LRRK2 PROTAC® degrader molecules induce robust translational biomarker responses in preclinical in vivo pharmacology studies following acute and chronic oral dosing

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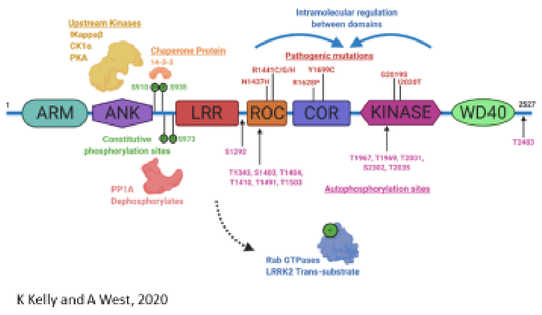
Objective: : Report on the non-clinical in vivo pharmacokinetic and pharmacodynamic biomarker response to PROteolysis TArgeting Chimera (PROTAC®) molecules designed to induce degradation of leucine rich repeat kinase 2 (LRRK2) for the potential treatment of Parkinson's disease (PD).

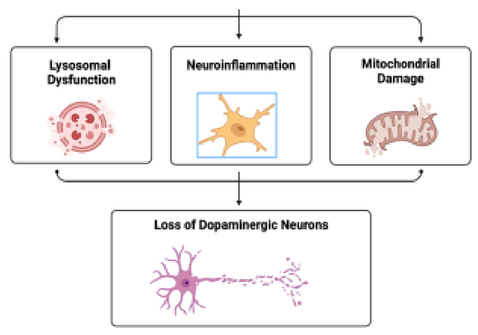
Human genetics and biology create a compelling rationale for developing LRRK2 PROTAC® degraders for the treatment of PD

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

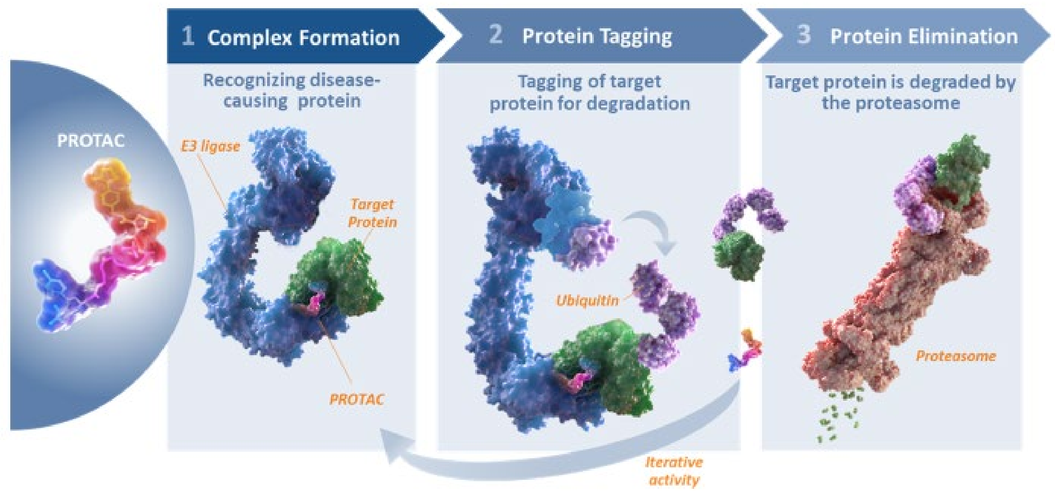


K Kelly and A West, 2020

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



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

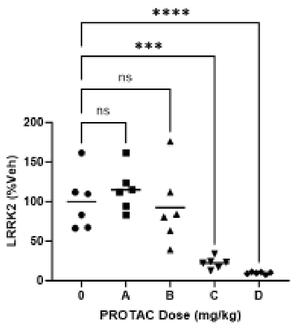


Background: Mutations in LRRK2, resulting in gain-of-function increase in kinase activity, are a common cause of familial and sporadic PD. In addition, an increase in LRRK2 expression driven by a single nucleotide polymorphism in the LRRK2 locus (rs76904798) is associated with a higher risk for developing PD. LRRK2 is a large multidomain protein and plays a role in diverse cellular processes including endolysosomal regulation, autophagy, mitophagy and neuroinflammation. Human genetics and preclinical animal model data suggest that reduction of 50% of LRRK2 protein may impact pathology and dysfunction in PD. Therefore, degradation of LRRK2 in the brain may be beneficial for the treatment of PD. We have identified potent, selective, orally bioavailable LRRK2 PROTAC degraders that cross the blood brain barrier in preclinical species and biodistribute to deep brain regions impacted in PD.

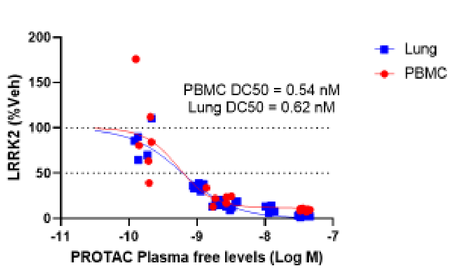
Methods: Orally bioavailable LRRK2 PROTAC degraders were assessed in non-clinical in vivo pharmacology studies in rodents and non-human primates to evaluate LRRK2 degradation and pathway engagement in samples relevant for clinical biomarker analysis. LRRK2 levels and phospho-RAB10 were measured in peripheral blood mononuclear cells (PBMCs) by Meso Scale Discovery assay and capillary immunoassay, respectively. LRRK2 levels in cerebrospinal fluid (CSF) were measured by single molecule array assay. Urine di-22:6 bis(monoacylglycerol) phosphate (BMP) levels were measured by LC-MS/MS. PROTAC plasma and CSF exposure levels were measured by LC-MS/MS.

PROTAC® molecules induce degradation of LRRK2 in mouse PBMCs consistent with pharmacology in lung tissue

LRRK2 degradation in PBMCs



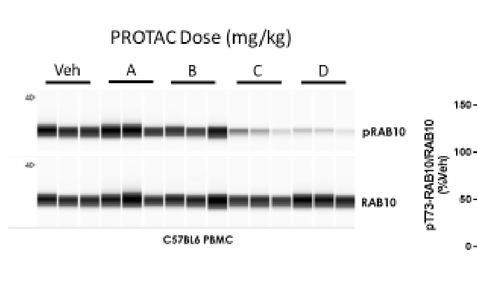
PK/PD of LRRK2 degradation in PBMC and lung



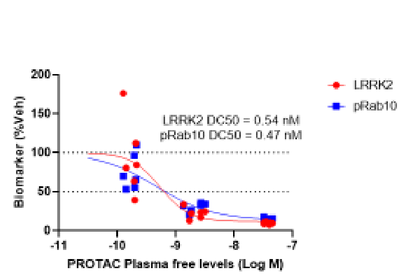
- PROTAC®–induced LRRK2 degradation was assessed in PBMCs collected from C57BL6 mice after a single oral dose. Quantitation of LRRK2 normalized to total protein was determined and reported as % of vehicle-treated controls.
- PK/PD analysis based on free plasma PROTAC levels vs LRRK2 degradation in PBMCs or lung tissue. Results demonstrate that LRRK2 degradation pharmacology in PBMCs is consistent with lung and support the utility of PBMC LRRK2 as surrogate biomarker

PROTAC® -induced reductions of pT73 Rab10 in mouse PBMCs confirm pathway engagement

pT73 Rab10/Rab10 reduction in PBMCs

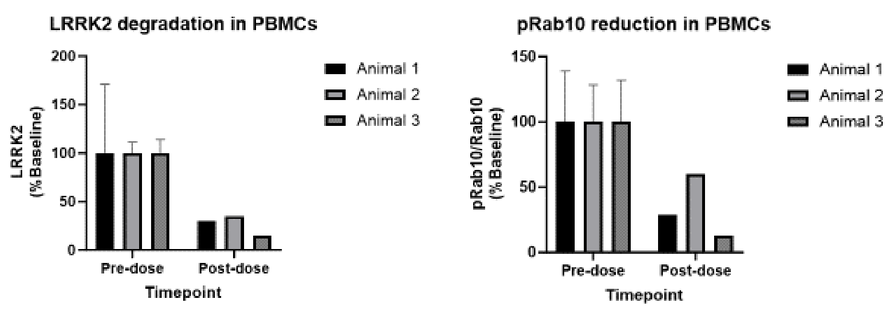


PK/PD of pRab10 and LRRK2



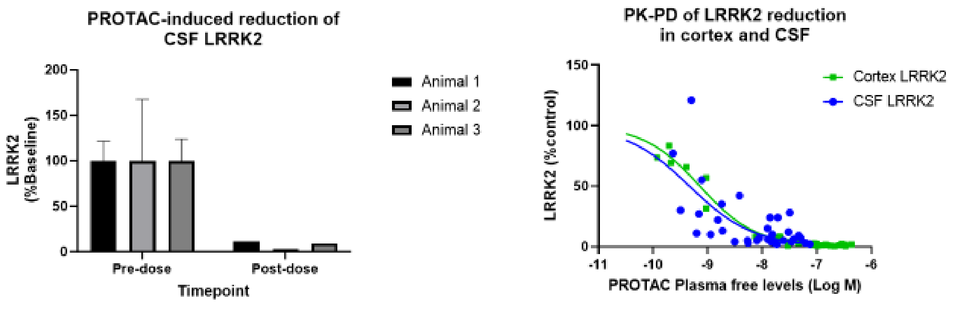
- PROTAC®–induced reduction of pT73 Rab10/RAb10 was assessed in PBMCs collected from C57BL6 mice after a single oral dose. pT73 Rab10 and Rab10 levels were measured by capillary immunoassay, the ratio calculated and reported as % vehicle-treated controls.
- Evaluation of PK/PD for plasma free drug levels vs LRRK2 degradation and pRAb10 reduction in mouse PBMCs. PK/PD analysis demonstrates equivalent pharmacology for both endpoints

Degradation of LRRK2 and reduction of pT73 Rab10 in PBMCs observed following oral dosing of PROTAC® in cynomolgus monkeys



- PROTAC®–induced LRRK2 degradation was assessed in PBMCs collected from cynomolgus monkeys (n=3) following a single oral dose. LRRK2 levels were measured and normalized to total protein. pRab10 and Rab10 levels were measured by capillary immunoassay and the pRab10/Rab10 ratio calculated. Baseline levels for each endpoint were calculated using mean data from 2 pre-dose samples and then used for baseline normalization for each animal. Data for one post-dose timepoint shown

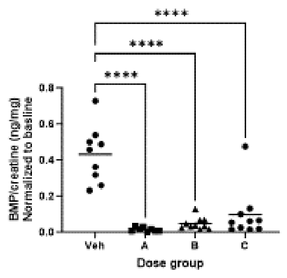
PROTAC ®-induced reductions of CSF LRRK2 consistent with brain LRRK2 reductions in cynomolgus monkeys



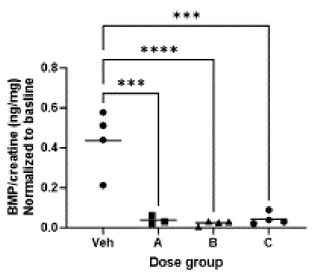
- A LRRK2 SIMOA assay was developed and used to analyze CSF samples collected from a study with a LRRK2 PROTAC® administered once daily via oral gavage to cynomolgus monkeys (n=3) ported for longitudinal CSF collection. Baseline samples collected at 3 timepoints prior to dosing were used to established baseline levels for each animal. LRRK2 levels were normalized to the mean of the baseline for each animal. CSF LRRK2 reductions are shown for a single post-dose timepoint after dosing to steady-state
- Evaluation of PK/PD at steady-state for plasma free drug levels vs LRRK2 reductions in CSF are shown and compared to a separate study comparing plasma free drug levels vs LRRK2 reduction in cortex tissue. Equivalent PK/PD supports the utility of measuring CSF LRRK2 as a surrogate for monitoring LRRK2 reductions in brain tissue

PROTAC® -induced reductions of urine di-22:6 BMP observed in rat and cynomolgus monkey

BMP reductions in Wistar Han Rats



BMP reductions in cynomolgus monkeys



- LRRK2 PROTAC® was administered once daily via oral gavage to Wistar Han rats or cynomolgus monkeys. Urine samples were collected predose and following dosing to steady-state. Di-22:6 BMP and creatinine were measured by UPLC-MS/MS (Nextcea, MA). BMP levels were normalized to creatinine and then expressed relative to baseline.
- Results consistent with findings from preclinical and clinical studies with LRRK2 kinase inhibitors and with results from LRRK2 knock-out mice (Fuji et al., 2015; Baptista et al., 2020: Jennings et al., 2022, 2023).

Results: Acute and chronic oral administration of LRRK2 PROTAC molecules induced robust reductions of LRRK2 protein in CSF, confirming PROTAC brain exposure and pharmacology. LRRK2 and phospho-RAB10 reductions were observed in PBMC extracts, demonstrating peripheral target and pathway engagement. Reductions of urine BMP levels following chronic dosing were observed, confirming effects of LRRK2 reduction on lysosome biology.

Conclusions These results support translational approaches for the future assessment of LRRK2 PROTAC molecules as a potential disease modifying therapy for the treatment of PD.

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