LRRK2 Antisense Oligonucleotides Ameliorate α-Synuclein Inclusion Formation and Provide Neuroprotection in a Parkinson's Disease Mouse Model

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ABSTRACT - POSTER #S183

Objective: LRRK2 mutations are the major cause of familial late-onset Parkinson's disease (PD). However, the interplay between LRRK2 and alpha-synuclein (aSyn) PD pathophysiology is still of debate and undergoing extensive research. To determine whether LRRK2 expression modifies aSyn pathology spreading, we lowered endogenous LRRK2 by antisense oligonucleotide (ASO) in mice injected with pre-formed aSyn fibrils (PFF), a model of PD.

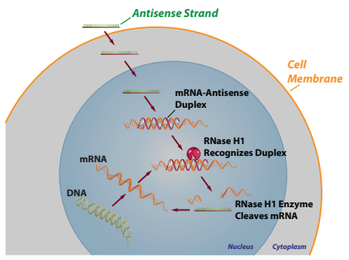
Methods: In short term study, wildtype mice were injected intracerebroventrically (ICV) with LRRK2 ASOs 14 days before intra-striatal inoculation of aSyn PFF, and were sacrificed at 56 days post ICV. In long term study, mice were also pretreated with ASO before PFF inoculation as short term study. However, mice received a 2nd ICV dose at 90 days, and were sacrificed at 180 days post 1st ICV treatment. LRRK2 mRNA, protein, and phosphorylated aSyn pathology were assessed by RT-QPCR, western blots, and immunohistochemical methods, respectively.

Results: Preventive ASO-mediated suppression of endogenous LRRK2 reduced pathological spread of αSyn pathology in both short and long term studies. Furthermore, mice were protected against aSyn pathology-induced wirehang deficit in αSyn PFF inoculation mouse model.

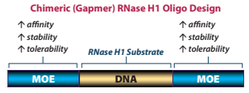
Conclusions: LRRK2 may play an important role in αSyn pathology formation and progression. Thus, ASO targeting LRRK2 is of potential therapeutic use for PD and other synucleinopathies.

BACKGROUND

Antisense Mechanism of Action
RNase H-mediated Degradation



2'-MOE Gapmer Chemistry
Affinity, Stability, Tolerability and Easy Formulation

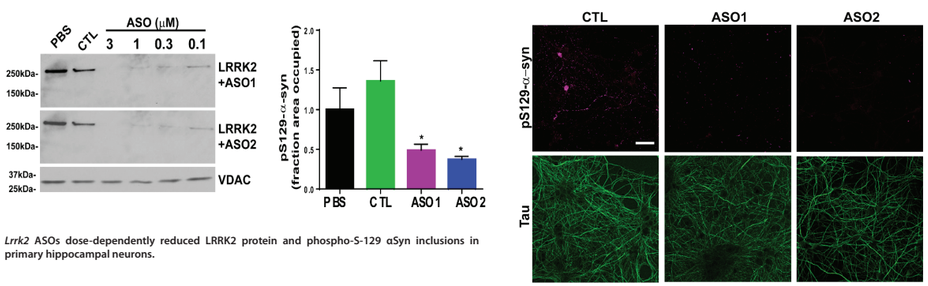


- ▲ Simple water based formulation with excellent stability profile
- ▲ Subcutaneous once/week or less frequent administration
- ▲ Onset of action 5-7 days; Long duration of action (14-21 days)
- ▲ No anticipated drug-drug interactions
- ▲ Clinical experience
  - ➤ Over 7000 subjects dosed
  - ➤ >150 clinical studies
  - ➤ Many therapeutic indications
  - ➤ > 140 patients dosed for > 1 year
  - ➤ Some patients dosed for > 4 years
  - ➤ Attractive tolerability profile

LRRK2 in Parkinson's Disease (PD) and Antisense Oligonucleotides (ASOs) as a Potential Therapy

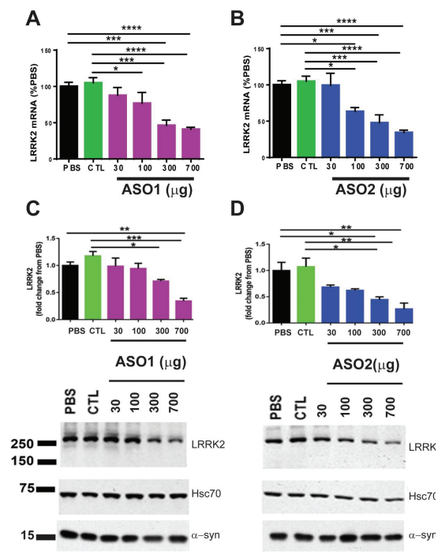
- ▲ LRRK2 is a member of the leucine-rich repeat kinase family
- ▲ Human data validate LRRK2 as a PD target
  - ➤ LRRK2 mutations are the most common genetic cause of PD, accounting for 5-10% of familial cases, and 1-2% of sporadic PD cases, depending on population (Berg et al. 2005, Healy et al. 2008)
  - ➤ Pathogenic mutations in the GTPase, COR, and kinase domains of LRRK2 lead to dominantly inherited late-onset PD (Paisan-Ruiz et al. 2004, Zimbrick et al. 2004), while genetic Genetic variants in LRRK2 gene confer risk for sporadic PD (Di Fonzo et al. 2006, Ross et al. 2008)
  - ➤ LRRK2 protein is increased in sporadic PD compared to controls (Cho et al. 2013, Guerreiro et al. 2013)
  - ▲ LRRK2 is involved in multiple pathways implicated in PD such as alpha synuclein (αSyn), tau, inflammatory response, oxidative stress, and mitochondrial dysfunction (MacLeod et al. Neuron 2006, Matta et al. *Neuron* 2012, Beilina et al. *PNAS* 2014, Gillardon et al *J Neurochem* 2009, for review see Rudenko et al *Neurotherapeutics* 2014)
- ▲ Thus, ASOs targeting LRRK2 will lower production of total LRRK2 and may be a potential therapy for PD

Figure 1: LRRK2 ASOs reduce formation of αSyn inclusions in primary neurons



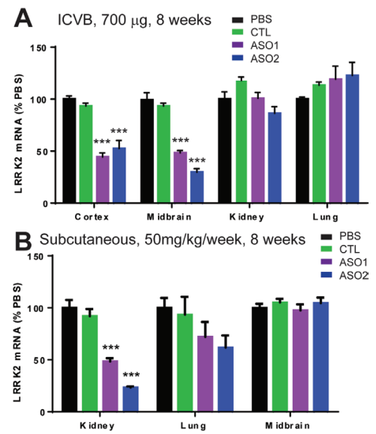
Lrrk2 ASOs dose-dependently reduced LRRK2 protein and phospho-S-129 αSyn inclusions in primary hippocampal neurons.

Figure 2: Central administration of LRRK2 ASOs reduce LRRK2 levels in the midbrain



Lrrk2 ASOs dose-dependently reduced *Lrrk2* mRNA and protein in the midbrain at 2 weeks post intracerebral ventricular bolus (ICVB) administration.

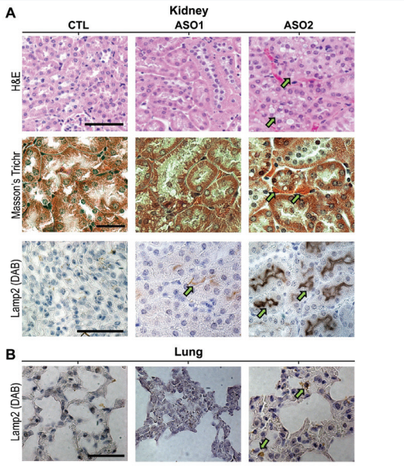
Figure 3: Effects of ICVB ASO injections compared to systemic ASO injections on LRRK2 levels in brain, kidney and lung



A. LRRK2 ASOs reduced *Lrrk2* mRNA in the cortex and midbrain for up to 8 weeks post ICVB administration, but had no effects on *Lrrk2* mRNA levels in the kidney and lung.

B. Subcutaneous injections of LRRK2 ASOs weekly for 8 weeks reduced *Lrrk2* mRNA in kidney and lung but had no effect in midbrain *Lrrk2* mRNA level.

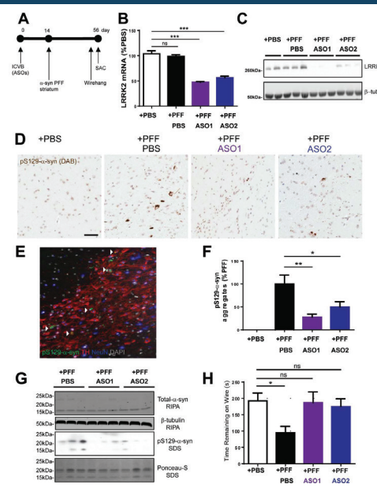
Figure 4: Systemic administration of LRRK2 ASOs recapitulates some LRRK2 knockout phenotypes



A. Masson's Trichrome stain revealed protein deposits (orange coloration, indicated by green arrows) and LAMP2 immunohistochemistry indicated accumulation of late endosomes/lysosomes in kidney tubule cells (green arrows) following systemic LRRK2 ASO administration.

B. LAMP2 immunohistochemistry revealed abnormal lysosomes accumulations in pneumocytes in lung (green arrows) following systemic LRRK2 ASO administration.

Figure 5: Centrally administered LRRK2 ASOs reduce formation of αSyn inclusions



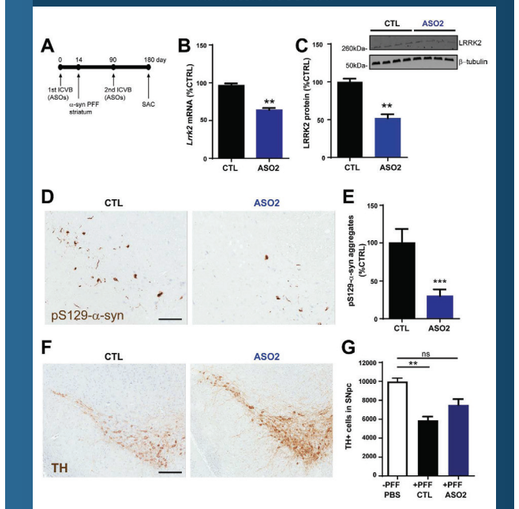
A. Schematic of ASO injection, PFF injections and time point at which LRRK2 levels and αSyn pathology was assessed.

B-C. *Lrrk2* mRNA and protein were reduced in ASO-treated mice.

D-G. LRRK2 ASOs treatment reduced phospho-S-129 αSyn inclusions in the substantia nigra pars compacta and insoluble αSyn in the contra-lateral cortex of PFF-injected mice.

H. LRRK2 ASOs treatment rescued wire-hang deficits in PFF-injected mice.

Figure 6: LRRK2 ASOs preserve dopamine neurons in the SNpc after PFF exposure compared to control ASOs



A. Schematic of ASO injection, PFF injections and time point at αSyn pathology, dopamine neurons counts were analyzed.

B-C. *Lrrk2* mRNA and protein were reduced in LRRK2 ASO-treated mice.

D-E. LRRK2 ASO reduced phospho-S-129 αSyn inclusions in the substantia nigra pars compacta of PFF-injected mice.

F-G. LRRK2 ASO ameliorated dopamine TH-positive cells loss in PFF-injected mice.

CONCLUSIONS

- ▲ LRRK2 ASOs dose-dependently reduced LRRK2 mRNA and protein, and exhibited long duration of action *in vivo*
- ▲ Preventive ASO-mediated suppression of endogenous LRRK2 reduced pathological spread of αSyn pathology and protected mice against a Syn pathology-induced wirehang deficit in αSyn PFF inoculation mouse model
- ▲ Thus, ASO targeting LRRK2 may be a potential therapy for PD

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