One-Step SAR: Using High-Content Imaging to Drive Small Molecule Protein Degraders from Hit to Lead

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Introduction

High-content imaging (HCI) techniques have revolutionized the landscape of drug discovery, offering powerful tools to accelerate the identification and characterization of potential drug candidates. By combining the capabilities of automated microscopy, image analysis, and multiplexing, HCI enables the simultaneous quantification of numerous cellular features within a single experiment. This approach provides a wealth of information on cellular morphology, protein content, localization, and functional readouts, all of which are crucial for understanding disease mechanisms and evaluating drug responses. These techniques not only enhance the speed and efficiency of screening large compound libraries but also offer deeper insights into the mechanisms of action of potential therapeutics, ultimately accelerating the drug discovery process from target identification to lead optimization. Here, Curia describes a cost-effective high-content immunofluorescence workflow developed in an immortalized patient cell line for the identification of small molecule protein degraders using the Revvity Opera Phenix™ confocal imaging system. With maximized content and careful assay design, the resulting data provides a rich description of compound effects including on-target and off-pathway phenotypes along with cytotoxicity information – all in a single assay.

Assay Design

Primary Screen of 100k Compounds from Curia's CSCC Library

- 2 compounds per well at a single concentration
- 1 target protein and 3 pathway-relevant targets
- **100k+ compounds from Curia's CSCC library**
- **~200 hits for non-toxic target protein degradation**

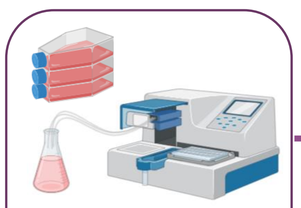
Hit Deconvolution and Confirmation

- Hits from primary screen tested in 5-point dose response format, 1 compound per well
- Assessed 1 target protein and 4 pathway-relevant targets in hit confirmation
- **Hit Confirmation Rate: 85%**

Structure-Activity Relationship (SAR) Testing

- Confirmed hits tested in 9-point dose response
- Assessed 1 target protein and 4 pathway-relevant targets for SAR campaign
- Performed phenotypic classification of each compound for structural comparison

Workflow



Cell Plating

Thermo Scientific® Multidrop ® Combi Dispenser

Compound Addition

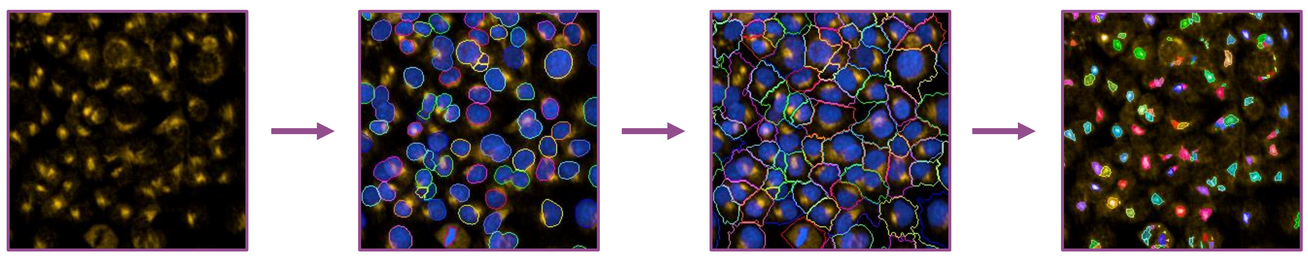
Echo™ 555 Acoustic Liquid Dispenser

Immunocytochemistry Staining and High-Content Imaging

BioTek® EL406 Washer Dispenser
Revvity Opera Phenix®

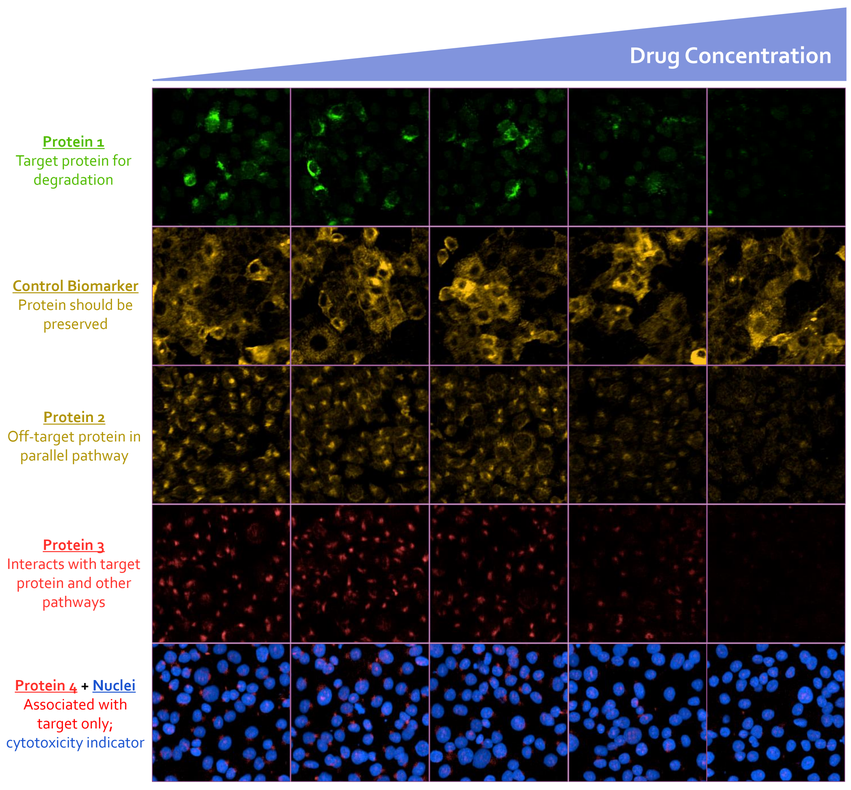
Automating High-Content Imaging and Analysis: High-content imaging assays were optimized to run in 384-well microplates. Cells were seeded using the Thermo Scientific ® Multidrop® Combi dispenser and incubated at 37°C and 5% CO_2 overnight. Assay plates were then moved to the Revvity Cell::Explorer™ robotics platform and treated with CSCC library compounds using the Echo™ 555 Acoustic Liquid Dispenser. After 24 hours, assay plates were removed from incubation for immunocytochemistry processing and were fixed, permeabilized, and processed for optimal epitope accessibility for all antibody targets. Primary antibodies were added in two separate mixes on different sections of each plate using the BioTek® EL406 Washer Dispenser and Multidrop® Combi to label all protein targets. Upon secondary antibody and Hoechst staining, plates were stored at 4°C prior to automated imaging on the Revvity Opera Phenix™ imaging system.

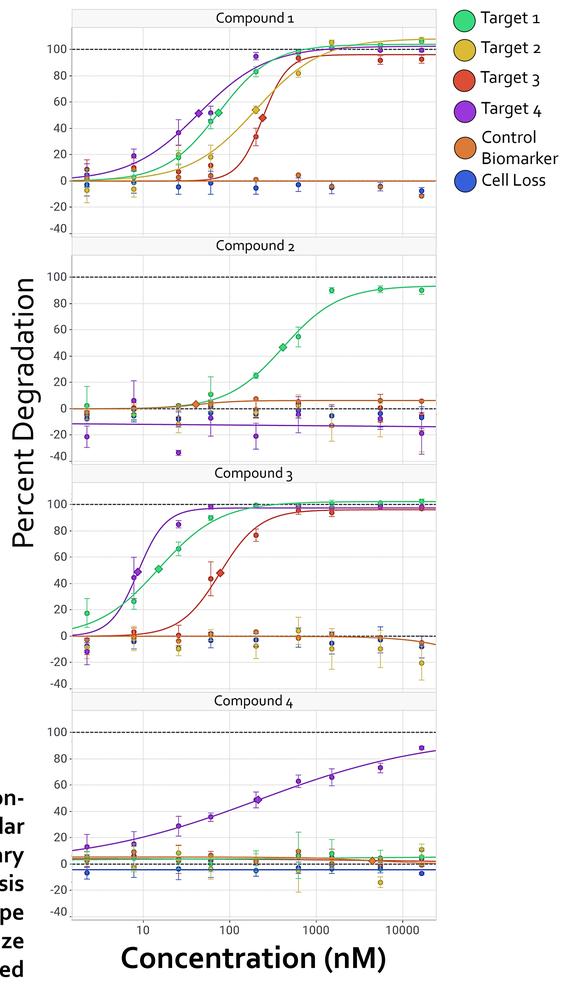
Image Acquisition and Analysis



Multiparametric Approach to Imaging Analysis: The Revvity Opera Phenix™ is a 4-channel dual spinning disc confocal imaging system capable of capturing 4 laser-excited fluorophores simultaneously. The instrument is integrated into a Revvity® Cell::Explorer™ automation system containing liquid handlers, plate sealers, and incubators, making it a valuable high-content imaging and screening platform to identify protein degraders. Utilizing an image analysis software pipeline including Harmony, Image Artist, and TIBCO® Spotfire® software, Curia delivers highly customized analysis workflows customized to the needs of the project. For this assay, 384-well plates were imaged using a 20x water objective, with individual protein targets identified across three fluorescent channels using spot detection algorithms optimized for each target with morphological and intensity-based metrics collected in parallel. While the most representative features were determined for each protein, inclusion of additional features enabled identification of more subtle and even novel phenotypes.

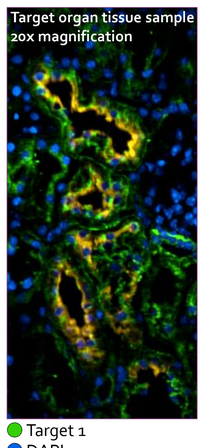
Assessing Protein Degraders by High-Content Imaging

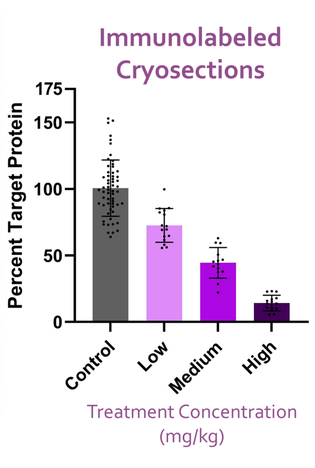


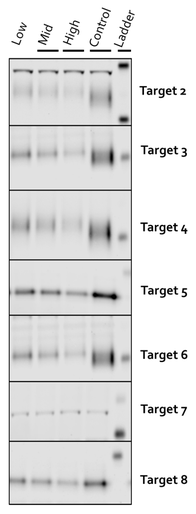


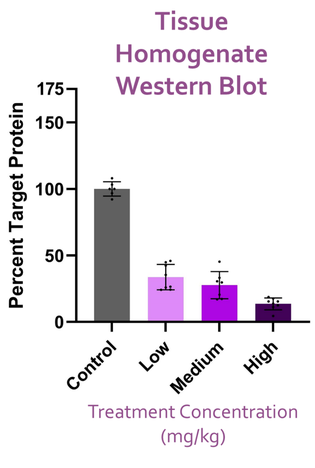
Hit Finding by High-Content Imaging: This approach allows for the assessment of on-target potency, off-target effects, and pathway specificity while tracking cellular health and morphology from a single well of a 384-well plate. Multiple primary antibody cocktails were utilized across each assay plate, and single image analysis methods were optimized to track degradation of each target protein. Phenotype classifications were assembled using a combination of target phenotypes to categorize each library and control compound tested, however several compounds exhibited phenotypes outside of predicted classifications. Capturing valuable phenotypic classification data across several on-target and off-target proteins increases the efficiency of compound screening and uncovers new mechanisms of action that furthers our understanding of the underlying biology of the target protein or pathway.

Validating *In Vitro* Hits with *In Vivo* Models for Disease









Hits Identified in High-Content Imaging Screen Demonstrated *In Vivo* Efficacy: The candidates identified from the small molecule protein degradation screen and SAR campaign have shown promising efficacy in both immortalized patient cell models and *in vivo* efficacy studies. These results suggest that conclusions from our high-content platform can correlate with therapeutic benefits. The preliminary success of these candidates indicates their potential for further development as novel treatments. Thanks to the depth of content offered, this platform remains a relevant SAR tool for future lead optimization stages.

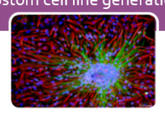
Curia Integrated Drug Discovery Services

Cell Based Assay Technology

Oncology, immunology and neurobiology in vitro models

Potency Assays: ADCC, CDC, ADCP

Custom cell line generation



Assay Development & Screening

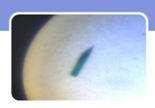
Biochemical, biophysical, and label-free assays

High throughput screening and SAR testing

Protein Expression, Crystallization & Analysis

Recombinant protein expression & purification

Structure determination & structure-based drug design



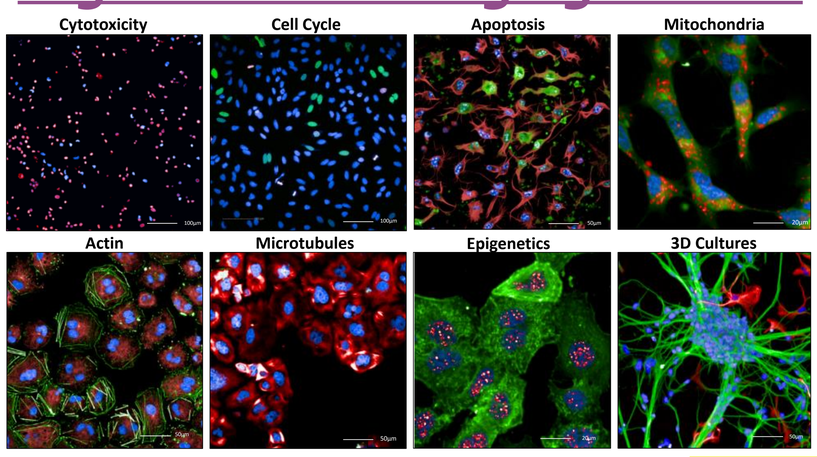
Antibody Discovery

Hybridoma development & B-cell / Beacon strategies

Maturation, humanization

Rapid recombinant mAb production

High-Content Imaging at Curia



Conclusions

Assessing targeted protein degraders by high-content imaging proved an effective tool to accelerate the hit-to-lead process. The ability to track up to five different biomarkers in dose response provided a level of content that permitted prudent SAR decisions within days of new compounds arriving at Curia. In addition, the ability to identify compounds with novel specificities for target degradation opened up new potential mechanisms for protein clearance and a chance to better understand the underlying biology.

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