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REVIEW

Leucine-rich repeat kinase 2 (LRRK2): an update on the potential therapeutic target for Parkinson's disease

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ABSTRACT

Mutations in Leucine-rich repeat kinase 2 (LRRK2) are a risk factor for and a cause of sporadic and familial Parkinson's disease (PD), respectively. These mutations are some of the most common genetic contributors to PD and render the kinase hyperactive. Increasingly within the past decade, there has been substantial effort investigating LRRK2 as a target for therapeutics in preclinical studies, and currently, small-molecule inhibitors and antisense oligonucleotides are being assessed in clinical trials as therapies to reduce the toxic hyperactivity of its kinase and/or reduce total levels of the protein in healthy individuals and people with PD.

Areas covered: In this review, we will provide an update on the current status of drugs and other technologies that have emerged in recent years and provide an overview of their efficacy in ameliorating LRRK2 kinase activity and overall safety in animal models and humans.

Expert opinion: The growth of both target discovery and innovative drug design has sparked a lot of excitement for the future of how we treat Parkinson's disease. Given the immense focus on LRRK2 as a therapeutic target, it is expected within the next decade to determine its therapeutic properties, or lack thereof, for PD.

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1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder affecting voluntary movement with cardinal features being bradykinesia, muscle rigidity, and resting tremor and most often including a slew of other non-motor symptoms including depression, constipation, hyposmia, postural instability, and insomnia [1]. A definitive diagnosis of PD is confirmed by loss of dopaminergic neurons within the substantia nigra pars compacta of the brain as well as the protein and lipid inclusions known as Lewy bodies observed through autopsy. Thus, a prehumous diagnosis relies on clinical observation of the presence of the cardinal features [2,3]. Additionally, a series of supportive data from motor and cognitive tests, and, in some cases dopamine transporter (DAT) positron emission tomography scans, can also be used to exclude other diseases or disorders. One such test is the observation of a positive response, or lack thereof, to a dopamine replacement therapy such as levodopa [1]. Levodopa, a soluble precursor of dopamine, helps to increase dopamine levels in the brain and diminish bradykinesia and rigidity. However, levodopa-induced motor complications will occur such as dyskinesias after a few years of continued use that impede daily activities and reduce patient quality of life [4]. Other medications that may be prescribed in the early stages of disease in order to prolong the onset of levodopa-induced complications are those curbing dopamine metabolism such as monoamine oxidase type b (MAO-B) inhibitors or medications that bypass dopamine by stimulating dopamine receptors directly such as dopamine agonists [4,5]. While useful in curtailing some of the symptoms of PD, currently there are no medications available that aid in slowing or stopping the progression of the disease.

Two decades ago, linkage analysis helped to identify coding variants in *Leucine-rich repeat kinase 2* (LRRK2) as a genetic cause of PD [6,7], and since then extensive genetic analyses and genome-wide association studies have confirmed mutations in *LRRK2* as one of the most common causes of PD, with non-coding variants associated with enhanced lifetime risk of developing idiopathic PD [8–10]. More recently, it has become clear that non-coding variants at the *LRRK2* locus are associated with the rate of progression of the primary tauopathy Progressive Supranuclear Palsy (PSP) [11]. PD-associated mutations in LRRK2 produce a toxic hyperactive kinase, as observed in *in vitro* and *in vivo* models utilizing a LRRK2 autophosphorylation site at serine 1292, a indirect but kinase conformation-dependent phosphorylation site at serine 935, and a subset of small Rab GTPase substrates [12–16]. LRRK2 activity has been associated with a variety of organellar membranes, demonstrating the protein's wide influence on many pathways including the endolysosomal system, autophagy, ciliogenesis,

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Article highlights

- Mutations in LRRK2 are some of the most common causes and risk factors for both familial and sporadic Parkinson's disease
- Pathogenic mutations of LRRK2 cause hyperactivity of its kinase that leads to cellular toxicity
- LRRK2 inhibition is an attractive therapeutic target that has been given much attention within the last decade
- Clinical trials are underway to test the efficacy of reducing kinase activity using a small-molecule inhibitor as well as reducing total protein levels using an antisense oligonucleotide
- Preclinical data suggest that LRRK2 inhibitors are safe in various animal models and new alternative methods for reducing LRRK2 kinase activity are currently under development
- Whether or not LRRK2 inhibitors are clinically efficacious in stopping or slowing disease progression remains to be determined

trans-Golgi integrity, vesicle sorting, and mitochondrial integrity, presumably via its interactions with Rab proteins and other as of yet unidentified substrates [17–28]. Therefore, inhibiting hyperactive LRRK2 is potentially beneficial in altering PD pathogenesis.

A complex protein with both GTPase and kinase domains, LRRK2 is conceptually a strong candidate for the development of targeted therapeutics. Currently, many structurally distinct small-molecule LRRK2 kinase inhibitors are commercially available for experimental purposes and one inhibitor is soon to start phase II of clinical trials. In this review, we will give an update on efforts to employ LRRK2 kinase inhibitors, the potential difficulties of targeting LRRK2 systemically based on the results of preclinical studies, and alternative methods currently being explored to lower LRRK2 kinase activity as a therapy for PD.

2. Type I and type II LRRK2 kinase inhibitors

Since the development of the first small-molecule kinase inhibitor used as an alternative to chemotherapy in cancer patients in 2001, the use of kinase inhibitors as a targeted treatment in a variety of diseases and illnesses have skyrocketed, with currently over 50 kinase inhibitors approved by the US Food and Drug Administration (FDA) available on the market [29,30]. Similarly, LRRK2 kinase inhibitors have gained a large focus as a potential treatment for PD. Years before the initial announcements of a LRRK2 clinical trial, commercially available LRRK2 kinase inhibitors were and continue to be a vital tool in basic biology research. Most LRRK2 kinase inhibitors are orthosteric, thus belonging to the type I class of inhibitors and bind to the ATP-binding pocket of LRRK2 with an 'in' orientation of the DYG activation loop (Figure 1), and range widely in structure and potency. Currently, the most used are the third-generation, structurally distinct, and brain penetrant molecules MLi-2 [31], PF-06685360 (PFE-360), and GNE-7915 for basic research purposes [32]. Each have been observed to strongly inhibit LRRK2 kinase activity via significant decreases in autophosphorylation at S1292 LRRK2 as well as dephosphorylation of downstream Rab substrates in varying models *in vitro* and *in vivo* [13,31–36]. Interestingly, a cluster of phosphorylation sites near the N-terminus of LRRK2, S910/S935/S955/S973 were found to promote 14-3-3 binding to the monomeric form of LRRK2 and are also dephosphorylated after acute and chronic administration of ATP-competitive inhibitors [37,38]. Thus, this cluster of phosphorylation sites have played an important role as readouts for LRRK2 inhibition, although indirect, as direct autophosphorylation readouts can be challenging to detect robustly due to low stoichiometry in some tissues, CSF, and blood [31,39,40].

Alternatively, type II kinase inhibitors, classified as binding to the ATP-binding pocket with an 'out' orientation of the activation loop (Figure 1), such as GZD-824, Rebastinib and Ponatinib, all of which were developed to counteract resistance against the popular drug for chronic myelogenous leukemia, imatinib, have also been found to target LRRK2 kinase activity [43–45]. When compared to the type I molecule MLi-2, all three type II drugs similarly were able to dephosphorylate downstream Rabs such as Rab10 and Rab12 at sites T73 and S105, respectively, in mouse embryonic fibroblasts [45]. Interestingly, type II inhibitors do not dephosphorylate residues S910/S935/S955/S973, however, require upwards of ~30-300x higher concentration compared to type I inhibitors in *in vitro* kinase assays utilizing wild-type LRRK2 [45]. Interestingly, when comparing the potency of type II inhibitors between wildtype and G2019S LRRK2 mutant proteins in a kinase assay using the synthetic LRRK2 substrate Nictide [46], Tasegian and colleagues observed that all three drugs had a lower potency when inhibiting mutant G2019S LRRK2, and this was recapitulated when measuring Rab10 dephosphorylation in both G2019S and R1441C LRRK2 knock-in MEFs [45]. This phenomenon was also observed by Kelly et al. when using the type I inhibitor MLi-2 in rats expressing human G2019S LRRK2 [36]. This suggests that the G2019S mutation may be more resilient to kinase inhibition, regardless of the conformation of LRRK2, although earlier data on the previous generation orthosteric inhibitor LRRK2-IN-1 reported higher sensitivity in G2019S LRRK2 cells compared to wild-type cells, suggesting that this may be drug-specific rather than class-specific [47].

To combat this G2019S resilience, a recent study introduced the synthesis of another indazole-based type I LRRK2 inhibitor with 2000-fold selectivity to G2019S LRRK2 compared to wild-type LRRK2 [48]. This inhibitor, named Compound 38, has also been shown to cross the blood–brain barrier, a first for G2019S-specific molecules, and provides a promising alternative specific for patients carrying the G2019S mutation. An additional compound, EB-42168, is reported to be 100x more selective for G2019S LRRK2 and been shown to ameliorate its hyperactive effects while sparing wild-type LRRK2 in peripheral blood mononuclear cells taken from G2019S-positive PD patients [49]. Thus, the creation of molecules with selective affinity to G2019S LRRK2 may be helpful when considering the drug efficacy of targeting hyperactive kinase specifically in G2019S-positive PD patients.

With regards to molecule affinity, Ponatinib, GZD-824, and Rebastinib have been found to inhibit 14–40 other kinases identified in a small drug screen of 140 different kinases [45], wheresas type I compounds such as MLi-2 and Compound 38 are highly selective for LRRK2 [31]. As of yet, there are no

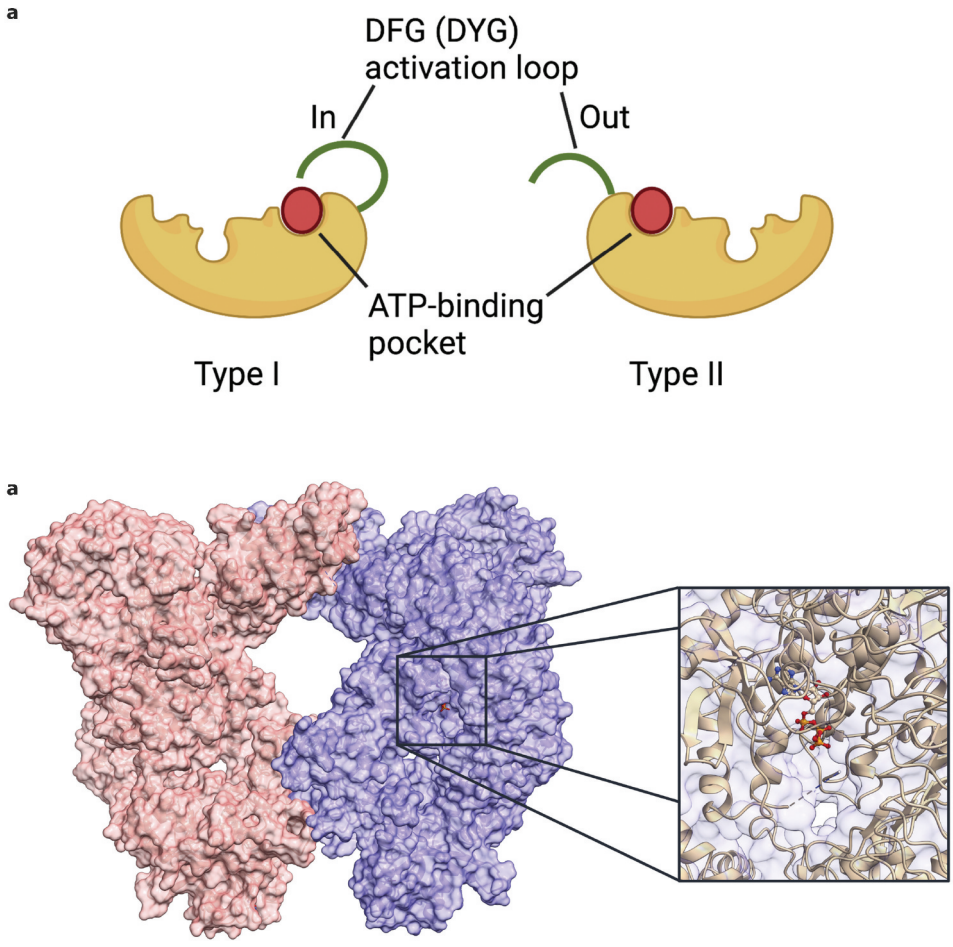

Figure 1. Structural differences between type I and II small molecule kinase inhibitors.

A generic enzyme (yellow) is shown depicting the difference in structural conformation of the DFG/DYG motif (green) when bound to type I and II class kinase inhibitors (red), respectively (A). Dimeric structure for the LRRK2 complex, showing the active site of the kinase with ATP bound (B). Image derived from PDB 7LHT [41] using UCSF chimera [42].

LRRK2-specific type II inhibitors that have been developed. Based on these data, type I inhibitor drugs would seem the better fit when considering chemical compounds for therapeutic use at this time. However, as both classes render LRRK2 in different structural conformations, with type I inhibitor binding favoring a closed, active conformation, which has been associated with microtubule interaction [50–52], and type II binding favoring an open, inactive conformation, both classes hold equal purpose in the continuing efforts to understand the relationship between LRRK2 structure, activity, and its downstream consequences.

2.1. Ongoing clinical trials using kinase inhibitors

Currently, there is one ongoing clinical trial using an orally administered, type I small molecule LRRK2 kinase inhibitor made by Denali Therapeutics (clinicaltrials.gov ID: NCT04056689). This compound, denoted as DNL151, and later BIIB122/DNL151 after a partnership between Denali and Biogen was announced mid-2020, was reported to have met all goals in phase 1 of the trial, in which 184 healthy individuals received a range of doses for up to 28 days. These goals included on-target engagement utilizing phosphorylation sites S935 LRRK2 (greater than or equal to 80% dephosphorylation) and T73 Rab10 (up to 50% dephosphorylation) as readouts in whole blood and PBMCs, respectively [53]. In addition, this trial reported a dose-dependent reduction in 22:6-bis[monoacylglycerol] phosphate (BMP) in urine, a lysosomal lipid and marker of lysosomal function. Phase 1b completed in late 2020 reported similar results and included 36 patients with Parkinson's disease [53]. So far, BIIB122/DNL151 has been well tolerated with minimal adverse events, namely nausea and headaches, which were quickly reversed after cessation of the drug [53]. Development of late-stage clinical trials using BIIB122/DNL151 are currently ongoing.

3. Complications of systemic employment of LRRK2 kinase inhibitors: what preclinical studies tell us

Some of the most successful kinase inhibitors on the market today are those that have systemic targets as on-target effects are easily achieved via oral administration, for example, Janus kinase inhibitors (JAKs) in the treatment for rheumatoid arthritis. What can be particularly challenging for any drug delivered systemically (i.e. oral, intravenous, or intramuscular administration) for neurodegenerative diseases is to design a molecule that can cross the blood–brain barrier, as well as have limited peripheral side effects with chronic use.

Since the generation of third wave LRRK2 inhibitor molecules, a few preclinical studies have been conducted across various animal models to gain insight into the efficacy and safety of targeting LRRK2 kinase chronically. Here, we will provide an update on the effects of type I kinase inhibitors at the molecular and tissue levels across mice, rats, and cynomolgus monkeys.

3.1. Molecular effects of acute and chronic LRRK2 inhibition

Recent studies have shown both consistent results as well as discrepancies between animal species treated with LRRK2 kinase inhibitors. First, several studies in mice and cynomolgus monkeys have shown that total LRRK2 levels in lung and kidney tissues diminish with type I inhibitors GNE-7915, GNE-0877, PFE-360, and MLi-2 within 7 days of daily PO dosing [38,39,54], as had been previously suggested by *in vitro* and kinase-dead knock-in mice studies [55–57]. However, one of these studies suggests that decreased levels are readily restored in peripheral tissues with cessation of treatment, as was observed in mice dosed PO with 120 mg/kg of MLi-2, the dose needed to achieve maximal dephosphorylation of S935, after a seven-day washout period [54]. This loss of total LRRK2 was not observed in rats in the same peripheral tissues, however total levels in brain tissue showed a dose-dependent decrease using in-diet dosing of PFE-360 [36]. Taken together, these data suggest that kinase activity may play a role in the stability of the protein, and thus dosing may need to be carefully monitored when considering LRRK2 kinase inhibitor use in PD patients.

Moreover, wild-type mice given a 60 mg/kg daily in-diet dose of MLi-2 for 6 months showed increased levels of pro-surfactant protein C (proSP-C) in lungs, with an observed peak at 28 days, followed by a gradual decrease back to levels comparable to control groups [54]. This suggests that surfactant trafficking can adapt to chronic high levels of LRRK2 inhibition. Further investigation showed that surfactant secretion is not affected by LRRK2 kinase inhibition across species as measured by surfactant D and A levels in bronchoalveolar lavage fluid [38,39,54]. These studies, executed in wild-type animals, show relatively modest effects on lung tissue after high doses of MLi-2. Interestingly, in G2019S LRRK2 knock-in mice given a 60 mg/kg/day in-diet dose of MLi-2 for 2.5 months showed no difference in proSP-C levels at both 10 days of dosing and at endpoint compared to untreated wildtype animals, suggesting that inhibition of hyperactive LRRK2 kinase does not develop the same molecular effects in lung tissue as wild-type LRRK2 [33]. This is an important distinction, as patients carrying mutant LRRK2 may then have less of a risk in developing secondary lung effects when drugs target hyperactive LRRK2 compared to those without a LRRK2 mutation.

Additionally, in-diet MLi-2 dosing of G2019S LRRK2 knock-in mice at 60 mg/kg/day was sufficient to lower hyperactive LRRK2 back to wild-type levels of activity, as concluded by comparable levels of S1292 autophosphorylation, at both 10 day and 10-week timepoints [33]. Interestingly, phospho- and total proteomic analyses of kidney tissue showed significant changes in endolysosomal proteins comparative to LRRK2 knockout animals [33,58]. This suggests that LRRK2 inhibition can mimic some loss-of-function (LOF) effects in the endolysosomal system in peripheral tissues, as these proteins remained unaffected in the brain tissue of these animals. However, modest changes in proteins of mitochondrial integrity were observed in brain tissue, such that treated G2019S LRRK2 knock-in animals resembled wildtype animals, suggesting that G2019S LRRK2 mutant animals have slight mitochondrial defects that are ameliorated with chronic LRRK2 inhibition [33].

What remains to be shown is whether any of these molecular effects are translatable to humans. Studies have shown that people with LRRK2 haploinsufficiency have reduced levels of total LRRK2 protein; however, there have been conflicting reports on whether reduced levels are associated with phenotypes or disease states in these individuals, with one claiming an increased risk for lung adenocarcinoma [59–61]. The caveat here is that chronic LRRK2 inhibition is not the same as LOF variants. In the former, the body must alter its normal mechanisms to account for reduced LRRK2 kinase activity, whereas the latter already developed mechanisms to cope with lower LRRK2 levels *in utero*. Lower LRRK2 protein levels may also not produce the same effects as lower kinase activity. Thus, it will be imperative to monitor the effects of LRRK2 kinase inhibitors as it relates to normal protein function.

3.2. Morphological changes in peripheral tissues

The most prominent morphological effects observed *in vivo* following LRRK2 kinase inhibitor treatments over a period of time have been an increase of vacuolation in lung type-II pneumocytes across wildtype mice and cynomolgus monkeys [39,54]. This returned to normal levels after a 1-week washout period. Studies using GNE-7915, GNE-0877, PFE-360, and MLi-2 all reported this vacuolation, suggesting that this is a result of on-target effects [31,38,39,54,62]; however, pulmonary function was also shown to be unaffected in monkeys receiving a 50 mg/kg PO MLi-2 daily dose for 28 days [39]. One potential concern here is that vacuolation has not been reported to regulate with continued treatment use. Thus, if translatable to humans, this phenotype may have the potential to harbor long-term adverse effects in PD patients using a LRRK2 inhibitor treatment.

Additionally, rats given 7.5 mg/kg of PFE-360 BID for 10 weeks showed hyperpigmentation of the kidneys, a phenotype seen in LRRK2 KO rats and mice [58,63]. It is worth noting that this was not observed in the treatments of mice nor monkeys, suggesting this is a species-specific phenotype [33,38,39,54]. Overall, it is currently unknown whether any lung or kidney complications will arise in humans, and thus careful monitoring of these organs will be critical in current and future clinical trials testing the efficacy of LRRK2 kinase inhibitors.

4. Alternative approaches to reduce LRRK2 kinase activity

Aside from type I and II kinase inhibitors, additional approaches have been suggested and are currently in

development. Here, we will briefly highlight some of these approaches as alternative therapies targeting LRRK2 kinase activity.

4.1. Antisense oligonucleotides

In recent years, there has been much excitement centered around the practicality and potential therapeutic use of antisense oligonucleotides (ASOs) in neurodegenerative diseases. This synthetic technology is aimed at reducing mutant or pathogenic proteins by marking the target's mRNA for degradation, or at producing more functional proteins via modulation of the splicing of RNA. The most successful use of an ASO in the neurodegenerative space has been for Spinal Muscular Atrophy (SMA), in which large deletions or LOF mutations found in the *SMN1* gene result in terminal disease [64]. The ASO nusinersen, approved by the FDA in 2016, works to modulate the splicing of the homologue gene *SMN2* such that full-length SMN transcripts are increased two-to six-fold and thereby increase its wild-type protein [65]. Patients who have received this treatment in clinical trials reported no serious adverse effects and observed significant improvements in motor function, survival rate, and independence from permanent ventilation [65]. This is a huge milestone in the treatment for SMA that sparked hope for many other neurodegenerative diseases targeting mutant proteins and LOF alleles. Clinical trials evaluating the therapeutic efficacy of ASOs for Huntington's, Alzheimer's, Amyotrophic Lateral Sclerosis (ALS), and Parkinson's diseases are currently underway, with varying degrees of success [66–70].

In August 2019, a phase 1 clinical trial using an ASO designated BIIB094, to target LRRK2 through intrathecal administration began, with the primary and secondary outcomes measuring safety/tolerability and pharmacokinetics in the blood of people with and without LRRK2 mutations (NCT03976349). The aim of reducing LRRK2 mRNA is to reduce the synthesis of LRRK2 protein in order to ameliorate the toxic effects of gain-of-function mutations in patients with LRRK2 mutations and reduce kinase activity in patients without LRRK2 mutations. Phase 1 is expected to conclude in 2023.

A preclinical study in mice showed that ASOs were able to successfully reduce LRRK2 mRNA in the brain, leaving peripheral tissues unaffected and thus potentially bypassing the peripheral toxicity observed in models using small-molecule inhibitors [71]. However, clinical relevant efficacy in the reduction of total LRRK2 protein has yet to be determined in humans, i.e. slowing, stopping, or reversing the progression of the disease. As LRRK2 haploinsufficiency is not associated with human diseases [59,60], this approach is predicted to be safe long term.

4.2. LRRK2 GTPase modulators

Both LRRK2 GTPase and kinase domains interact and depend on one another for proper functioning at the cytosol and organellar membranes. In fact, common mutations found within the GTPase domain have been shown to alter the affinity of GTP and decrease GTP hydrolysis, affecting LRRK2 localization and kinase activity [14,72–78]. Recently, two studies revealed that the Roc GTPase domain exists in a dynamic dimer-monomer equilibrium, and that the R1441C/G/H mutations alter this equilibrium [79,80]. Therefore, modulating its GTPase activity may impart therapeutic benefits by allosterically reducing kinase activity.

Two GTP-binding inhibitors, Compound 68 and FX2149, have been shown to decrease LRRK2 kinase activity, utilizing kinase assays *in vitro* as well as measuring a reduction in S935 phosphorylation *in vivo* [75,81–83]. Moreover, an increase in LRRK2 ubiquitination was observed after GTP-binding inhibitor treatment in the brains of mice receiving two doses of 10 mg/kg FX2149 intraperitoneally for 3 days [82]. This suggests GTP-binding inhibition may reduce total levels of LRRK2 similarly to kinase inhibitors. With longer treatment time points, it will be interesting to determine whether similar peripheral effects on kidneys and lungs of animals occur as observed in those treated with kinase inhibitors.

Additionally, recent studies suggest a role of LRRK2 in inflammatory response mechanisms across various PD models and PD patients [84–87]. Interestingly, LRRK2-dependent inflammatory effects, such as TNF-alpha secretion, have been shown to be reduced in human lymphoblasts treated with Compound 68 prior to incubation with LPS for up to 4 hours *in vitro*, suggesting that the GTPase domain is critical in regulating inflammatory stimuli, and thus GTPase modulation may have protective effects against neuroinflammation that is often observed in PD pathology [83]. Alternatively, methods to increase the GTP hydrolysis of LRRK2, especially when dealing with mutations in the ROC-COR domains, should also be considered when developing GTPase modulators for therapeutics.

4.3. Nanobodies

Nanobodies, small single-domain antigen-binding fragments originating from camelid heavy chain-only antibodies, have recently made their way into clinical trials as potential therapeutics in autoimmune disorders and cancer with one, caplacizumab, receiving approval from the European Medicines Agency (EMA) and the FDA for treatment of patients with thrombotic thrombocytopenic purpura [88–90]. Nanobodies have strong affinity for their targets and can act as antagonists, agonists, allosteric inhibitors or activators. Additionally, nanobody affinities are conformation-specific. Taken together, nanobodies provide high modulability and versatility, with the therapeutic potential to target many wide-ranging disorders and diseases.

LRRK2-targeting nanobodies have recently been developed by Singh et al. to determine their binding capabilities and potential therapeutic properties [91]. They reported the generation of 10 distinct nanobodies that show a strong affinity for both over-expressed human and endogenous mouse LRRK2 as detected with immunoprecipitation assays *in vitro*. Interestingly, some nanobodies worked to allosterically inhibit or activate LRRK2 kinase, depending on their affinity for GDP-, GTP-bound or unbound LRRK2, as measured by *in vitro* kinase assays with LRRK2-specific AQT0615 peptide substrate, as well as Western blot analyses of pS1292 LRRK2 and pT73 Rab10 in HEK293FT cells over-expressing G2019S LRRK2 [91]. Overall,

nanobodies provide a promising and exciting alternative avenue for LRRK2 kinase modulation. As the generation of nanobodies targeting LRRK2 is brand new, it will be important for future studies to test the safety and efficacy of these tools *in vivo*, as well as the evaluation of their capacity to cross the blood–brain barrier.

5. Conclusion

The current development of such a wide array of tools targeting LRRK2 has indeed provided much hope for the field of PD therapeutics. We are now within the liminal space determining this target's efficacy to either revert, halt, or slow disease progression. As we await the outcomes of kinase inhibitor and ASO trial studies, we can reflect on the significant genetic and mechanistic gains the field has made which has provided a clearly defined target, with a stratified population and obvious routes to modulating activity of LRRK2. There is also evidence for the potential to generalize this target to other patients should it be successful in the genetically defined population.

6. Expert opinion

LRRK2 is a large protein, encompassing a GTPase and kinase catalytic core, flanked by WD40, ankyrin, and leucine-rich repeat protein–protein interaction and stabilization domains. Most disease-causing mutations have been identified within the enzymatic domains and all of which contribute to the hyperactivity of its kinase function [92–94]. Thus far, ATP-competitive kinase inhibitors have been the most focused avenue as a therapeutic intervention in reducing LRRK2 kinase activity; however, other tools such as ASOs, GTPase modulators, and nanobodies pose interesting and creative alternatives that are worth investigation (Figure 2). An important perspective to consider, aside from the clinical relevant efficacy of any of these therapeutic tools, is of their logistical merit as a long-term therapy for patients, i.e. cost of treatment, ease of administration, and potential adverse side effects. Small molecules as well as nanobodies are highly stable, low molecular weight compounds. Additionally, they are cheap to produce and thus can keep costs relatively low for patients [95,96]. Alternatively, ASOs cost more to produce and require large volumes of hazardous materials and energy [97]. A single intrathecal injection of the SMA ASO nusinersen costs $125,000 USD, creating a huge wealth gap between families that can and cannot afford life-saving treatment [98]. Therefore, in terms of patient accessibility, cheaper alternative drugs would be much more inclusive for the majority, however, considering the economies of scale for a common disease such as PD may allow for cheaper costs for ASO treatments. Additionally, orally administered small-molecule drugs also lend themselves to patient accessibility, as patients and caretakers would not need to schedule in-person visits every few months for intrathecal injection, not to mention is much more comfortable, without risk of developing adverse effects at the sight of insertion such as tenderness, bruising or infection. In contrast, intrathecal administration of ASOs allows for little to no risk of peripheral side effects as observed with kinase inhibitors in preclinical models.

The ongoing clinical trials for LRRK2 kinase inhibitors and ASOs will, over the coming years, clarify whether targeting LRRK2 will be therapeutically advantageous in PD, opening up the possibility for treatment to be patient stratified based on mutation, risk status, and/or drug toleration [99]. In addition to those with specific LRRK2 mutations, studies have shown that enhanced LRRK2 kinase activity can also be found in patients with idiopathic PD and therefore suggest that LRRK2-modifying treatments may be useful to a broader scope of PD patients rather than mutation carriers only [100,101]. Aside from PD, recent advances in PSP genetics have identified a lead single-nucleotide polymorphism (SNP)

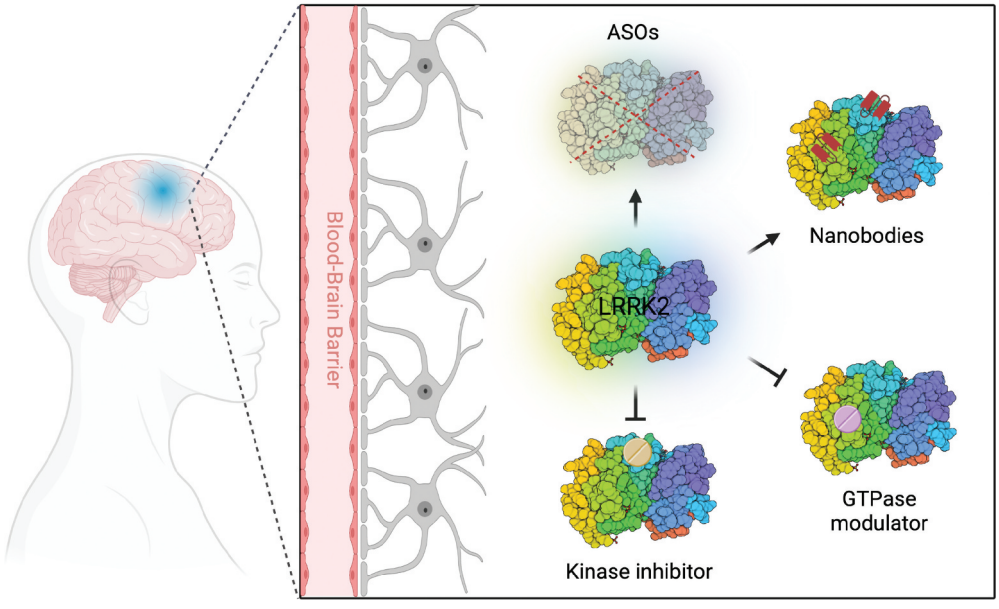

Figure 2. Schematic of potential LRRK2-targeting treatments. This schematic was created in Biorender, utilizing the PDB Builder feature for the LRRK2 structure (ID: 5U6I).

that regulates LRRK2 expression as a factor for survival outcome in people with PSP [11]. Therefore, intervention with therapies targeting LRRK2 could potentially serve as an umbrella therapy, benefiting those with PSP as well. In addition to PSP, some case studies have reported a potential link between LRRK2 and multiple-system atrophy (MSA), whereas others have reported no association [102–105]. Therefore, it will require additional investigation in order to confirm a true mechanistic link between LRRK2 and MSA.

For nearly the last six decades, the treatment of PD has been centered on dopamine replacement therapies, as progress in the development of disease-modifying treatments for PD has been limited. Thus, it is a particularly exciting time for PD therapeutics, with multiple clinical trials underway for mechanistically targeted therapies, i.e. LRRK2, GBA and alpha-synuclein [106]. However, there is still a long way to go in the development of disease-modifying treatments for PD. In comparison, there have been over 200 clinical trials to date to assess treatments for Alzheimer's disease (AD), of which 99% have failed to be efficacious, most probably due to the complexity of the disease and its aging component that most disease models lack [107]. This has both positive and negative implications for PD. The failures of AD clinical trials highlight what hurdles may be on the horizon for PD and give us the opportunity to prepare for them. Realistically however, it would not come as a surprise if PD clinical trials mirror a similar path, as PD is just as complex, and the most commonly used models still lack an aging component. It is just the beginning for PD therapeutics; however, the path to effective treatments is now more hopeful than at any other time in the past half century.

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Jankovic J. Parkinson's disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatry. 2008 Apr;79(4):368–376.
2. Postuma RB, Berg D, Stern M, et al. MDS clinical diagnostic criteria for Parkinson's disease. Mov Disord. 2015;30(12):1591–1601.
3. Hughes AJ, Daniel SE, Kilford L, et al. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry. 1992 Mar;55(3):181–184.
4. Schapira AHV. Present and future drug treatment for Parkinson's disease. J Neurol Neurosurg Psychiatry. 2005 Nov 1;76 (11):1472–1478.
5. Nemade D, Subramanian T, Shivkumar V. An update on medical and surgical treatments of Parkinson's disease. Aging Dis. 2021 Jul 1;12(4):1021–1035.
6. Paisán-Ruíz C, Jain S, Evans EW, et al. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. Neuron. 2004 Nov 18;44(4):595–600.
7. Zimprich A, Müller-Myhsok B, Farrer M, et al. The PARK8 locus in autosomal dominant Parkinsonism: confirmation of linkage and further delineation of the disease-containing interval. Am J Hum Genet. 2004 Jan;74(1):11–19.
8. Satake W, Nakabayashi Y, Mizuta I, et al. Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. Nat Genet. 2009 Dec;41(12):1303–1307.
9. Simón-Sánchez J, Schulte C, Bras JM, et al. Genome-wide association study reveals genetic risk underlying Parkinson's disease. Nat Genet. 2009 Dec;41(12):1308–1312.
10. Nalls MA, Blauwendraat C, Vallerga CL, et al. Identification of novel risk loci, causal insights, and heritable risk for Parkinson's disease: a meta-analysis of genome-wide association studies. Lancet Neurol. 2019 Dec 1;18(12):1091–1102.
11. Jabbari E, Koga S, Valentino RR, et al. Genetic determinants of survival in progressive supranuclear palsy: a genome-wide association study. Lancet Neurol. 2021 Feb 1;20(2):107–116.
12. Sheng Z, Zhang S, Bustos D, et al. Ser1292 autophosphorylation is an indicator of LRRK2 kinase activity and contributes to the cellular effects of PD mutations. Sci Transl Med. 2012 Dec 12;4 (164):164ra161–164ra161.
13. Kluss JH, Conti MM, Kaganovich A, et al. Detection of endogenous S1292 LRRK2 autophosphorylation in mouse tissue as a readout for kinase activity. Npj Park Dis. 2018 Apr 19;4(1):13.
14. West AB, Moore DJ, Choi C, et al. Parkinson's disease-associated mutations in LRRK2 link enhanced GTP-binding and kinase activities to neuronal toxicity. Hum Mol Genet. 2007 Jan 15;16 (2):223–232.
15. Steger M, Tonelli F, Ito G, et al. Phosphoproteomics reveals that Parkinson's disease kinase LRRK2 regulates a subset of Rab GTPases. eLife. 2016 Jan 29;5. DOI:10.7554/eLife.12813.
16. Greggio E, Jain S, Kingsbury A, et al. Kinase activity is required for the toxic effects of mutant LRRK2/dardarin. Neurobiol Dis. 2006 Aug 1;23(2):329–341.
17. Greggio E, Zambrano I, Kaganovich A, et al. The Parkinson disease-associated leucine-rich repeat kinase 2 (LRRK2) is a dimer that undergoes intramolecular autophosphorylation. J Biol Chem. 2008 Jun 13;283(24):16906–16914.
18. Manzoni C, Mamais A, Dihanich S, et al. Inhibition of LRRK2 kinase activity stimulates macroautophagy. Biochim Biophys Acta BBA - Mol Cell Res. 2013 Dec 1;1833(12):2900–2910.
19. Bonet-Ponce L, Beilina A, Williamson CD, et al. LRRK2 mediates tubulation and vesicle sorting from lysosomes. Sci Adv. 2020 Nov 1;6(46):eabb2454.
20. Liu Z, Bryant N, Kumaran R, et al. LRRK2 phosphorylates membrane-bound Rabs and is activated by GTP-bound Rab7L1 to promote recruitment to the trans-Golgi network. Hum Mol Genet. 2018 15;27(2):385–395.
21. Purlyte E, Dhekne HS, Sarhan AR, et al. Rab29 activation of the Parkinson's disease-associated LRRK2 kinase. EMBO J. 2019 Jan 15;38(2):e101237.
22. Steger M, Diez F, Dhekne HS, et al. Systematic proteomic analysis of LRRK2-mediated Rab GTPase phosphorylation establishes

a connection to ciliogenesis. eLife. 2017 10;6. DOI:10.7554/eLife.31012.
23. Herbst S, Campbell P, Harvey J, et al. LRRK2 activation controls the repair of damaged endomembranes in macrophages. EMBO J. 2020 Sep 15;39(18):e104494.
24. Beilina A, Bonet-Ponce L, Kumaran R, et al. The Parkinson's disease protein LRRK2 interacts with the GARP complex to promote retrograde transport to the trans-golgi network. Cell Rep. 2020 May 5;31(5):107614.
25. Bonello F, Hassoun SM, Mouton-Liger F, et al. LRRK2 impairs PINK1/Parkin-dependent mitophagy via its kinase activity: pathologic insights into Parkinson's disease. Hum Mol Genet [Internet]. 28(10):1645–1660. [cited 2019 Feb 4]; Available from: https://academic.oup.com/hmg/advance-article/doi/10.1093/hmg/ddz004/5281397
26. Korecka JA, Thomas R, Christensen DP, et al. Mitochondrial clearance and maturation of autophagosomes are compromised in LRRK2 G2019S familial Parkinson's disease patient fibroblasts. Hum Mol Genet. 2019 Oct 1;28(19):3232–3243.
27. Dhekne HS, Yanatori I, Vides EG, et al. LRRK2-phosphorylated Rab10 sequesters Myosin Va with RILPL2 during ciliogenesis blockade. Life Sci Alliance [Internet]. 2021 May 1;4(5). [cited 2022 Jan 9]; Available from: https://www.life-science-alliance.org/content/4/5/e202101050
28. Kluss JH , Bonet-Ponce L, Lewis, PA, et al. Directing LRRK2 to membranes of the endolysosomal pathway triggers RAB phosphorylation and JIP4 recruitment. Neurobiology of disease.2022 May;170:0969–9961.
29. Jeon J, Sparreboom A, Baker S. Kinase inhibitors: the reality behind the success. Clin Pharmacol Ther. 2017 Nov;102(5):726–730.
30. Roskoski R. Properties of FDA-approved small molecule protein kinase inhibitors: a 2020 update. Pharmacol Res. 2020 Feb;152:104609.
31. Fell MJ, Mirescu C, Basu K, et al. MLi-2, a potent, selective, and centrally active compound for exploring the therapeutic potential and safety of LRRK2 kinase inhibition. J Pharmacol Exp Ther. 2015 Dec;355(3):397–409.
•• **This is the first in-depth in vivo dosing study using MLi-2.**
32. Estrada AA, Liu X, Baker-Glenn C, et al. Discovery of highly potent, selective, and brain-penetrable leucine-rich repeat kinase 2 (LRRK2) small molecule inhibitors. J Med Chem. 2012 Nov 26;55(22):9416–9433.
33. Kluss JH, Mazza MC, Li Y, et al. Preclinical modeling of chronic inhibition of the Parkinson's disease associated kinase LRRK2 reveals altered function of the endolysosomal system in vivo. Mol Neurodegener. 2021 Mar 19;16(1):17.
• **This is the first study to employ chronic in vivo dosing of MLi-2 to G2019S KI mice to ameliorate hyperactive LRRK2 levels back to wildtype levels. This study includes total and phosphoproteomic analyses of brain and peripheral tissues known to be affected by LRRK2 inhibitors.**
34. Wang S, Kelly K, Brotchie JM, et al. Exosome markers of LRRK2 kinase inhibition. NPJ Park Dis. 2020 Nov 13;6(1):32.
35. Ito G, Katsemonova K, Tonelli F, et al. Phos-tag analysis of Rab10 phosphorylation by LRRK2: a powerful assay for assessing kinase function and inhibitors. Biochem J. 2016 01;473(17):2671–2685.
36. Kelly K, Wang S, Boddu R, et al. The G2019S mutation in LRRK2 imparts resiliency to kinase inhibition. Exp Neurol. 2018 Nov 1;309:1–13.
37. Nichols RJ, Dzamko N, Morrice NA, et al. 14-3-3 binding to LRRK2 is disrupted by multiple Parkinson's disease-associated mutations and regulates cytoplasmic localization. Biochem J. 2010 Sep 15;430(3):393–404.
38. Fuji RN, Flagella M, Baca M, et al. Effect of selective LRRK2 kinase inhibition on nonhuman primate lung. Sci Transl Med. 2015 Feb 4;7(273):273ra15.
•• **This study compares three structurally distinct LRRK2-specific type I inhibitors in non-human primates. An in-depth characterization of pulmonary function is included.**
39. Baptista MAS, Merchant K, Barrett T, et al. LRRK2 inhibitors induce reversible changes in nonhuman primate lungs without measurable pulmonary deficits. Sci Transl Med [Internet]. 2020 Apr 22;12(540). [cited 2020 Apr 26]; Available from: https://stm.sciencemag.org/content/12/540/eaav0820
40. Dzamko N, Deak M, Hentati F, et al. Inhibition of LRRK2 kinase activity leads to dephosphorylation of Ser910/Ser935, disruption of 14-3-3 binding and altered cytoplasmic localization. Biochem J. 2010 Aug 27;430(3):405–413.
41. Myasnikov A, Zhu H, Hixson P, et al. Structural analysis of the full-length human LRRK2. Cell. 2021 Jun 24;184(13):3519–3527.e10.
42. Pettersen EF, Goddard TD, Huang CC, et al. UCSF Chimera–a visualization system for exploratory research and analysis. J Comput Chem. 2004 Oct;25(13):1605–1612.
43. Liu M, Bender SA, Cuny GD, et al. Type II kinase inhibitors show an unexpected inhibition mode against Parkinson's disease-linked LRRK2 mutant G2019S. Biochemistry. 2013 Mar 12;52(10):1725–1736.
44. O'Hare T, Shakespeare WC, Zhu X, et al. AP24534, a Pan-BCR-ABL inhibitor for chronic myeloid leukemia, potently inhibits the T315I mutant and overcomes mutation-based resistance. Cancer Cell. 2009 Nov 3;16(5):401–412.
45. Tasegian A, Singh F, Ganley IG, et al. Impact of type II LRRK2 inhibitors on signaling and mitophagy. Biochem J. 2021 Oct 6;478(19):3555–3573.
46. Nichols RJ, Dzamko N, Hutti JE, et al. Substrate specificity and inhibitors of LRRK2, a protein kinase mutated in Parkinson's disease. Biochem J. 2009 Oct 23;424(1):47–60.
47. Deng X, Dzamko N, Prescott A, et al. Characterization of a selective inhibitor of the Parkinson's disease kinase LRRK2. Nat Chem Biol. 2011 Apr;7(4):203–205.
48. Leśniak RK, Nichols RJ, Schonemann M, et al. Discovery of G2019S-Selective leucine rich repeat protein kinase 2 inhibitors with in vivo efficacy. Eur J Med Chem. 2022 Feb 5;229:114080.
49. Bright JM, Carlisle HJ, Toda AMA, et al. Differential inhibition of LRRK2 in Parkinson's disease patient blood by a G2019S selective LRRK2 inhibitor. Mov Disord. 2021;36(6):1362–1371.
50. Deniston CK, Salogiannis J, Mathea S, et al. Structure of LRRK2 in Parkinson's disease and model for microtubule interaction. Nature. 2020 Dec;588(7837):344–349.
51. Watanabe R, Buschauer R, Böhning J, et al. The in situ structure of Parkinson's disease-linked LRRK2. Cell. 2020 Sep 17;182(6):1508–1518.e16.
52. Schmidt SH, Weng JH, Aoto PC, et al. Conformation and dynamics of the kinase domain drive subcellular location and activation of LRRK2. Proc Natl Acad Sci U S A. 2021 Jun 8;118(23):e2100844118.
53. Press Release [Internet]. Denali. [cited 2021 Nov 30]. Available from: https://www.denalitherapeutics.com/investors/press-release
•• **The most recent update on results of the Phase I trials of DNL151/BIIB122.**
54. Bryce D, Ware CM, Woodhouse JD, et al. Characterization of the onset, progression, and reversibility of morphological changes in mouse lung following pharmacological inhibition of LRRK2 kinase activity. J Pharmacol Exp Ther [Internet]. 2021 Jan 1;377(1):11–19. [cited 2021 Feb 4]; Available from: https://jpet.aspetjournals.org/content/early/2021/01/28/jpet.120.000217
• **In line with studies in non-human primates, this study confirms morphological changes to lung type II pneumocytes in wildtype mice treated with LRRK2 kinase inhibitors.**
55. Lobbestael E, Civiero L, De Wit T, et al. Pharmacological LRRK2 kinase inhibition induces LRRK2 protein destabilization and proteasomal degradation. Sci Rep [Internet]. 2016 Sep 23;6. [cited 2020 May 8]; Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5034242/
56. Zhao J, Molitor TP, Langston JW, et al. LRRK2 dephosphorylation increases its ubiquitination. Biochem J. 2015 Jul 1;469(Pt 1):107–120.
57. Herzig MC, Kolly C, Persohn E, et al. LRRK2 protein levels are determined by kinase function and are crucial for kidney and

lung homeostasis in mice. Hum Mol Genet. 2011 Nov 1;20 (21):4209–4223.
58. Pellegrini L, Hauser DN, Li Y, et al. Proteomic analysis reveals co-ordinated alterations in protein synthesis and degradation pathways in LRRK2 knockout mice. Hum Mol Genet. 2018 15;27(18):3257–3271.
59. Blauwendraat C, Reed X, Kia DA, et al. Frequency of loss of function variants in LRRK2 in Parkinson disease. JAMA Neurol. 2018 Nov 1;75 (11):1416–1422.
60. Whiffin N, Armean IM, Kleinman A, et al. The effect of LRRK2 loss-of-function variants in humans. Nat Med. 2020 Jun;26(6):869–877.
61. Lebovitz C, Wretham N, Osooly M, et al. Loss of Parkinson's susceptibility gene LRRK2 promotes carcinogen-induced lung tumorigenesis. Sci Rep. 2021 Jan 22;11(1):2097.
62. Scott JD, DeMong DE, Greshock TJ, et al. Discovery of a 3-(4-Pyrimidinyl) Indazole (MLi-2), an orally available and selective leucine-rich repeat kinase 2 (LRRK2) inhibitor that reduces brain kinase activity. J Med Chem. 2017 Apr 13;60(7):2983–2992.
63. Andersen MA, Wegener KM, Larsen S, et al. PFE-360-induced LRRK2 inhibition induces reversible, non-adverse renal changes in rats. Toxicology. 2018 15;395:15–22.
64. Lunn MR, Wang CH. Spinal muscular atrophy. Lancet Lond Engl. 2008 Jun 21;371(9630):2120–2133.
65. Wurster CD, Ludolph AC. Nusinersen for spinal muscular atrophy. Ther Adv Neurol Disord. 2018 Mar 13;11:1756285618754459.
66. Kingwell K. Double setback for ASO trials in huntington disease. Nat Rev Drug Discov. 2021 May 19;20(6):412–413.
67. Miller T, Cudkowicz M, Shaw PJ, et al. Phase 1–2 trial of antisense oligonucleotide tofersen for SOD1 ALS. N Engl J Med. 2020 Jul 9;383(2):109–119.
68. Grabowska-Pyrzewicz W, Want A, Leszek J, et al. Antisense oligonucleotides for Alzheimer's disease therapy: from the mRNA to miRNA paradigm. EBioMedicine [Internet]. 2021 Dec 1;74. [cited 2022 Jan 5]; Available from: https://www.thelancet.com/journals/ebiom/article/PIIS2352-3964(21)00485-0/fulltext#seccesectitle0003
69. Cole TA, Zhao H, Collier TJ, et al. α-Synuclein antisense oligonucleotides as a disease-modifying therapy for Parkinson's disease. JCI Insight. 2021;6(5):e135633.
70. Kingwell K. Zeroing in on neurodegenerative α-synuclein. Nat Rev Drug Discov. 2017 Jun 1;16(6):371–373.
71. Zhao HT, John N, Delic V, et al. LRRK2 antisense oligonucleotides ameliorate α-synuclein inclusion formation in a Parkinson's disease mouse model. Mol Ther Nucleic Acids. 2017 Sep 15;8:508–519.
72. Lewis PA, Greggio E, Beilina A, et al. The R1441C mutation of LRRK2 disrupts GTP hydrolysis. Biochem Biophys Res Commun. 2007 Jun 8;357(3):668–671.
73. Li C, Ting Z, Qin X, et al. The prevalence of LRRK2 Gly2385Arg variant in Chinese han population with Parkinson's disease. Mov Disord Off J Mov Disord Soc. 2007 Dec;22(16):2439–2443.
74. Xiong Y, Coombes CE, Kilaru A, et al. GTPase activity plays a key role in the pathobiology of LRRK2. PLOS Genet. 2010 Apr 8;6(4): e1000902.
75. Liao J, Wu CX, Burlak C, et al. Parkinson disease-associated mutation R1441H in LRRK2 prolongs the "active state" of its GTPase domain. Proc Natl Acad Sci U S A. 2014 Mar 18;111(11):4055–4060.
76. Soliman A, Cankara FN, Kortholt A. Allosteric inhibition of LRRK2, where are we now. Biochem Soc Trans. 2020 Oct 20;48(5):2185–2194.
77. Blanca Ramírez M, Ordóñez AJL, Fdez E, et al. GTP binding regulates cellular localization of Parkinson's disease-associated LRRK2. Hum Mol Genet. 2017 Jul 15;26(14):2747–2767.
78. Biosa A, Trancikova A, Civiero L, et al. GTPase activity regulates kinase activity and cellular phenotypes of Parkinson's disease-associated LRRK2. Hum Mol Genet. 2013 Mar 15;22(6):1140–1156.
79. Wu CX, Liao J, Park Y, et al. Parkinson's disease-associated mutations in the GTPase domain of LRRK2 impair its nucleotide-dependent conformational dynamics. J Biol Chem. 2019 Apr 12;294(15):5907–5913.
80. Huang X, Wu C, Park Y, et al. The Parkinson's disease–associated mutation N1437H impairs conformational dynamics in the G domain of LRRK2. FASEB J. 2019 Apr;33(4):4814–4823.
81. Li T, He X, Thomas JM, et al. A novel GTP-Binding inhibitor, FX2149, Attenuates LRRK2 toxicity in Parkinson's disease models. PLoS ONE. 2015 Mar 27;10(3):e0122461.
• **This study characterizes a LRRK2 GTP-binding inhibitor in vitro.**
82. Thomas JM, Wang X, Guo G, et al. GTP-binding inhibitors increase LRRK2-linked ubiquitination and Lewy body-like inclusions. J Cell Physiol. 2020;235(10):7309–7320.
83. Li T, Ning B, Kong L, et al. A LRRK2 GTP binding inhibitor, 68, reduces LPS-induced signaling events and TNF-α release in human lymphoblasts. Cells. 2021 Feb 23;10(2):480.
84. Dzamko NL. LRRK2 and the immune system. Adv Neurobiol. 2017;14:123–143.
85. Brockmann K, Apel A, Schulte C, et al. Inflammatory profile in LRRK2-associated prodromal and clinical PD. J Neuroinflammation. 2016 May 24;13(1):122.
86. Wallings RL, Tansey MG. LRRK2 regulation of immune-pathways and inflammatory disease. Biochem Soc Trans. 2019 Dec 20;47 (6):1581–1595.
87. Kluss JH, Mamais A, Cookson MR. LRRK2 links genetic and sporadic Parkinson's disease. Biochem Soc Trans. 2019 Apr 30;47(2):651–661.
88. Jovčevska I, Muyldermans S. The therapeutic potential of nanobodies. BioDrugs Clin Immunother Biopharm Gene Ther. 2020 Feb;34(1):11–26.
89. Fernandes JC. Therapeutic application of antibody fragments in autoimmune diseases: current state and prospects. Drug Discov Today. 2018 Dec;23(12):1996–2002.
90. Scully M, Cataland SR, Peyvandi F, et al. Caplacizumab treatment for acquired thrombotic thrombocytopenic purpura. N Engl J Med [Internet]. 2019 Jan 9;380(4):335–346. [cited 2022 Jan 9]; Available from: https://www.nejm.org/doi/10.1056/NEJMoa1806311
91. Singh RK, Soliman A, Guaitoli G, et al. Nanobodies as allosteric modulators of Parkinson's disease–associated LRRK2. Proc Natl Acad Sci. 2022 Mar;119(9):e2112712119.
• **This study describes the development and characterization of the first LRRK2-targeting nanobodies.**
92. Berwick DC, Heaton GR, Azeggagh S, et al. LRRK2 biology from structure to dysfunction: research progresses, but the themes remain the same. Mol Neurodegener. 2019 Dec 21;14(1):49.
93. Greggio E, Cookson MR. Leucine-rich repeat kinase 2 mutations and Parkinson's disease: three questions. ASN NEURO. 2009 Apr 14;1(1):e00002.
94. Cookson MR. The role of leucine-rich repeat kinase 2 (LRRK2) in Parkinson's disease. Nat Rev Neurosci. 2010 Dec;11(12):791–797.
95. Makurvet FD. Biologics vs. small molecules: drug costs and patient access. Med Drug Discov. 2021 Mar 1;9:100075.
96. Muyldermans S. A guide to: generation and design of nanobodies. FEBS J. 2021;288(7):2084–2102.
97. Andrews BI, Antia FD, Brueggemeier SB, et al. Sustainability challenges and opportunities in oligonucleotide manufacturing. J Org Chem. 2021 Jan 1;86(1):49–61.
98. Jalali A, Rothwell E, Botkin JR, et al. Cost-Effectiveness of nusinersen and universal newborn screening for spinal muscular atrophy. J Pediatr. 2020 Dec 1;227:274–280.e2.
99. Bandres-Ciga S, Diez-Fairen M, Kim JJ, et al. Genetics of Parkinson disease: an introspection of its journey towards precision medicine. Neurobiol Dis. 2020 Apr;137:104782.
100. Di Maio R, Hoffman EK, Rocha EM, et al. LRRK2 activation in idiopathic Parkinson's disease. Sci Transl Med. 2018 Jul 25;10(451): eaar5429.
101. Rocha EM, Keeney MT, Maio RD, et al. LRRK2 and idiopathic Parkinson's disease. Trends Neurosci. 2022 Mar 1;45(3):224–236.

102. Heckman MG, Schottlaender L, Soto-Ortolaza AI, et al. LRRK2 exonic variants and risk of multiple system atrophy. Neurology. 2014 Dec 9;83(24):2256–2261.
103. Riboldi GM, Palma J, Cortes E, et al. Early-onset pathologically proven multiple system atrophy with LRRK2 G2019S mutation. Mov Disord. 2019 Jul;34(7):1080–1082.
104. Ozelius LJ, Foroud T, May S, et al. G2019S mutation in the leucine-rich repeat kinase 2 gene is not associated with multiple system atrophy. Mov Disord. 2007;22(4):546–549.
105. Katzeff JS, Phan K, Purushothuman S, et al. Cross-examining candidate genes implicated in multiple system atrophy. Acta Neuropathol Commun. 2019 Jul 24;7(1):117.
106. McFarthing K, Rafaloff G, Baptista MAS, et al. Parkinson's disease drug therapies in the clinical trial pipeline: 2021 update. J Park Dis. 2021;11(3):891–903.
107. Yiannopoulou KG, Anastasiou AI, Zachariou V, et al. Reasons for failed trials of disease-modifying treatments for alzheimer disease and their contribution in recent research. Biomedicines. 2019 Dec 9;7(4):97.

• **This comprehensive review provides an in-depth discussion on the reasons why clinical trials for Alzheimer's disease therapies have failed. This is particularly important, as it foreshadows what may be in store for those pursuing drug trial success in Parkinson's disease.**