Development of potent, orally bioavailable, and highly selective LRRK2 PROTAC® degrader molecules as potential disease modifying therapeutics for Parkinson's disease

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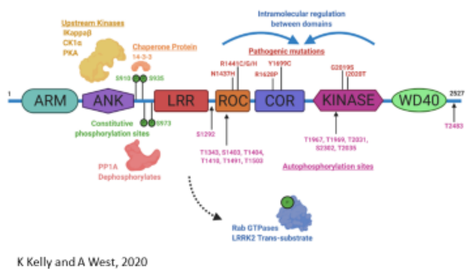
Objective: : Report on the pharmacological characteristics of small molecule PROteolysis TArgeting Chimera (PROTAC®) molecules designed to induce degradation of leucine rich repeat kinase 2 (LRRK2) for the potential treatment of Parkinson's disease (PD) and related neurodegenerative diseases.

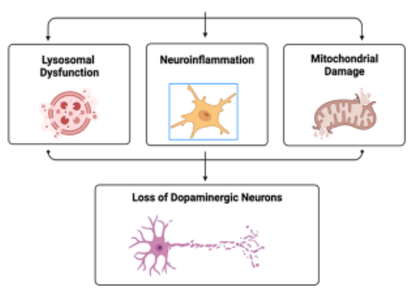
Human genetics and biology create a compelling rationale for developing LRRK2 PROTAC® degraders for the treatment of PD

LRRK2 is composed of two enzymatic domains flanked by multiple protein-protein binding domains



K Kelly and A West, 2020

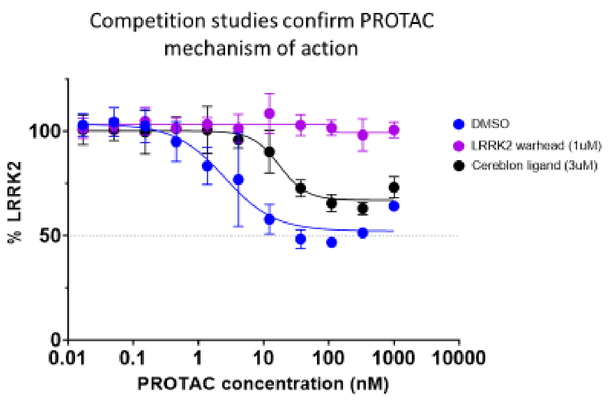
Mutations in and increased expression of LRRK2 are associated with key aspects of neurodegeneration in PD



Background: LRRK2 missense mutations are the most common cause of familial PD and LRRK2 variants are associated with increased risk for developing sporadic PD. LRRK2 mutations cause gain-of function increases in kinase activity and LRRK2 overactivation also has been observed in sporadic PD. Human genetics and pre-clinical studies have shown that reduction of LRRK2 kinase activity or expression is neuroprotective.

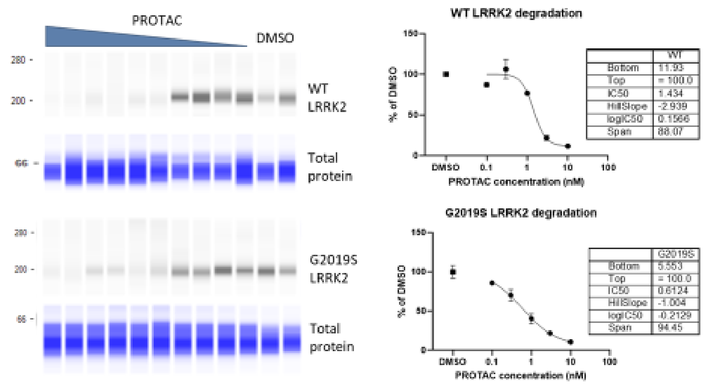
PROTAC®-mediated LRRK2 degradation is on-mechanism

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

LRRK2 PROTAC® molecules induce LRRK2 degradation in WT and G2019S human iPSC-derived microglia



	WT	G2019S
Bottom	11.93	5.553
Top	= 100.0	= 100.0
IC50	1.434	0.6124
HillSlope	-2.939	-1.004
logIC50	0.1566	-0.2129
Span	88.07	94.45

- 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

LRRK2 PROTAC® dose-dependently, and durably degrade target in mouse brain 24h following single oral administration, with rapid return to baseline

PROTAC-A and B Dose-Response PK/PD in mouse cortex 24h post single oral dose

PROTAC-B time course in mouse cortex

Differential Pharmacology of LRRK2 PROTAC® vs. LRRK2 Kinase Inhibitor in G2019S mouse model

G2019S LRRK2 Engagement
LRRK2 PROTAC® vs. Kinase inhibitor (Tmax)

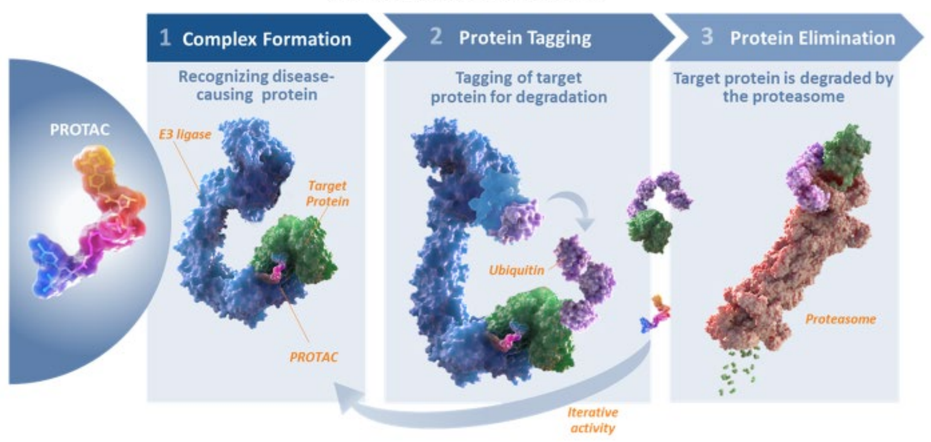
G2019S pRAB12 Pathway Engagement
(LRRK2 PROTAC® vs. Inhibitor)

- PROTAC® advantage (event-driven pharmacology) results in iterative activity compared to kinase inhibition
- 73-fold difference in target engagement and 53-fold difference in pathway engagement

Results: LRRK2 PROTAC® molecules demonstrate robust and selective LRRK2 degradation and pathway engagement in cell-based models and in vivo. Oral administration of LRRK2 PROTAC® molecules demonstrate concentration-dependent reductions of LRRK2 protein in the brain. Assessment of LRRK2 degradation kinetics indicate that pharmacodynamic responses are durable and reversible. We demonstrate LRRK2 degradation in mouse, rat, and non-human primate after oral dosing, with robust biodistribution to deep anatomic brain regions. Lastly, we show LRRK2 PROTAC® degraders more potently engage the target compared to kinase inhibitors.

References
- K. Kelly and A.B. West, (2020). Pharmacodynamic biomarkers for emerging LRRK2 therapeutics. Frontiers in Neuroscience, 14:807
- R Marwaha and M Sharma, (2017). DQ-Red BSA Trafficking Assay in Cultured Cells to Assess Cargo Delivery to Lysosomes, Bio-protocol 7 (19): e2571.

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



Methods: LRRK2 PROTAC® molecules were characterized pharmacologically in vitro and in vivo, across species, and in models of familial PD. LRRK2 degradation and pathway engagement were measured in different cellular systems and mechanism-of-action confirmed by ligand competition. In vivo characterization of PK/PD relationships were evaluated by relating LRRK2 PROTAC® plasma and brain exposure to LRRK2 degradation and downstream phospho-RAB pathway engagement in the brain. PK/PD for LRRK2 kinase inhibitors were also evaluated for comparison. Drug exposure was measured by LC-MS/MS. LRRK2 protein levels were measured using an MSD immunoassay and phospho-RAB pathway engagement was assessed by immunoblot. Target selectivity was assessed by TMT proteomics.

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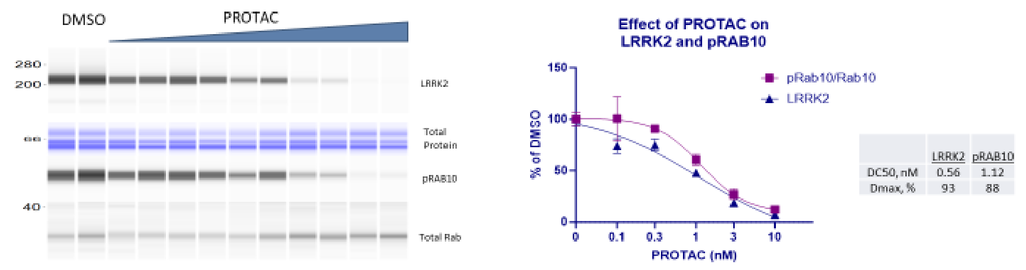
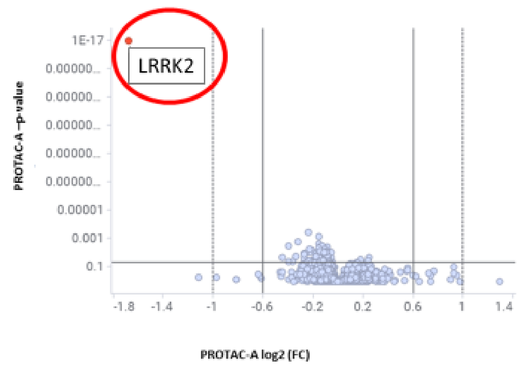
LRRK2 PROTAC® molecules induce LRRK2 degradation and pRab10 reduction in human PBMCs



	LRRK2	pRAB10
DC50, nM	0.56	1.12
Dmax, %	93	88

- Human PBMCs were treated with PRTOAC® and the effect on LRRK2 and pT73 Rab10 measured by capillary immunoassay
- Data demonstrates comparable pharmacology and pathway engagement

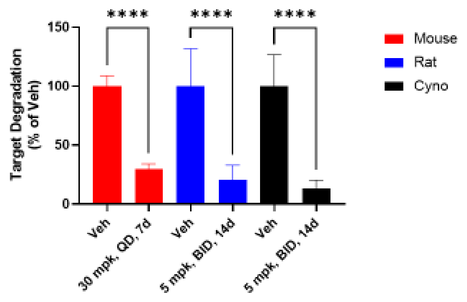
Oral PROTAC® degrader is highly selective for LRRK2 in mouse brain



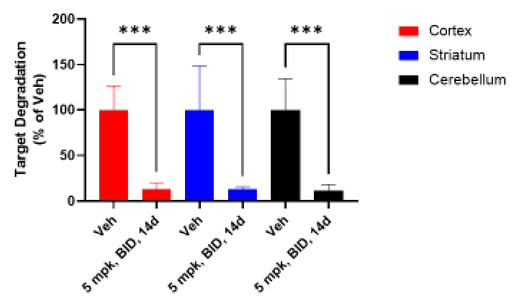
- C57BL6 mice were dosed PO with PROTAC®. Cortex was collected 24 hr post-dose and tissue extracted and subjected to TMT proteomic analysis
- Data demonstrates that LRRK2 is the most significantly changed protein (p> 10e-17) in the cortex

PROTAC®-induced LRRK2 degradation in brain following oral administration observed in mouse, rat and cyno, robust biodistribution in cyno

Target degradation in brain across species (mouse, rat, cyno) after oral PROTAC® dosing

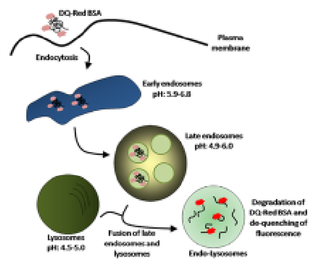


Robust biodistribution in cynomolgus monkey brain measured after oral dosing across brain (cortex, cerebellum, & striatum)

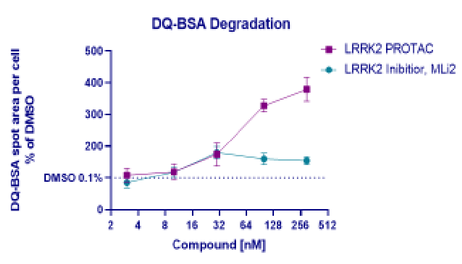


LRRK2 PROTAC® enhances lysosome-based degradation

DQ-Red BSA can be used to monitor lysosome-mediated degradation



More robust induction of DQ-Red BSA degradation with LRRK2 PROTAC compared to kinase inhibitor



- Comparable pharmacology for target engagement observed for LRRK2 PROTAC® and MLi2 kinase inhibitor (data not shown)
- No impact on transferrin uptake (data not shown)
- Data demonstrates that LRRK2 degradation induces enhanced lysosomal clearance

Conclusions LRRK2 PROTAC® molecules demonstrate excellent in vitro and in vivo LRRK2 degradation and downstream pathway engagement, compare favorably to LRRK2 kinase inhibitors, and may represent a new class of disease modifying therapeutics for PD.

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Presented at the 2023 International Congress of Parkinson's Disease and Movement Disorders in Copenhagen, Denmark