Molecular Glues – Developing a Robust Screening Cascade for CK1a Specific Degradation

Ryan Mordue, Alison Gordon, Jenny McLaughlan, Kathy Dodgson, Gary Allenby

Charnwood Discovery, Charnwood Campus, 9 Summerpool Road, Loughborough, LE11 5RD

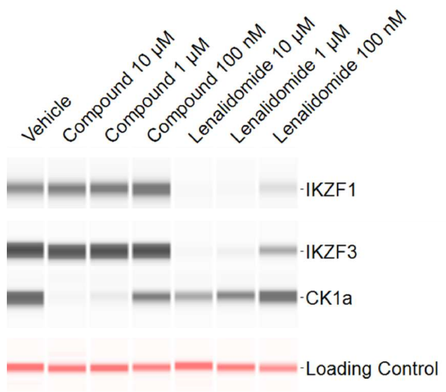
charnwood discovery

Introduction

Development of small molecule therapeutics require a robust screening cascade that can efficiently identify compound that engage the target, determine selectivity, and demonstrate activity in function and phenotypic assays.

Here we present data on the screening cascade successfully developed to identify molecular glues for the selective degradation of CK1a.

Identified through serendipity, this project was designed to develop new PROTAC® warheads that did not degrade known neo-substrates such as IKZF1, IKZF3, CK1a, GSPT1, and SALL4, while maintaining the binding of Cereblon (CRBN). Screening of new warheads against these neo-substrates identified a more potent and specific degrader of CK1a than the current market leader Lenalidomide. Once confirmed, the design-make-test-analyze (DMTA) cycle was used to develop the molecule in a robust screening cascade.



Identification of a novel CK1a degrader showing increased potency and greater selectivity than Lenalidomide.

Screening Cascade

RealTime-Glo® Proliferation assay

NanoBRET Target Engagement (CRBN)

Confirm desired phenotypic effect

Confirm E3 ligase target engagement

Flow cytometry
Single concentration against CK1α (on-target) and IKZF1 (off-target)

Determine activity against target and potential off-targets

Flow cytometry
Degradation DC_{50} and D_{max} (CK1α)

Orthogonal readouts

Simple Western™
Degradation DC_{50} and D_{max} (CK1α)

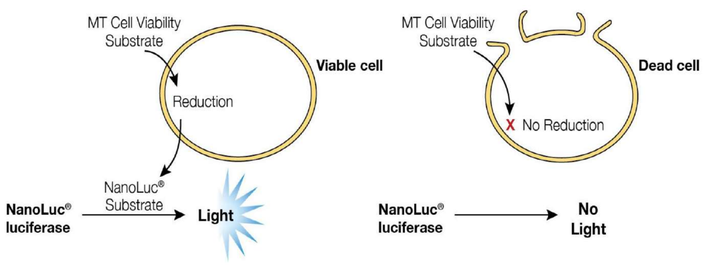
Calculate degradation constants such as DC_{50} and D_{max} to support compound development SAR.

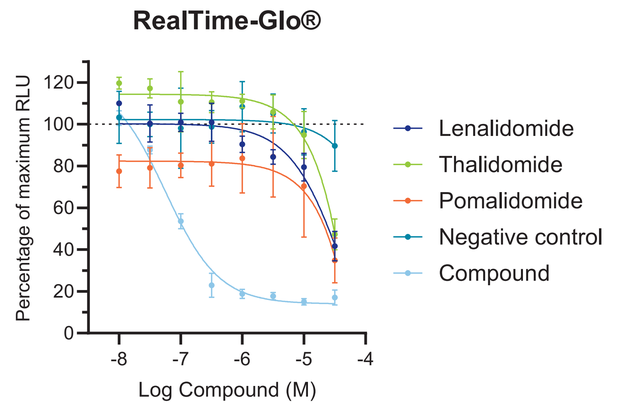
Summary and Conclusion

Here, we successfully identify molecular glue's with degradation properties by developing a screening cascade and suite of assays designed to identify compounds with appropriate properties. At Charnwood Discovery, the co-location of scientists from bioscience and chemistry under one roof and efficient implementation of the DMTA cycle progressed this candidate molecule in a timely manner from target validation to pre-clinical candidates.

RealTime-Glo® – Proliferation Assay

In the Promega RealTime-Glo® cell viability assay luminescence is directly proportional to live cell number. Run over a 72 hour period, this allows for the detection of anti-proliferative effect of compounds in a relevant cell background at high throughput.

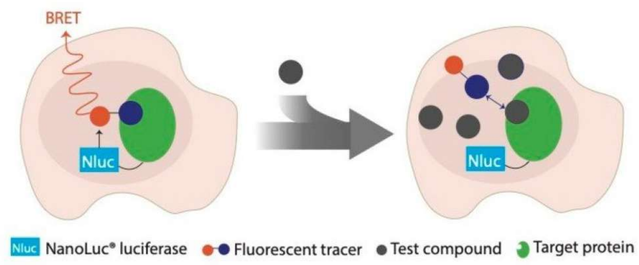




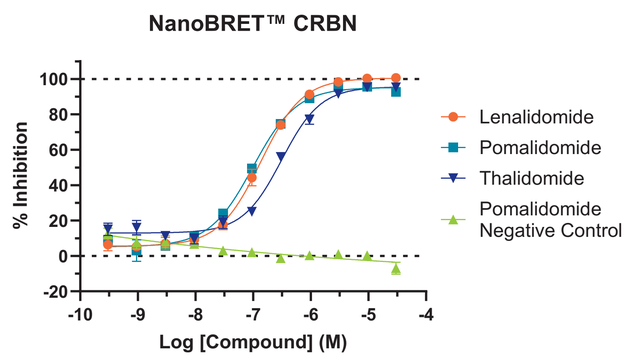
Compounds capable of degrading CK1a demonstrated an anti-proliferative effect in cells of a acute myeloid leukaemia background.

NanoBRET™ – Target Engagement

The Promega NanoBRET™ target engagement assay enables the determination of compound affinity for the full length target protein in a competent cellular background, resulting in data on cell permeability and residence time.

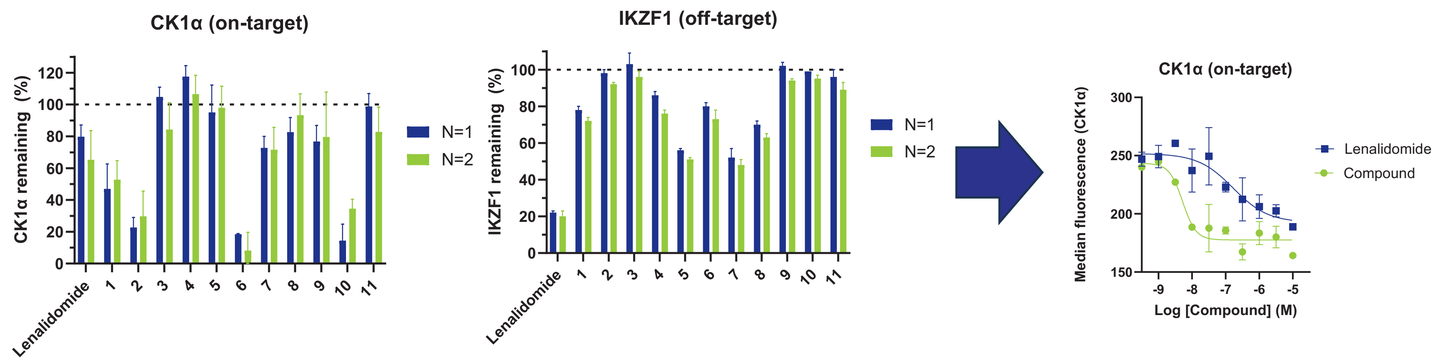


CK1a molecular glue degraders must bind CRBN to facilitate ubiquitination of the target protein, in this case CK1a. Engagement of new compounds to CRBN is measured by NanoBRET™.



Flow Cytometry – On-target Effect

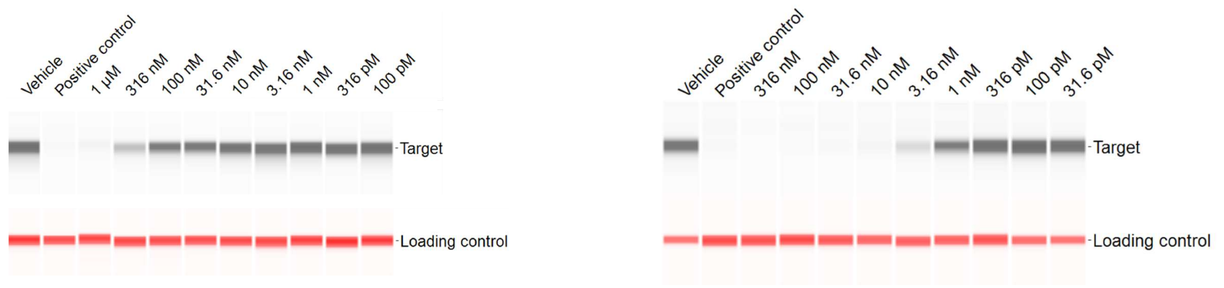
Flow cytometry enables increased throughput while maintaining physiological relevance, allowing target protein expression to be determined within individual cells. Multiple effects (both on- and off-target) can be measured simultaneously within a mixed cell population, together with other key cell health markers, such as apoptosis.

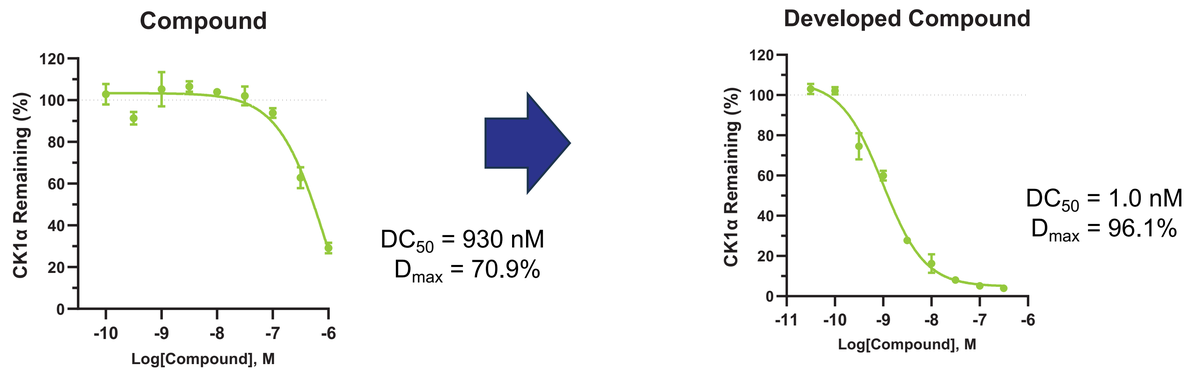


Compounds examined by flow cytometry for their ability to degrade CK1α (on-target) and IKZF1 (off-target) at a single concentration, the most promising compounds are screened for degradation constants DC_{50} and D_{max} by flow cytometry in an orthogonal assay.

Simple Western™ – Degradation Constant Determination

Simple Western Jess is used as an orthogonal assay to flow cytometry for CK1α degradation. The lower throughput denotes that only key molecules are assessed by this method as an orthogonal readout to flow cytometry. Nonetheless, the methodology allows for an accurate derivation of degradation constants DC_{50} and D_{max} to aid in understanding the structure-activity-relationship (SAR) of the molecules and directly influence the DMTA process.





CK1a degraders demonstrate a concentration dependant depletion of the target. Data demonstrates the development a starting molecule with a 3-magnitude order improvement in potency and 25% improvement in maximal degradation following development.