A step forward for LRRK2 inhibitors in Parkinson's disease

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Abstract

The results of a phase 1 clinical trial for kinase inhibitors targeting LRRK2 provide the foundations for moving towards testing the efficacy of LRRK2 kinase inhibition in Parkinson's disease.

In common with the majority of neurodegenerative diseases, there is an urgent need for disease-modifying therapies for Parkinson's disease (PD). PD is a progressive neurological disorder, characterised by declining brain function across a range of domains, most obviously (but not limited to) loss of motor control. Although there are a number of pharmacological and non-pharmacological therapies for treating the symptoms of PD, predominantly targeting the dopamine pathway, to date there are no drugs that slow, halt or reverse the progression of PD. Reporting the results of a first-in-human trial for a kinase inhibitor of Leucine Rich Repeat Kinase 2 (LRRK2), Jennings and co-workers present an important advance along the drug development pathway for a target that has long been a priority for the PD research community (*1*).

The expansion of our understanding of genetic variation that increases the risk of developing PD has provided an important source of putative disease modifying drug targets for the PD research community. Yet it is more than 18 years since coding mutations at the *LRRK2* locus on chromosome 12 were identified as a common cause of familial PD (*2, 3*). Since then, functional characterisation of LRRK2 and the impact of mutations has revealed a key role for altered kinase function in PD pathogenesis, with increased substrate phosphorylation observed for the majority of reported mutations. Importantly, several early studies demonstrated that neuronal

toxicity associated with mutations was reduced following genetic ablation of kinase activity, providing a clear rationale for targeting the enzymatic activity of LRRK2 as a therapeutic strategy in PD (*4, 5*). The human genetics of the *LRRK2* locus have, in the meantime, become even more interesting – with evidence accruing that non-coding variants around the *LRRK2* gene increase the risk for idiopathic PD. This raises the possibility that targeting LRRK2 potentially may have benefits for the more common sporadic forms of PD, as well as for those carrying mutations in the *LRRK2* gene (*6*).

One specific concern that arose from preclinical animal model analyses of LRRK2 was the observation that vesicular pathology was found in the lungs and kidneys of rodent and primate models either lacking LRRK2 or following treatment with LRRK2 kinase inhibitors. This concern was partly addressed by the demonstration that on target LRRK2 kinase inhibitor-associated pathology was reversible in non-human primates upon cessation of treatment (*7*). Intriguingly, and reducing concern about targeting LRRK2 in humans and increasing the incentive to investigate further, there are also heterozygous loss-of-function variants at the *LRRK2* locus with no apparent deleterious consequences (*8*). This suggests that it should be possible to reduce LRRK2 activity by half in humans without causing any on target side effects.

Building on the human genetic and target validation data, the outcome of a first-in-human clinical trial for LRRK2 kinase inhibitors has, therefore, been keenly anticipated by the PD community. Using the aminopyrazole LRRK2 kinase inhibitor DNL201 (previously called GNE-0877), Jennings et al. from the biotechnology company Denali Therapeutics, now report the outcome of both extensive preclinical testing, and a phase 1 and phase 1b human clinical trial (*1*). The preclinical data adds to the already extensive literature for LRRK2 kinase inhibitors, including DNL201. Indeed, it builds upon recent research that has revealed a key role for LRRK2 in responding to lysosomal damage and stress. The authors demonstrate that inhibition of LRRK2 kinase activity can reverse lysosomal dysfunction concomitant with expression of a mutant form of LRRK2 carrying the G2019S mutation, the most common PD-associated mutation in humans. Intriguingly, the authors also examined whether LRRK2 inhibition could benefit lysosomal damage caused by mutations in *GBA1*, which are a strong risk factor for PD when

heterozygous and cause Gaucher disease when homozygous. They showed a reduction in lysosomal pathology in fibroblasts from patients with Gaucher disease treated with DNL201 in vitro. Whether this finding has implications for a broader application of LRRK2 kinase inhibition in patients with genetic forms of PD where proteins other than LRRK2 are mutated requires further investigation. This is emphasised by the conflicting data in the literature relating to the interplay between LRRK2 and Glucocerebrosidase (the protein product of *GBA1*), with both upregulation and downregulation of glucocerebrosidase enzymatic activity reported in the presence of LRRK2 mutations.

At the heart of the new study is how LRRK2 responds to LRRK2 kinase inhibitors *in vivo* in humans, with important details presented on the pharmacokinetics and pharmacodynamics of these compounds. The Denali Therapeutics team demonstrate that DNL201 is able to enter the human central nervous system based on measurements of inhibitor concentrations in cerebrospinal fluid in human volunteers treated with DNL201, replicating work from animal models that had suggested that this compound could cross the blood brain barrier (**Figure 1**). The investigators also report a dose-dependent inhibition of LRRK2 kinase by DNL201 using blood-based readouts including phosphorylation of serine 935 of LRRK2 (an indirect measure of LRRK2 kinase activity) and phosphorylation of threonine 73 of Rab10 (thought to be a direct LRRK2 substrate). Their data from non-human primates, imply ---but do not prove---equivalent inhibition within the human central nervous system.

One of the abiding concerns around the use of LRRK2 kinase inhibitors in humans, deriving from rodent and non-human primate preclinical studies, is that there may be on-target consequences for pulmonary function. The results of the human volunteer studies in the Jennings et al. paper (*1*) did not reveal any impact upon lung function across the dose ranges investigated, allaying at least some of the concerns relating to targeting LRRK2. As the authors note, however, longer term monitoring beyond 10 days will be required to assess the impact of chronic dosing with LRRK2 kinase inhibitors upon human physiology. In order to assess whether LRRK2 kinase inhibition can modulate lysosomal function, the authors measured urine concentrations of bis(monoacylglycero)phosphate (BMP), a lysosomal cofactor, as a proxy for direct measures of lysosomal function. Although they provide

preclinical data linking urine BMP concentrations to LRRK2 inhibition, the precise mechanisms and events linking LRRK2, lysosomal function, and BMP require further investigation.

A final note of interest is provided by a direct comparison in the phase 1b trial of *LRRK2* mutation carriers with PD (harboring the G2019S mutation in the kinase domain of LRRK2) and those with idiopathic PD of unknown cause carrying a wildtype LRRK2 allele. The small numbers included in the phase 1b trial (a total of 8 LRRK2 G2019S mutation carriers) preclude a detailed analysis of whether lysosomal response to DNL201 varied among mutation carriers and those with a wildtype *LRRK2* allele. It will be important to elucidate these relationships more fully in future clinical trials.

Spanning preclinical and clinical data, the Jennings et al. study represents a major step forward for LRRK2 kinase inhibition as a therapeutic strategy in humans, demonstrating target engagement and, crucially, safety and tolerability (*1*). There are, however, many questions that remain relating to how to target and measure LRRK2 activity in humans. Despite considerable progress over the past decade, it is still not entirely clear how mutations in LRRK2 lead to neurodegeneration and thence to disease. This raises substantive questions in the context of targeting LRRK2, most notably relating to the appropriate aspect of LRRK2 biology to target and how best to measure this in an experimental medicine setting. For example, whereas there is abundant evidence that LRRK2 kinase activity is disrupted by coding mutations, it has not yet been demonstrated beyond doubt that it is this enzymatic change that drives pathology in PD given that LRRK2 also possesses an active GTPase domain, an activity that is also disrupted by mutations. This then begs the question as to whether LRRK2 kinase inhibitors will be able to address deficits in individuals with mutations outside of the kinase domain of LRRK2, or for that matter those who do not have disease-associated coding variants. In light of this, it is noteworthy that an alternative strategy using antisense oligonucleotide knockdown of LRRK2 has moved into human testing in a phase 1 trial (Clinical Trials identifier NCT03976349). Likewise, the disease relevance of direct and indirect phospho-substrates of LRRK2 such as Rab10, as well as functional readouts, for LRRK2 are a matter of some debate. Improving and optimising Rab phospho-substrates and lysosomal function as markers for the inhibition of LRRK2 kinase activity *in vivo* is a key unmet need for

future clinical trials, and is under intense investigation in both academic and industry-based research laboratories.

Another key question is whether there is broader applicability of LRRK2 kinase inhibition beyond *LRRK2* mutation carriers to include *GBA1* heterozygous mutation carriers and patients with idiopathic PD, and perhaps even other neurodegenerative diseases. . The identification of the *LRRK2* locus as a modulator of progression in the primary tauopathy Progressive Supranuclear Palsy by a recent genome-wide association study is of particular interest in this regard (*9*). The functional basis of this relationship, however, remains unknown.

Finally, there are the substantial challenges faced by clinical trials for all neurodegenerative diseases – measuring outcomes in chronic, slowly progressing disorders with variable rates of progression. A defined genetic cohort reduces, but does not dismiss, some of these challenges. However the only way to understand how to measure and target LRRK2 in PD will be to carry out further phase 2 and 3 clinical trials (*10*), with Denali Therapeutics already planning to initiate these in partnership with Biogen for a small molecule LRRK2 kinase inhibitor (DNL151/BIIB122) distinct from the inhibitor reported in Jennings et al.

There is much still to be done in terms of both clinical and fundamental investigation into the pathophysiology of LRRK2 in PD. However, the demonstration that small molecule LRRK2 kinase inhibitors can enter the human brain, and can achieve a reduction in LRRK2 activity in the absence of serious side effects, surmounts a major obstacle to further development in this area. The new work opens the door to testing the implications of almost 20 years of accumulated research into LRRK2 and PD, as well as sustaining a promising avenue of drug discovery for PD into the next phase of development.

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Competing interests

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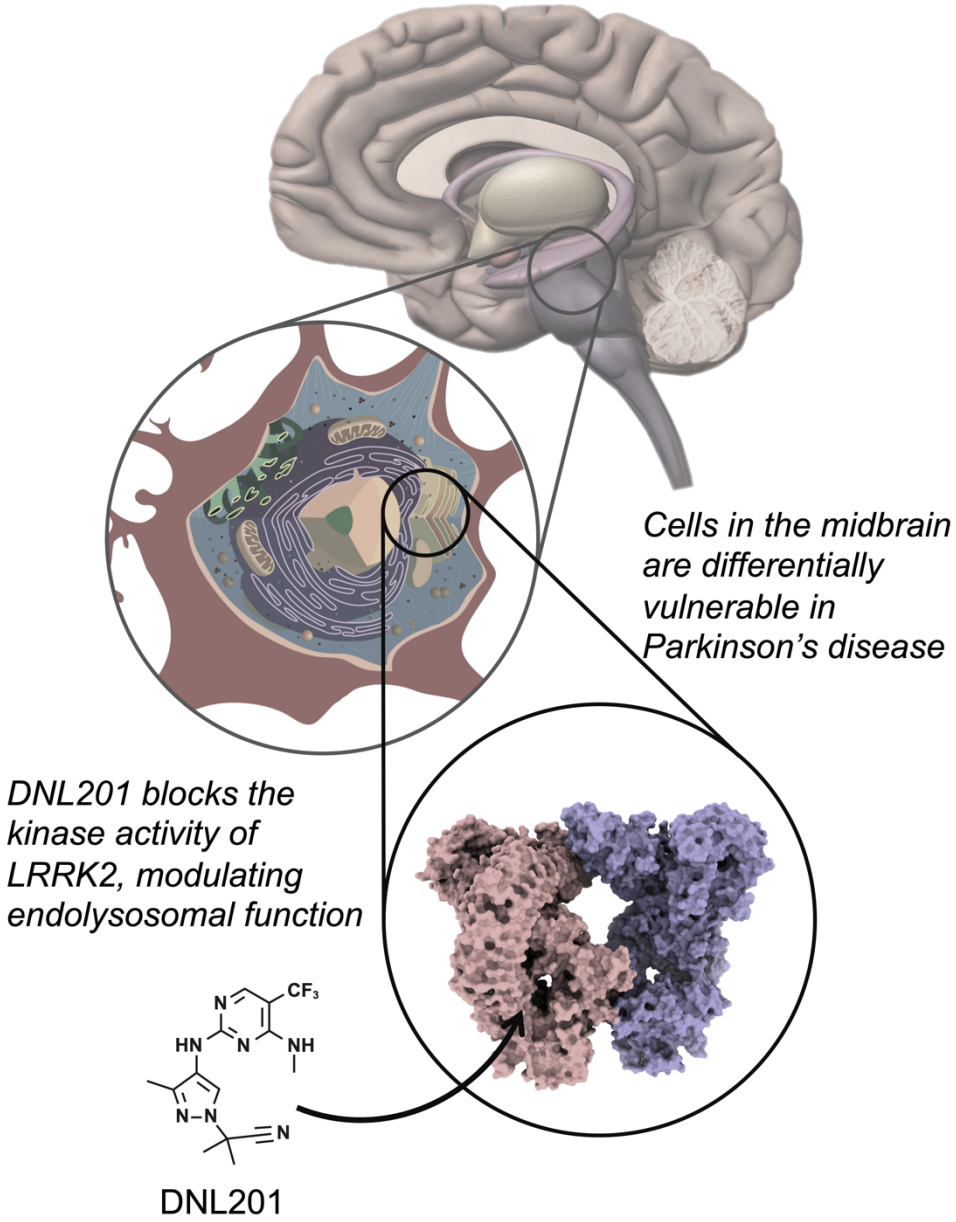

Figure 1. **Reducing LRRK2 kinase activity in the human brain.** Reducing the kinase activity of LRRK2 (circled cryoEM structure) is a therapeutic strategy for slowing the progress of PD, which has just entered clinical testing. The LRRK2 inhibitor DNL201 (shown) enters the human brain and reduces LRRK2 kinase activity in brain cells (1) including those of the nigrostriatal pathway, which preferentially degenerate in PD. DNL201 inhibits LRRK2, and is thought to modulate lysosomal function in brain cells in rodents, nonhuman primates and human volunteers. Image of the LRRK2 dimeric complex is derived from Protein Data Bank (PDB) 7LHT (https://www.rcsb.org/structure/7LHT) using UCSF ChimeraX.