Abstract
Thrombosis 4H KF3CT MyoF CASM3C MyoF BE3C BF4T KF3CT BT l l l Profiling of Targeted Protein Degraders Using the BioMAP Human Phenotypic Platform for Mechanistic and Biomarker Insights

Chemical Structures of Degraders and Inhibitors Used in this Study

Methods
BioMAP systems are composed of human primary cells pooled from healthy donors, in mono- or co-culture conditions, to stimulate the complexity of human disease biology, BRD-related degraders, small molecule inhibitors, and the two E3 ligase (cereblon) binders panellism and lenalidomide were assessed for their concentration-dependence impacts on a panel of 148 protein-based and clinically relevant biomarkers of inflammation, immune biology, tissue remodeling, hemostasis, proliferation, and cell cytotoxicity. Detailed protocols for the BioMAP Diversity Plus Panel of 12 disease-relevant systems are published [1-3].

From Figure 3.

Chemical Structures of Degraders and Inhibitors Used in this Study

Pillars of the BioMAP Human Phenotypic Platform

Figure 1. Each component of the targeted protein degrader chemical structure is open for development, bringing unique challenges, including how to assess the biological impacts of these components and how to find biomarkers indicative of the protein degradation response. Chemical structures of the BRD4 degrader MZ1 and its negative control, cisMZ1 (Figure 1), other BRD degrader inhibitors, and ARV255 (Figure 4) and the BioMAP small molecule inhibitor JQ1 (Figure 5) are shown. Incorporation into early stages of drug development, phenotypic screening, and profiling can positively guide developmental chemistry efforts.

Comparative Overlay Analysis Validates Biological Activities of MZ1 Relative to Negative Control Compound cisMZ1

Figure 3. Overlay analysis shows MZ1 is highly active while the negative control molecule cisMZ1 is weakly active. A BioMAP profile was generated for each test agent at 4 x EC50 values in protein biomarker panels normalized to vehicle controls. Common biomarker readouts are annotated where the majority of all profiles is outside of the significance envelope with an effect size >20%. (Blue, red = ±1 in the same direction. Biomarker readouts, selected for therapeutic and biological relevance, are predictive for disease outcomes or specific drug effects and are validated using assays with known mechanism of action (MOA). Selectivity counts were annotated on the x-axis in the BioMAP system with MZ1, but not cisMZ1 (thin black arrow, lower right), so this system was excluded from analysis and analysis. At higher concentrations, MZ1, but not cisMZ1, was potent across multiple human primary cell types in this Diversity Plus Panel. Two common activities annotated in two systems may reflect scalar or dual linear effects.

Figure 4. Comparative overlay analysis identifies common activities of three BRD degraders MZ1, ARV255, and ARV415 at concentrations that have similar effect size. SHG degraders represent a potential pharmacological signature for the BRD4 degrader class, with 16 common activities annotated within the following systems: SC [7T, 411 (P-selectin), LPS (CH50 -1, 5-1)], ST [EGFR, EGFR, NK1, NF-κB, TNF-α, KDH], KDH (TNFR-2), MZ1, MZ1, ARV255, 520B (positive, n = 11).

Network Clustering Discriminates BRD Inhibitors and Degraders

A BioMAP profile was generated for each

Figure 5. Pairwise correlation analysis identifies clustering between and among BRD inhibitors and degraders. Agents that do not cluster with one another are considered as mechanistically distinct. 1 - Compounds cluster based on their respective target-class profiles, with the handkerchief analogues distinct from the BRD analogues. 5. Within the BRD class, small molecule inhibitors do not cluster with degraders, reflecting different mechanistic signatures. Profiles of all small molecule inhibitors cluster, outlining a shared mechanistic similarity even at an elevated statistical threshold. Each colored circle represents the BRD profile of one agent in a specific non-cytotoxic concentration, with size corresponding dose. Profiles that are similar with a Pearson’s correlation coefficient of 0.1 (or a 0.05) are connected by lines.

Summary
Targeted protein degraders are multifunctional complexes composed of target, linker, and inhibitor elements. Using the BioMAP Platform of human cell-based disease models, we show:

- Although the BioMAP profile for the bromodomod (BRD) inhibitor MZ1 was significantly more active than its negative control cisMZ1 at equimolar concentrations, the negative control was not without biological activity.
- In multiple systems, BRD-targeting protein degraders were cytotoxic at concentrations close to reported EC50 values in assays that do not address cytotoxicity. This observed cytotoxicity emphasizes the value of human-validated degraders to advance candidates optimized for efficacy and safety, and prioritized for therapeutic superiority.

References

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Discover the power of BioMAP Human Phenotypic Platform.

Profiling of Targeted Protein Degraders Using the BioMAP Human Phenotypic Platform for Mechanistic and Biomarker Insights

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