High throughput screening techniques to inform on compound liability and solubility to advance the PROTAC® platform

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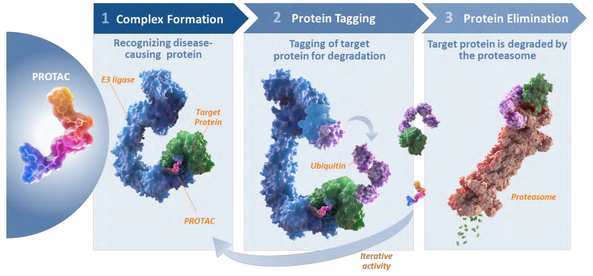
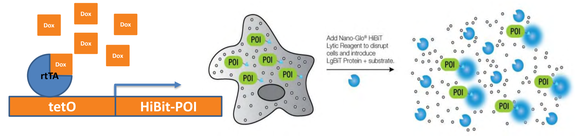
Abstract #XXXXX

PROteolysis Targeting Chimeras (PROTAC) are small molecules that exploit the ubiquitin−proteosome system (UPS) cellular machinery to achieve degradation of a target protein of interest (POI). PROTAC degraders are heterobifunctional molecules targeting POI via two distinct recognition elements combined by a linker moiety: one end of the PROTAC molecule incorporates a binding partner for the protein to be degraded, while the other end is comprised of an E3 ubiquitin ligase binding motif. While several E3-ligases have been utilized for targeted protein degradation (TPD) including Cereblon (CRBN), VHL, MDM2, and cIAP1, CRBN-based PROTAC degraders have entered and are progressing through the clinic. Optimization of PROTAC molecules presents several challenges, including low aqueous solubility and, for CRBN-based PROTAC degraders, recruitment and degradation of neomorphic protein substrates such as GSPT1, CK1α, SALL4, and others that present potentially cytotoxic profiles. To assess activity on their POI, PROTAC protein degraders are often tested in cell-based degradation assays where poor compound solubility and general toxicity can confound structure activity relationships (SAR) and lead to misinterpretation of data. Here we present a testing workflow to efficiently generate PROTAC SAR that addresses neomorphic degradation for CRBN-engaging PROTAC molecules, cellular toxicity, and aqueous solubility in a variety of commonly used cellular assay buffers. Furthermore, we present screening data on mutant Huntingtin (mHTT) targeting PROTAC degraders engaging different E3-ligases that show high potency, allelic selectivity, and no measurable toxicity utilizing mHTT-expressing cell lines.

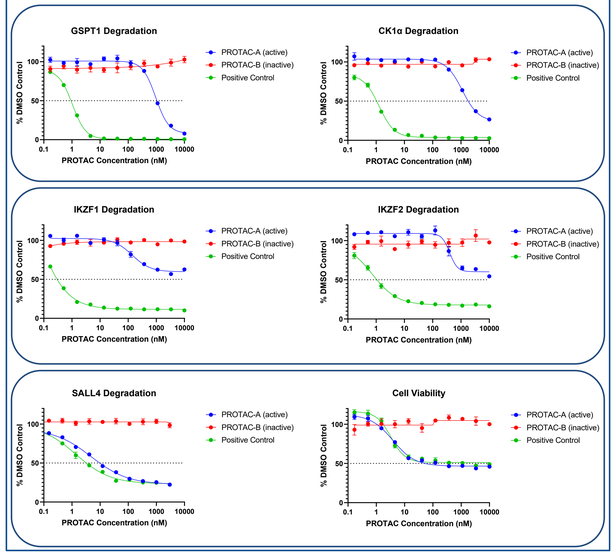
PROTAC® molecules harness the ubiquitin-proteasome system to degrade proteins



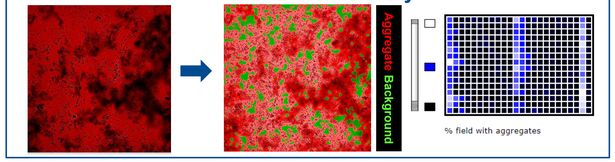
Constitutive and inducible HiBit tagged cell lines allow monitoring and measurement of protein degradation



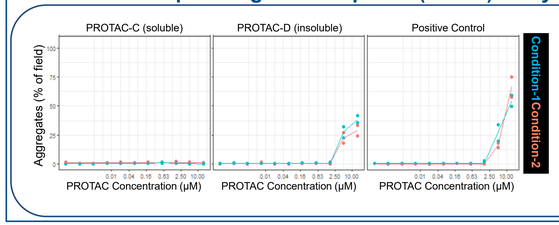
Quantifying degradation of neomorphic targets to de-risk CRBN based PROTAC® molecules



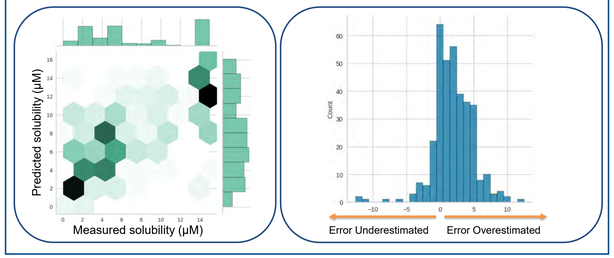
High content screening and machine learning to measure PROTAC® solubility



Determining maximum PROTAC® solubility using multivariate adaptive regressive splines (MARS) analysis



Utilizing PROTAC® solubility data: Generating a highly accurate predictive linear regression model



PROTAC® degraders: a differentiated opportunity vs. other modalities used for CNS diseases

PROTAC® degrader small molecules can overcome the limitations of other platforms

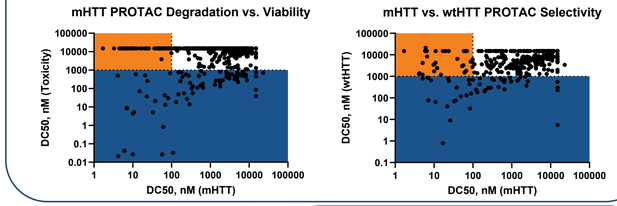
PROTAC *Potential vs. Other Modalities*

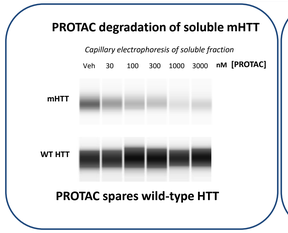
- Reduce intra- and extracellular pathologic protein
- Discriminate between wild type and pathologic protein
- Oral administration with BBB biodistribution

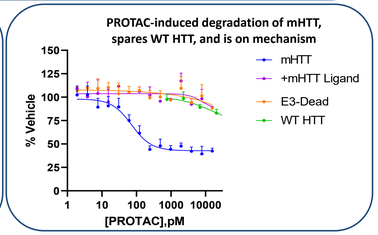
ASO	Ab
• Requires intrathecal dosing • Does not discriminate wt from pathologic protein	• Blocks only extracellular pathologic protein • IV dosing results in only 0.5% in CSF

Huntington's Disease: Ligand chemistry enables allele-selective PROTAC® degradation of mHTT

PROTAC degradation of cell-expressed mutant or WT HTT identifies numerous non-toxic, allele-selective degraders engaging different E3-ligases (highlighted in orange)







Conclusions

Our CRBN-based PROTAC® screening workflow:

- Quickly and efficiently identifies neomorphic activity to inform SAR.
- Allows de-risking of compounds prior to advancing within programs.
- Allows accurate interpretation of cell based TPD data.

Our aqueous solubility assay:

- Utilizes HCS and machine learning to accurately measure PROTAC® solubility in multiple relevant assay buffers.
- Generates SAR informing a predictive model of compound solubility prior to synthesis.

Our mHTT targeting PROTAC® degraders:

- Degrade mHTT *in vitro* with no measurable toxicity.
- Directly target and degrade mutant allele selectively, on mechanism, through engaging an undisclosed E3-ligase.
- Differentiate from conventional inhibitors and represent an exciting opportunity in treating CNS diseases

References

PROTAC review: Békés, M., Langley, D.R. & Crews, C.M. PROTAC targeted protein degraders: the past is prologue. Nat Rev Drug Discov 21, 181–200 (2022).

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Presented at the Society for Laboratory Automation and Screening 2023 Meeting, San Diego, CA