curia
Small Molecule

WHITE PAPER

Accelerating Small Molecule Protein Degrader Development Using High-Content Imaging

Curia Global, Integrated Drug Discovery

Targeted protein degradation has emerged as a transformative approach in drug discovery, enabling the selective elimination of disease-associated proteins using the ubiquitin-proteasome system. Modalities such as PROTACs® and molecular glues provide therapeutic solutions for previously "undruggable" targets and offer mechanisms to overcome resistance to traditional small molecule inhibitors.

A critical driver of this field's rapid progress is the integration of advanced screening technologies. 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 assay plate well. This high-content imaging approach enables comprehensive characterization of cellular morphology, protein expression and localization, as well as functional readouts, providing critical insights into disease mechanisms and the evaluation of drug responses.

Here, Curia describes a cost-effective high-content immunofluorescence workflow developed in an immortalized patient cell line used to identify small molecule protein degraders using the Revvity Opera Phenix confocal imaging system. With maximized content and careful assay design, the resulting data provide single-cell-level visualization of target protein degradation in addition to on-target and off-pathway phenotypes and cytotoxicity information–all from a single assay. 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. Upstream of more costly animal studies, this multiparametric approach provides critical insights into the mechanism of action, potency, and safety—key factors in translating targeted protein degraders into clinical success.

Challenge: Advancing a Protein Degrader Program from Hit to Lead Candidate for IND Filing

Curia scientists worked closely with a recent client who aimed to identify both a lead candidate and a structurally distinct backup target protein degrader candidate for IND submission. The program required a robust and scalable screening approach that could balance assay throughput with phenotypic resolution across multiple compound series and multiple targets.

Assay Evaluation: Choosing the Optimal Screening Strategy

To address the unique needs of this protein degrader program, the client evaluated three complementary assay formats to screen through candidate molecules, including high-content imaging, HiBiT, and ASMS. While both HiBiT and ASMS approaches provide unique and meaningful insights, the client ultimately chose a multiparametric high-content imaging approach, which allowed for the simultaneous quantification of target protein degradation and off-target phenotypic effects within a single experiment. A critical factor in this decision was the suitability of HCI as a primary screening tool, given its inherent ability to detect cytotoxicity and broader cellular perturbations, thereby reducing the risk of false positives.

Assay	Description	Pros	Limitations
HiBiT	Bioluminescent reporter-based assay using an epitope tagged target protein	High throughput, fast readout	Requires engineered cell lines; potential compound interference
ASMS	Cell-free affinity selection mass spectrometry	Rapid target engagement data	Lacks cellular context
High Content Imaging	Multiplexed immunofluorescence imaging and phenotypic analysis	Detailed, multiparametric insights in a cellular environment	Lower throughput, higher complexity

Table 1: Comparison of assay formats for screening targeted protein degraders. Table summarizes the relative strengths and limitations of HiBiT, affinity selection mass spectrometry (ASMS), and high-content imaging (HCI). While HiBiT and ASMS offer rapid and sensitive detection of protein content, HCI was ultimately selected as the primary screening approach because it provides multiplexed, spatially resolved measurements with scalability to content-rich workflows.

Identifying Small Molecule Protein Degraders using High-Content Imaging: Developing an Agile Screening Workflow

To mitigate time and throughput constraints inherent to high-content imaging, Curia established an automated workflow capable of screening up to23,000 compounds per week. The screening workflow was optimized to run on Curia's fully automated Revvity Cell::Explorer platform, which houses a Revvity Opera Phenix Plus Confocal Screening System. Cells were seeded using the ThermoFisher Multidrop Combi 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 Curia's CSCC Compound 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 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.

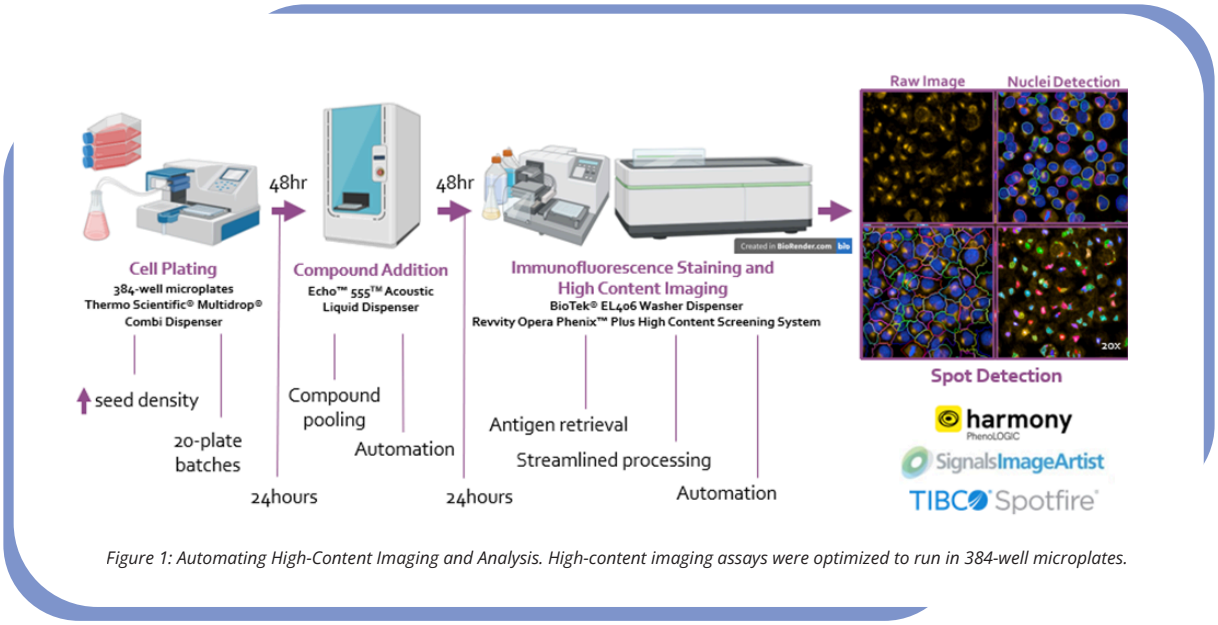

Figure 1: Automating High-Content Imaging and Analysis. High-content imaging assays were optimized to run in 384-well microplates.

Identifying Small Molecule Protein Degraders using High-Content Imaging: Screening Funnel

The screening workflow was broken down into three stages to accommodate the quick turnaround time requested by the client. Leading up to the screen, up to five protein targets were validated in Curia's HCI assay, and plate treatments were arranged to allow for a flexible number of labeled targets per assay. To increase the efficiency of the primary screen, two (2) library compounds were tested per assay well at a single concentration (5 µM). To maximize throughput, three protein targets were tracked in the primary screen. Hit deconvolution and hit confirmation steps were combined to further shorten the overall timeline of the 100,000-compound library screen, and a 5-point dose response format utilized during hit confirmation allowed for early IC_{50} determination, saving time in the SAR testing stage. Furthermore, assay plate layouts were rearranged to accommodate two additional targets to be tracked by imaging in the deconvolution and confirmation testing stage, adding further nuance to the confirmed hit phenotypes.

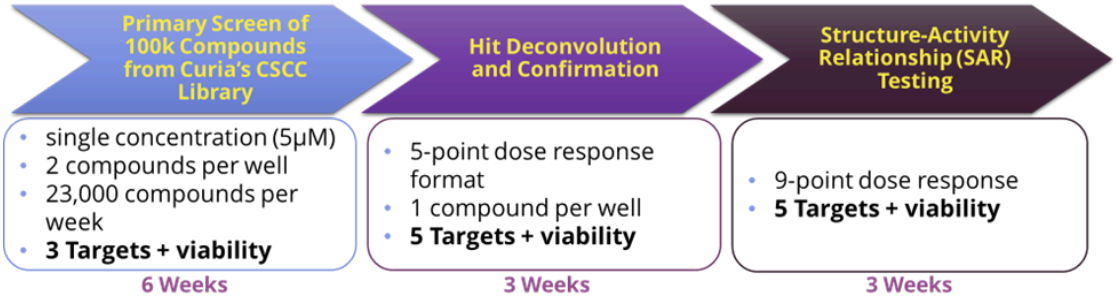

Figure 2: Three stages of the high-content imaging workflow.

Identifying Small Molecule Protein Degraders using High-Content Imaging: Screening of 100,000 Small Molecules from Curia's CSCC Compound Library Collection

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 assay plate. Multiple primary antibody cocktails were utilized across each assay plate, and single image acquisition and analysis methods were optimized to track the degradation status of each target protein.

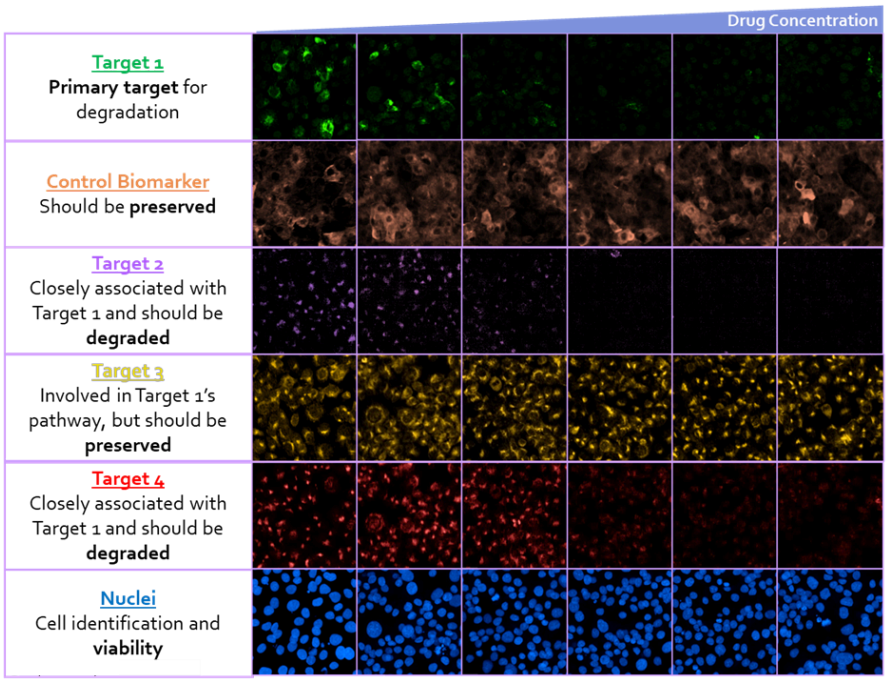

Figure 3. Identifying small molecule protein degrader using high-content imaging. Increasing test compound concentration is presented from left to right, where target 1 is the primary targeted protein for degradation, Targets 2 – 4 are proteins associated with the target protein, and the Control Biomarker should remain constant throughout treatment. We observe degradation of the target protein (top row, green) with increasing concentration of test compound, with varying degradation of off-target or in-pathway proteins. Nuclear DAPI staining is used to assess cytotoxicity of each test compound in the patient-derived cell line.

Hit classifications were assembled using a combination of five target phenotypes to categorize each library and control compound tested; however, several compounds exhibited phenotypes outside of the predicted classifications for the target protein and pathway. Capturing phenotypic data across multiple on-target and off-target proteins enhances the efficiency of compound screening while also revealing novel mechanisms of action, thereby advancing the understanding of the underlying biology of the target protein or pathway. From the primary screen of 100,000 CSCC Library compounds, 200 well hits for non-toxic target protein degradation were identified, equating to a 0.2% hit rate. As two library compounds were tested per assay well in the primary screen, 400 total compounds were tested in a 5-point dose response format to reveal 170 confirmed hits, equating to a notable 85% hit confirmation rate. Confirmed hits were further characterized in a 10-point dose response format before being classified into unique phenotypic profiles based on the relative degradation profiles of the 5 protein targets in the assay described in Figure 4.

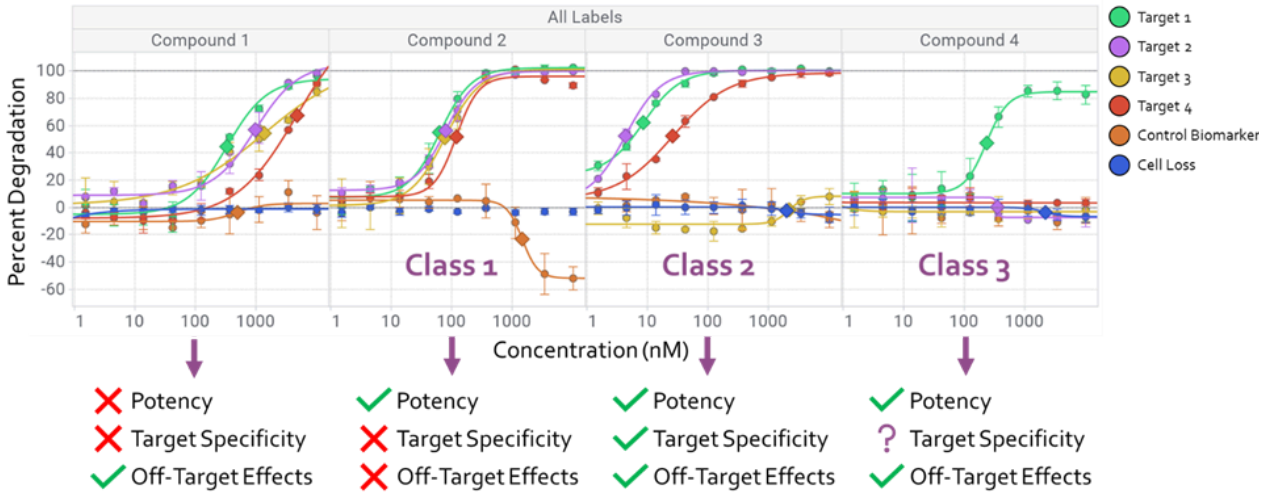

Figure 4: Hit Finding by High-Content Imaging. This approach allows for the assessment of on-target potency, off-target effects, and pathway specificity while tracking cellular health and morphology from a single well of a 384-well plate. Phenotype classifications were assembled using a combination of target phenotypes to categorize each library and control compound tested; however, several compounds exhibited phenotypes outside of predicted classifications.

Notably, phenotypically classified hits translated into therapeutic efficacy in patient-derived cell models and humanized animal studies—validating the predictive power of the high-content imaging platform. 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 correlate with therapeutic benefits in an in vivo system. The preliminary success of these candidates indicates their potential for further development as a novel treatment for targeted protein degradation. Thanks to the depth of content offered, this high-content imaging platform continues to be used as the primary SAR assay used for future lead optimization efforts.

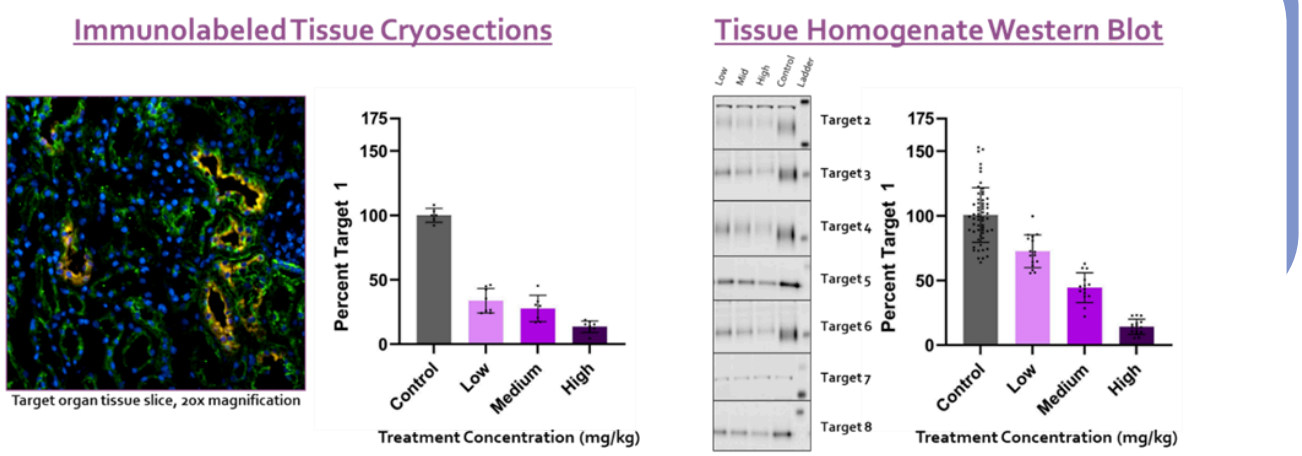

Figure 5: Hits Identified in High-Content Imaging Screen Demonstrated In Vivo Efficacy.

Identifying Small Molecule Protein Degraders using High-Content Imaging: Key Results

- **Hit Rate:** 0.2% (200 hits identified from Primary Screen)
- **Hit Confirmation Rate:** 85% (170 hits confirmed in Deconvolution and SAR Testing), lead compounds from in vitro primary screen were further validated using in vivo models for disease
- **Target Classification:** Hits were grouped into three mechanistic classes based on differential activity across 5 protein targets in the assay
- **Hit-to-Lead Timeline:** 4 Months; Prioritization of candidate series with confirmed in vitro and in vivo efficacy

Conclusion

Curia's high-content imaging platform offers a powerful and scalable solution for accelerating protein degrader discovery programs. By enabling deep phenotypic insights, multiplexed biomarker tracking, and rapid SAR iteration, high-content imaging bridges the gap between chemical design and biological response—driving lead optimization with precision.

Key Benefits

- Streamlined hit triage with fewer false positives
- Mechanism of action insights within a cellular context
- Efficient translation from hit-finding to IND-enabling studies

To learn more about Curia's capabilities in high content imaging and targeted protein degradation, contact us at Marketing@Curiaglobal.com