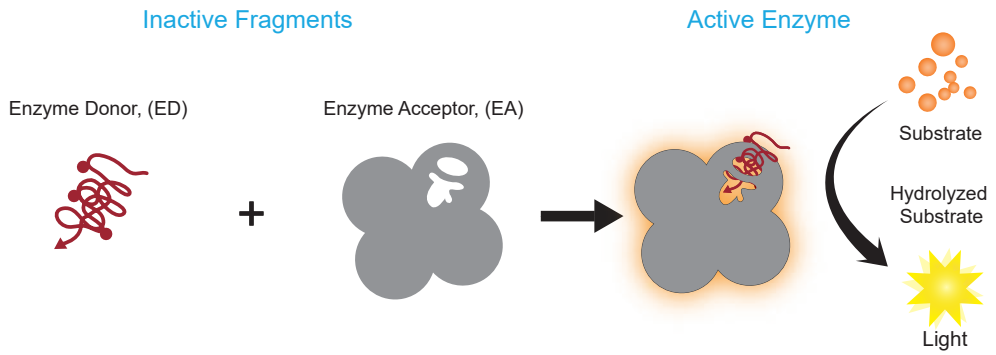
ASSAY PLATFORM BENEFITS:

- Robust and Sensitive – Quantify the kinetics of endogenous protein turnover in the discovery of disease-relevant therapeutic agents.
- Rapid Kinetics – High assay platform sensitivity allows detection of target protein turnover induced by PROTACs with more rapid kinetics than traditional phenotypic endpoint assays such as cell proliferation.
- Enables Discovery – Cell-based biosensor assays provide a screening platform to identify new molecular entities that modulate oncogenic protein levels for therapeutic development.
- Simple to Run – Homogeneous format and high sensitivity of the EFC assay allow direct and rapid quantitation of drug-induced changes in endogenous protein levels, making it well suited for medium to high throughput screens.

ASSAY PRINCIPLE:

Using advanced CRISPR technology combined with the well-established EFC system, a small β-galactosidase fragment (ED) is introduced into the BRD4 locus in a physiologically relevant blood cancer cell line.

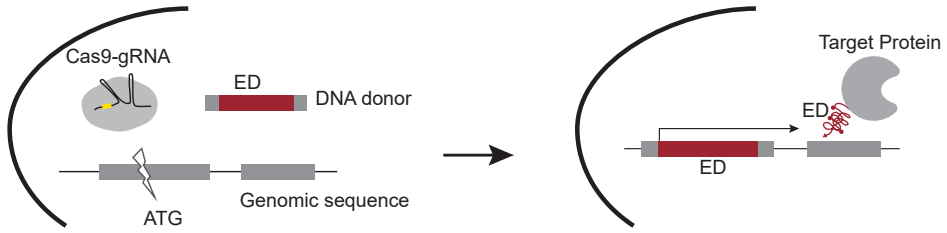
As shown in Figure 4 below, the ED fragment of the EFC reporter is introduced via gene editing into the endogenous cellular target. In the absence of drug, this ED-tagged protein produces a robust EFC signal upon the addition of and binding to a complementing EA. Treatment of these biosensor cells with a PROTAC specific to the ED-tagged protein results in proteasome-mediated degradation and subsequent loss of EFC signal upon addition of the complementing EA.



Learn more about SPRINTer assays by visiting discoverx.com/turnover

CRISPR-MEDIATED KNOCK-IN OF ED TAG INTO ENDOGENOUS BRD4 OR C-MYC LOCI

CRISPR-mediated homologous knock-in of ED tag



Assay for quantitation of endogenous protein turnover induced by PROTACs

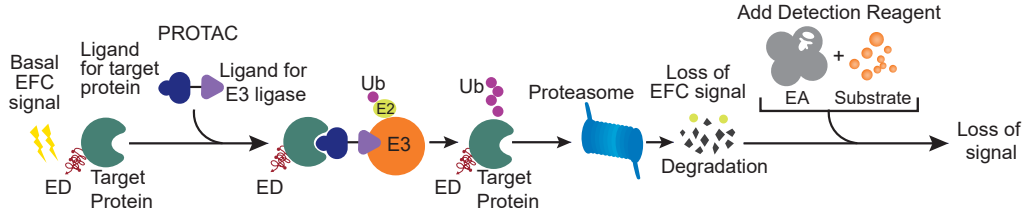
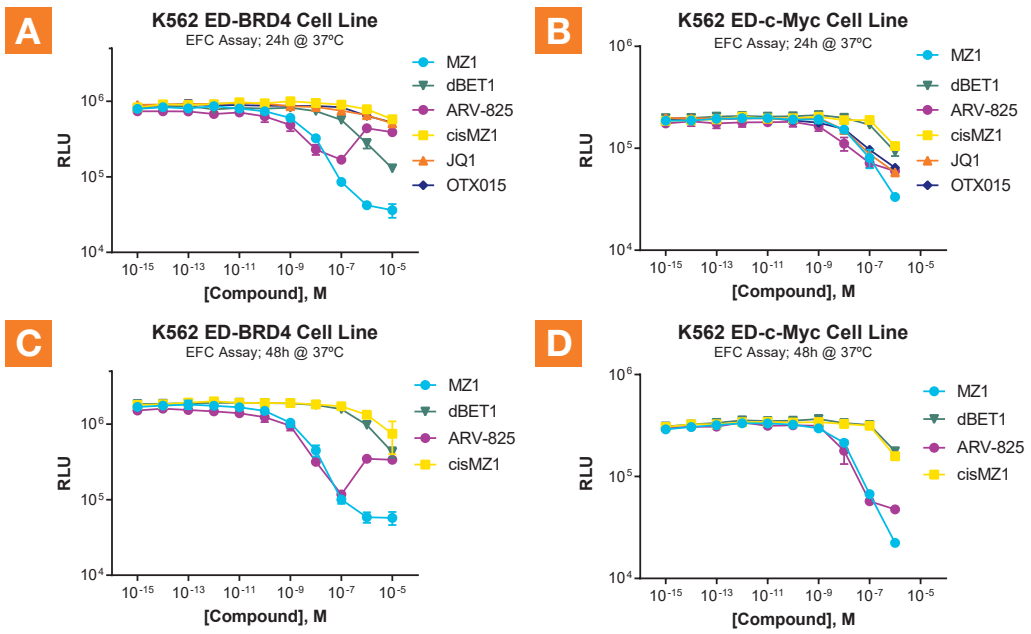


Figure 4. Biosensor technology combines EFC with CRISPR genome editing to quantify endogenous protein degradation induced by PROTACs.

The SPRINTer™ Protein Turnover Biosensor Cell Lines were applied for the detection of drug-induced changes in endogenous BRD4 levels and its downstream target c-Myc in a blood cancer cell model (K562) (Figure 5). A panel of BRD4 inhibitors (JQ1 and OTX015), PROTACs (MZ1, dBET1 and ARV-825) or a control molecule (cisMZ1) targeting BRD4 was tested in this system to provide a rank order of efficacies (ARV-825>MZ1>>dBET1). In contrast to measuring direct activity on BRD4 degradation, differentiation of BRD4 PROTACs by measuring c-myc degradation was less distinct until 48 hrs of incubation but provides equivalent rank order to that of K562 ED-BRD4. In conclusion, EFC-based BRD4 and c-Myc biosensors displayed high sensitivity and more rapid kinetics than commonly used phenotypic endpoint assays such as proliferation and provide a screening platform to identify new molecular entities that modulate oncogenic protein levels for therapeutic development.



Highlights of the kinetics of BRD4 and c-Myc protein degradation induced by MZ1		
	24 hr treatment	
	EC_{50}	S/B
ED-BRD4 cell line	4.3 nM	18
ED-c-Myc cell line	51 nM	5.6
	48 hr treatment	
	EC_{50}	S/B
ED-BRD4 cell line	1.6 nM	28
ED-c-Myc cell line	27 nM	13

Figure 5. Time course of endogenous ED-BRD4 and ED-c-myc protein degradation induced by BRD4-targeted PROTACS. (A. and B. are 24 hr time points and C. and D. are 48 hr time points) To date, several cell lines with ED-labeled BRD4 or c-Myc have been generated with more in development.

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COMPARISON OF E3 LIGASE SUBSTRATE RECRUITMENT MODULATORS
with BioMAP Phenotypic Profiling

IDENTIFY IMPACTS

Human cell-based disease models, a comprehensive reference database, and powerful data analytics provide an unbiased, orthogonal approach to testing inhibitors, degraders, and substrate recruitment modulators.

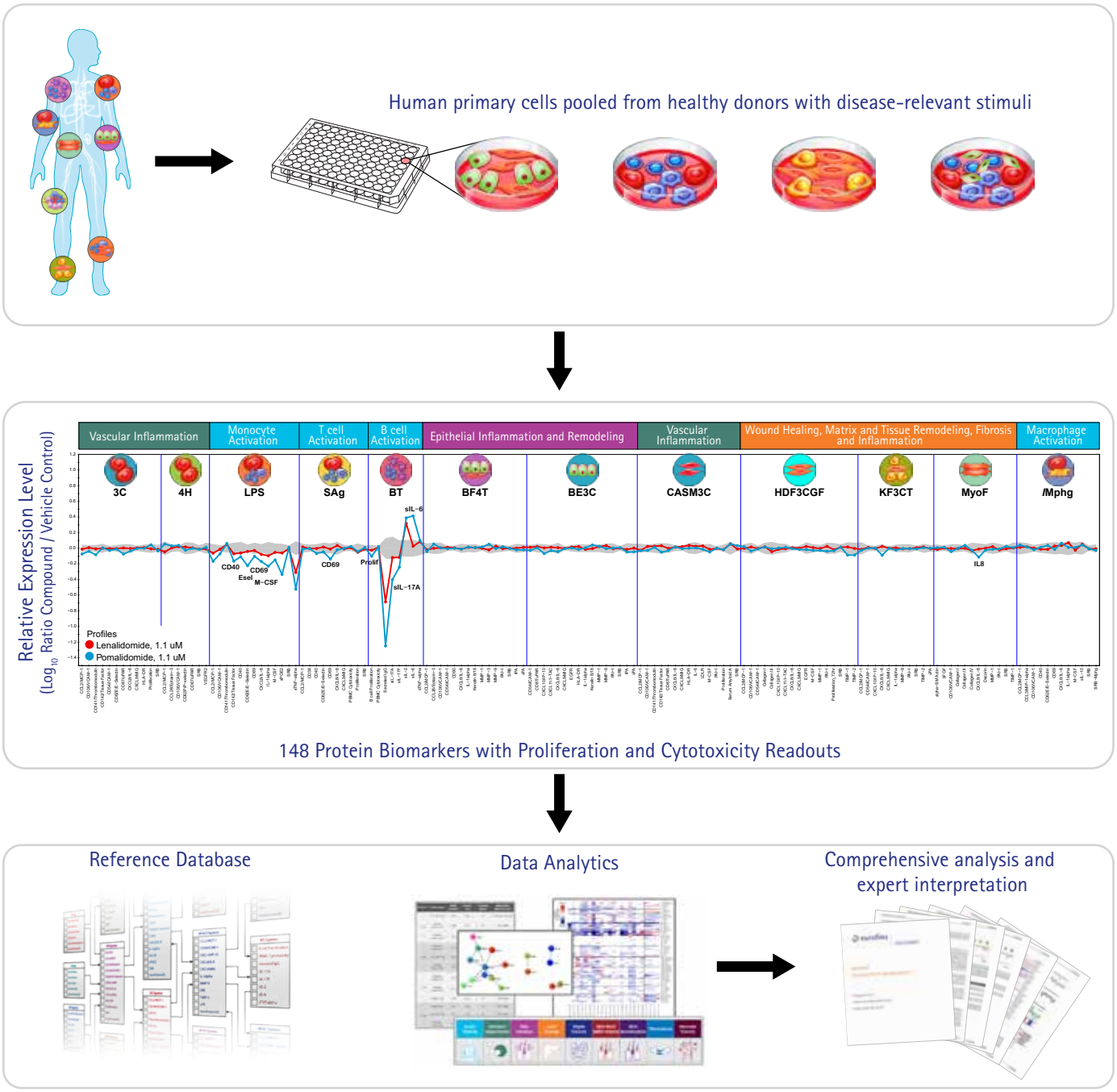

Figure 6. The BioMAP® Phenotypic Platform provides translational Insights with the speed and ease of *in vitro* evaluation. Top panel: BioMAP Systems. Middle panel: Phenotypic profiling using the broad BioMAP Diversity PLUS® Service shows that thalidomide analogues lenalidomide and pomalidomide have both shared and differential activities, with pomalidomide the more active substrate recruitment modulator. Bottom panel: Advantages of Eurofins Discovery BioMAP Phenotypic Profiling include a profile database of 4,500+ compounds, proprietary data analytics, and expert interpretation.

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Profiling in the 12 systems of the BioMAP Diversity PLUS® Panel reveals - in an unbiased manner - test agent impact on protein biomarker readouts within individual system environments. Biomarkers 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). Using custom-designed software and advanced data mining tools, BioMAP profiles for test agents are compared against a proprietary reference database of > 4,500 profiles (including approved drugs, chemicals, biologics, and experimental agents) to computationally identify the most similar activity profiles. Comparative overlay analysis of test agents can reveal common or differentiating biomarker activities (Figure 6).

BioMAP profiling can identify dose-dependent biomarker activities and anti-proliferative effects for the primary target (efficacy), as well as cytotoxicity and potential off-target secondary effects (safety). Multiparameter BioMAP profiles have been used to distinguish compounds based on MoA and target selectivity, and provide insights on selectivity, biomarker identification, competitive benchmarking, indication guidance, and combination approaches. BioMAP profiling with Toxicity Signature Analysis evaluates for pharmacology/toxicity signatures predictive for *in vivo* adverse outcomes (e.g., vascular toxicity, skin toxicity, organ toxicity, immunosuppression, thrombosis) across diverse physiological systems.

PROTEOME-WIDE CHARACTERIZATION OF PROTAC ACTIVITY

Due to the pleiotropic cellular effects of modifying protein levels through targeted degradation, PROTACs as a therapeutic approach have potential to cause unforeseen toxicities (i.e. the well-known teratogenic effects of thalidomide, which binds to and reprograms cereblon E3 ligase). It is thus important to characterize the effects of PROTACs by phenotypic profiling in physiologically relevant systems and proteome wide studies in relevant cell types. To complete the offering capability for characterization of protein degradation on a holistic level, Eurofins Discovery will soon add proteomics to our binding and cellular functional approaches. This addition will complement existing strategies and provide a global readout at the cellular level on changes in protein synthesis and degradation after small molecule treatment.

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