Plate-based screening approaches and mechanistic insights to inform optimization of PROTAC® degraders for the treatment of neurodegenerative disease

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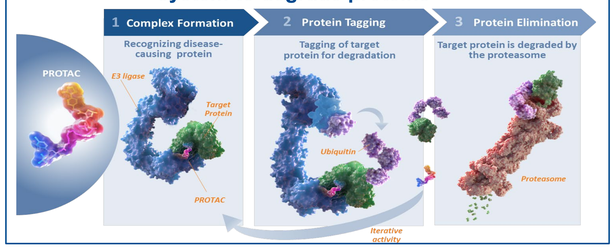
Abstract

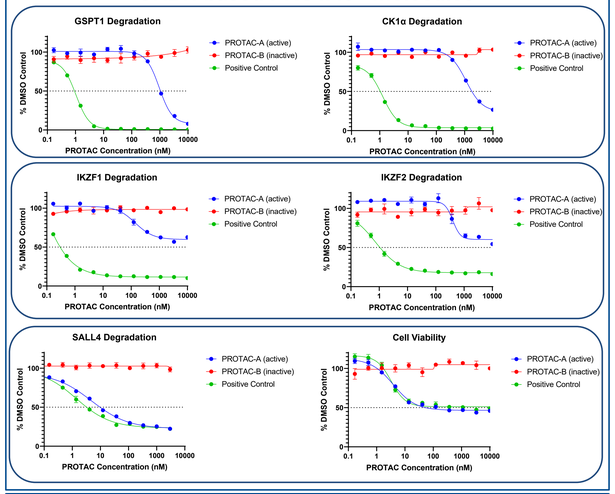
PROteolysis Targeting Chimeras (PROTAC) are small molecules that employ the ubiquitin-proteosome system (UPS) to achieve degradation of a target protein of interest (PoI). PROTAC degraders are heterobifunctional molecules targeting a PoI via two distinct recognition elements joined together by a linker moiety: one end of the PROTAC incorporates a ligand to bind the PoI, while the other end of the molecule incorporates an E3 ubiquitin ligase binding ligand. While several E3-ligases have been utilized for targeted protein degradation (TPD), cereblon (CRBN)-based PROTAC degraders have entered and are progressing through the clinic for the potential treatment of cancer, inflammatory, and most recently neurology conditions. Neurodegenerative diseases represent an area of high unmet clinical need where PROTAC degrader molecules could have distinct advantages over other therapeutic modalities, including the opportunity for oral administration to remove toxic gain of function proteins from the central nervous system. Two neurodegenerative proteins we describe here are Leucine-rich repeat kinase 2 (LRRK2), a kinase whose increased activity and human genetics are associated with Parkinson's Disease (PD) and Progressive Supranuclear Palsy (PSP); and mutant Huntingtin (mHTT), a protein whose polyglutamate expansion leads to Huntington's disease after exceeding a threshold polyQ length.

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 other potentially cytotoxic profiles. To assess activity on their PoI, PROTAC 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 Structure-Activity and Liability Relationships (SAR/SLR) that addresses neomorphic degradation for CRBN-engaging PROTAC molecules, cellular toxicity, and aqueous solubility. Furthermore, we present in vitro screening data for both mHTT- and LRRK2-targeting PROTAC degraders engaging different E3 ligases that show high potency and no measurable toxicity utilizing mHTT- or LRRK2-expressing cell lines. We demonstrate that our mHTT-targeting PROTAC molecules also exhibit allelic selectivity, binding mHTT over the wild-type protein. Lastly, utilizing competition experiments with either an E3 binding ligand or PoI binding competitor, we demonstrate that both our LRRK2- and mHTT-targeting PROTAC molecules are functioning on mechanism, binding the correct PoI, form appropriate ternary complexes, and subsequently degrade the PoI via the UPS.

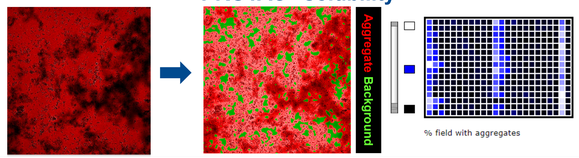
PROTAC® molecules harness the ubiquitin-proteasome system to degrade proteins



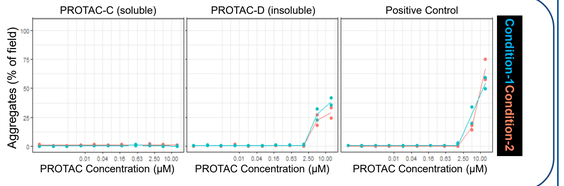
Quantifying degradation of neomorphic targets to de-risk CRBN based PROTAC® molecules



High content screening and machine learning to measure PROTAC® solubility



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



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

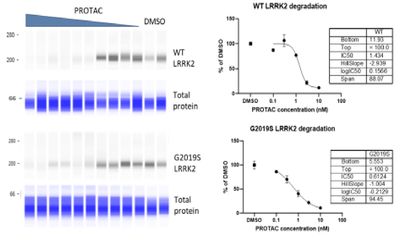
PROTAC® degrader small molecules can provide unique benefits compared to other modalities

PROTAC *Potential vs. Other Modalities*

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

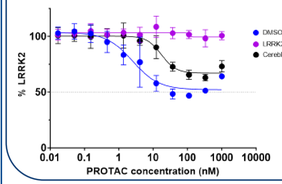
ASO	Ab
• Requires intrathecal dosing • Does not discriminate wt from pathologic protein	• Blocks only extracellular pathologic protein • IV dosing results in only 0.5% in CSF

Parkinson's Disease: LRRK2 PROTAC® degrades both WT and common mutant G2019S protein and is on mechanism



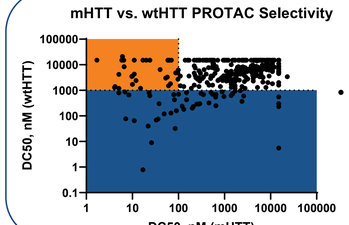
- Human iPSC-derived microglia expressing WT or G2019S LRRK2 were treated with PROTAC for 24 hr and the effect on LRRK2 measured by capillary immunoassay
- Data demonstrates equivalent potency against the G2019S mutant and LRRK2 reduction in a disease-relevant cell type

Competition studies confirm PROTAC mechanism of action

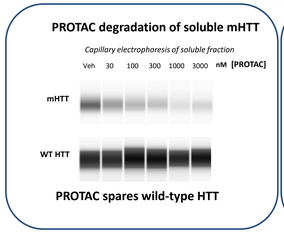


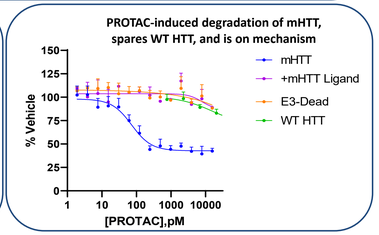
- HEK cells expressing HiBit-tagged LRRK2 were treated with PROTAC® in the presence or absence of 1 uM LRRK2 warhead, 3 uM cereblon ligand and effect on LRRK2 levels determined
- Data confirms that LRRK2 degradation is dependent on a PROTAC® mechanism linked to the cereblon E3 ligase

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



- *In vitro* screening tier identifies allele selective, non-toxic PROTAC degraders for HTT (*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 compound solubility and aiding interpretation of cell-based degradation and viability data

Our LRRK2 and mHTT targeting PROTAC® degraders differentiate from conventional inhibitors and represent an exciting potential opportunity for treating CNS diseases:
- LRRK2 PROTAC degrades both WT and G2019S LRRK2 with no apparent preclinical toxicity and
- Directly targets and degrades LRRK2 on mechanism through engaging an undisclosed E3-ligase
- mHTT PROTAC directly targets and degrades mHTT allele selectively, on mechanism, through engaging an undisclosed E3-ligase.

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