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The development of inhibitors of leucine-rich repeat kinase 2 (LRRK2) as a therapeutic strategy for Parkinson's disease: the current state of play

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Current therapeutic approaches for Parkinson's disease (PD) are based around treatments that alleviate symptoms but do not slow or prevent disease progression. As such, alternative strategies are needed. A promising approach is the use of molecules that reduce the function of leucine-rich repeat kinase (LRRK2). Gain-of-function mutations in *LRRK2* account for a notable proportion of familial Parkinson's disease cases, and significantly, elevated LRRK2 kinase activity is reported in idiopathic Parkinson's disease. Here, we describe progress in finding therapeutically effective LRRK2 inhibitors, summarising studies that range from *in vitro* experiments to clinical trials. LRRK2 is a complex protein with two enzymatic activities and a myriad of functions. This creates opportunities for a rich variety of strategies and also increases the risk of unintended consequences. We comment on the strength and limitations of the different approaches and conclude that with two molecules under clinical trial and a diversity of alternative options in the pipeline, there is cause for optimism.

KEYWORDS
Kinase inhibitor, LRRK2, Neurodegeneration, Parkinson's disease

1 | PARKINSON'S DISEASE (PD)

Parkinson's disease (PD) is a movement disorder that has been observed in humans since ancient times but was first studied as a distinct condition by the English physician James Parkinson, who published his 'essay on the shaking palsy' in 1817 (Parkinson, 1817). Today, PD is recognised as the second most common neurodegenerative disease, affecting up to 10 million individuals worldwide, with a lifetime risk estimated around 2% (Elbaz et al., 2002; Gan-Or et al., 2015). Despite so many years of study and so many affected individuals, PD is an incurable condition.

PD is predominantly a disease of the elderly, with most sufferers over the age of 60 (Reeve et al., 2014). The condition is usually considered both sporadic and idiopathic, with contributions to disease risk coming from an individual's genetics and their lifetime exposure to environmental factors, but with age by far the most important factor (Farrer, 2006; Reeve et al., 2014). Nonetheless, clear patterns of Mendelian inheritance are apparent in around 10% of patients (Rocca et al., 2004; Sveinbjörnsdottir et al., 2000). Patients typically present with a tetrad of motor features, namely, resting tremor, muscle rigidity, posture instability and bradykinesia (Postuma et al., 2015). Of these, resting tremor is often the first symptom to present and is perhaps the most distinctive of the condition when compared with other motor disorders. It is now well established that these oftentimes debilitating symptoms are due to the degeneration of dopaminergic neurons within the substantia nigra pars compacta, which leads to a

depletion of the neurotransmitter dopamine (Postuma et al., 2015). However, PD is not limited to motor dysfunction (Pfeiffer, 2016). Several neuronal networks are affected, causing patients to also suffer from psychiatric and dysautonomic problems, and as the disease progresses, cognitive problems can develop (Pfeiffer, 2016). More common nonmotor symptoms include anosmia, depression and constipation. Because these nonmotor symptoms can occur more than 10 years prior to the onset of motor symptoms and diagnosis, prodomal PD is currently a research area attracting great attention.

From a pharmacological perspective, it is most important to emphasise that existing PD treatments only alleviate symptoms and do not delay or halt progression of the disease (AlDakheel et al., 2014). Current therapeutic approaches for PD are summarised in Table 1. The majority of these treatments aim to restore motor function by re-establishing dopamine signalling (AlDakheel et al., 2014) and are derived from pioneering studies by Arvid Carlsson who demonstrated that injection of the dopamine biosynthesis precursor L-dehydroxyphenylalanine (L-DOPA) rescued motor function in animal models (Carlsson et al., 1957). It took the best part of a decade to find a suitable dosing and delivery regimen for human PD patients (Cotzias et al., 1967), but oral L-DOPA and conceptually similar dopamine receptor agonists have been the mainstay of PD treatment ever since. For any drug, such enduring popularity would usually indicate that it is a highly efficacious and problem-free compound, and it is certainly true that after starting L-DOPA PD patients often experience a honeymoon period lasting several years, where motor symptoms may be in almost complete abeyance. But symptoms inevitably return as the disease progresses and the dopamine replacement therapies themselves can cause some fairly serious side effects, not least

L-DOPA-induced dyskinesias (Tran et al., 2018). Furthermore, these treatments do not address the majority of nonmotor symptoms, which typically do not involve loss of dopamine neurones (Chaudhuri et al., 2006; Postuma et al., 2015). PD patients will require additional medications to treat these symptoms, such as selective 5-HT (serotonin) reuptake inhibitors for the treatment of depression (AlDakheel et al., 2014). But perhaps the most fundamental limitation of dopamine receptor agonists is that these compounds do not affect the progression of the underlying neurodegeneration.

The need for new therapeutic strategies for PD is clear. Moreover, because the world's population is ageing, it is also clear that this need is growing (Reeve et al., 2014). Over recent years, a great deal of research has therefore been directed towards the genetic causes of PD, in the hope that these will point the way to novel drug targets. Therapeutic strategies that target proteins involved in PD aetiology offer great potential. In principle, such approaches may not only arrest PD progression but also may even be sufficient to prevent disease in the first place.

The rest of this article deals with the current state of play regarding pharmacological inhibitors of leucine-rich repeat kinase 2 (LRRK2), the product of a gene that is strongly implicated as both a cause of familial PD and an important risk factor contributing towards the more common sporadic form of the condition (Kluss et al., 2019; Nalls et al., 2014; Paisan-Ruiz et al., 2004; Zimprich et al., 2004). For reasons we outline below, targeting LRRK2 may be fruitful not only for individuals with pathogenic *LRRK2* mutations but potentially also for the majority of PD patients whose *LRRK2* status is likely to be normal. Nonetheless, LRRK2 inhibitors are far more likely to form part of a next generation of PD treatments, alongside therapies targeting

TABLE 1 Overview of the current treatments for Parkinson's disease

Target	Strategy	Treatment
Motor symptoms (loss of dopaminergic neurons and striatal dopamine depletion)	Dopamine replacement	Dopamine precursor (e.g. L-DOPA), dopamine agonists (e.g. ropinirole)
	Inhibition of dopamine degradation	MAO-B inhibitors (e.g. rasagiline) and catecholamine-*O*-methyltransferase inhibitors (e.g. entacapone)
	Glutamatergic activity decrease (promote dopamine synthesis) and other neurotransmitters	Amantadine, NMDA receptor antagonist and cholinesterase inhibitors
	Surgical intervention	For example, deep brain stimulation
	Nonpharmacological approaches	Exercise, rehabilitative therapy and physiotherapy
Nonmotor symptoms	Approaches used in the general population	Psychiatric symptoms (e.g. depression, anxiety and psychosis): dopamine-related (quetiapine), antidepressants, 5-HT (serotonin) and noradrenaline reuptake inhibitors, and cholinesterase inhibitors
		Cognitive impairments: cholinesterase inhibitors and MAO-B inhibitors

Note: This table summarises the most commonly used PD treatments that target motor dysfunction by re-establishing the physiological dopamine pathway. However, some nonmotor symptoms also respond to the treatments (AlDakheel et al., 2014; Armstrong & Okun, 2020; Chaudhuri & Schapira, 2009; Duty & Jenner, 2011; Jankovic, 2008; Pfeiffer, 2016; Postuma et al., 2015).
Abbreviations: L-DOPA, L-dehydroxyphenylalanine; PD, Parkinson's disease.

other genes involved in PD aetiology. For example, clinical trials are currently underway aiming to restore the function of the lysosomal enzyme **glucosylceramidase beta** (product of the *GBA* gene). Strategies employed include gene therapy (NCT04127578; clinicaltrials.gov) and compounds such as **ambroxol** (NCT02941822, NCT04388969 and NCT02914366; clinicaltrials.gov) that are expected to act as molecular chaperones to improve the delivery of glucosylceramidase beta to the lysosome (Maegawa et al., 2009; Migdalska-Richards et al., 2017). Direct targeting of α-synuclein is also being pursued. For example, the aggregation inhibitor anle138b (Wagner et al., 2013) is in clinical trials (NCT04685265; clinicaltrials.gov), as is the **c-Abl** inhibitor **nilotinib** (NCT02281474; http://clinicaltrials.gov), which is expected to remove α-synuclein by promoting autophagy (Karuppagounder et al., 2014). It is hoped that these compounds—in particular when allied with methods to identify at-risk individuals, such as genetic screens and research into prodromal PD, to allow earlier intervention—will become the next generation of PD treatments that are so desperately needed.

2 | LEUCINE-RICH REPEAT KINASE 2

Leucine-rich repeat kinase 2 protein (LRRK) is the product of the *LRRK2* gene. *LRRK2* was originally discovered independently by two groups as the gene located within the so-called *PARK8* locus—a region on chromosome 12 associated with autosomal dominant late-onset parkinsonism (Paisan-Ruiz et al., 2004; Zimprich et al., 2004). Mutations in *LRRK2* are now accepted as one of the most common causes of familial PD (Kluss et al., 2019; Nalls et al., 2014). Pathogenic LRRK2 mutations give rise to phenotypes that, at the individual level, are clinically indistinguishable from those of sporadic PD, including age of onset, symptoms and patterns of neurodegeneration in nigrostriatal dopaminergic neurons. Post-mortem *LRRK2* brains also display accumulation of Lewy bodies—proteinaceous aggregates typically enriched in α-synuclein and ubiquitin—that are a pathological hallmark of PD (Kalia et al., 2015). Curiously, *LRRK2* brains also frequently display Tau protein pathology; although the gene encoding **Tau**, *MAPT* has been strongly implicated in sporadic PD via genome-wide association studies, the accumulation of Tau is usually associated with other neurodegenerative conditions including Alzheimer's disease (Iqbal et al., 2010; Nalls et al., 2014). Thus, LRRK2 may sit at the top of neurodegenerative cascades involving both α-synuclein and Tau. Complicating matters further, TAR DNA-binding protein 43 kDa (TDP-43) pathology, more usually associated with amyotrophic lateral sclerosis, has been reported in a small number of *LRRK2* brains (Ling et al., 2013; Wider et al., 2010), as have post-mortem brains with no apparent proteinopathies (Gaig et al., 2007; Takanashi et al., 2018).

Importantly, genome-wide association studies (GWAS) have also shown that *LRRK2* has a major influence on an individual's risk of developing sporadic PD (Do et al., 2011; Lill et al., 2012; Satake et al., 2009; Simon-Sanchez et al., 2009). Thus, with gene variants of different severity either causing familial PD or contributing risk of sporadic PD, *LRRK* is considered a pleiomorphic risk locus for this condition (Cookson, 2015) and it is clear that the study of LRRK2 may have wider implications for other forms of neurodegenerative disease.

Adding an extra layer of complexity, genome-wide association studies have also implicated *LRRK2* as a factor in the genetic risk of Crohn's disease (Barrett et al., 2008; Franke et al., 2010) and leprosy (Wang et al., 2015; Zhang et al., 2009), whereas other studies have linked LRRK2 to tuberculosis (Hartlova et al., 2018). LRRK2 has also been associated with the risk of cancer but with conflicting results. Some studies find that the pathogenic LRRK2 variant G2019S increases the risk of cancer (Agalliu et al., 2015; Inzelberg et al., 2012; Saunders-Pullman et al., 2010), but others do not (Allegra et al., 2014; Ruiz-Martinez et al., 2014). Interestingly, loss of LRRK2 appears to increase risk of lung cancer (Lebovitz et al., 2021), an observation that may be germane to the long-term use of LRRK2 inhibitors (see later in this article). In any case, the observations linking LRRK2 to Crohn's disease, tuberculosis and leprosy are curious, because these conditions share some clear similarities with each other (e.g. they are all chronic inflammatory conditions with other genetic loci in common), but similarities with PD are less obvious. Whether LRRK2 dysfunction has a common role in all four conditions or whether the molecular mechanism in PD is distinct remains to be seen, but it is interesting to note that although that no shared pathogenic variants have been identified, the protective R1398H variant appears to be common to risk of Crohn's disease and PD (Hui et al., 2018; Ross et al., 2011; Tan et al., 2010). As such, it is easy to imagine that, at the very least, research into LRRK2 in PD could also have important implications for these non-neurodegenerative conditions.

LRRK2 itself is a large (2527 amino acid) and very unusual protein that belongs to the ROCO family of GTPases and contains multiple functional domains (Gotthardt et al., 2008; Paisan-Ruiz et al., 2004; Zimprich et al., 2004). The structure of LRRK2 is depicted in Figure 1. In addition to the four protein–protein interaction domains, LRRK2 contains domains conferring both serine–threonine kinase activity and GTPase activity, the latter via the combination of a Ras of complex proteins (Roc) domain and a C-terminal of Roc (COR) domain, which are often collectively referred to as a Roc–COR tandem domain (Gotthardt et al., 2008). A number of pathogenic mutations have been found, all of which encode single amino acid substitutions with the Roc, COR and kinase domains, although a relatively common variant associated with increased risk encodes a G2385R amino acid substitution with the WD40 domain, at the C-terminus of the protein (Berwick et al., 2019; Kluss et al., 2019). Typically, LRRK2 exists in cells as both homodimers and monomers, with dimerisation suggested to regulate LRRK2 subcellular location and function (Ho et al., 2018). Fascinatingly, monomers are generally cytosolic, whereas dimers are predominantly located on organelle membranes (Berwick & Harvey, 2012; Nichols et al., 2010), and membrane-bound LRRK2 has been reported to possess a higher kinase activity (Berger et al., 2010). Thus, LRRK2 dimerisation and membrane recruitment have been suggested to play a role in kinase activation, perhaps in a mechanism governed by the membrane-bound small GTPase Rab29, which has been shown to bind LRRK2 and increase its kinase activity in cells (Liu et al., 2018; Purlyte et al., 2019).
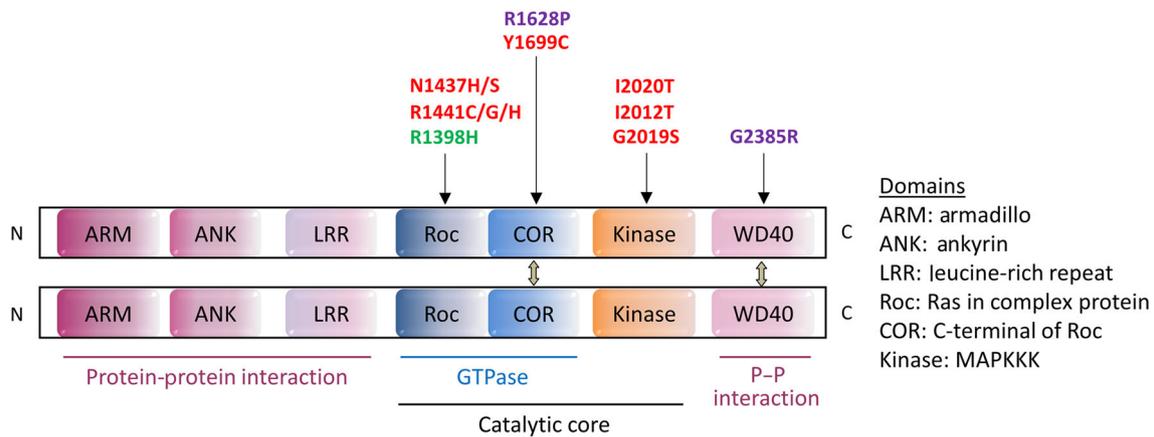
FIGURE 1   Leucine rich repeat kinase 2 (LRRK2) structure and mutations. LRRK2 is a homodimer belonging to the ROCO family of GTPases. LRRK2 contains multiple domains: protein–protein interaction domains ARM (armadillo), ANK (ankyrin-like), LRR (leucine-rich repeat) and WD40 (all in pink); a serine–threonine kinase domain (in orange); and a Roc (Ras of complex proteins)–COR (C-terminal of Roc) tandem domain conferring GTPase activity (in blue). The arrows indicate the putative intramolecular interactions. The locations of the pathogenic mutations are indicated in red; the risk variants are indicated in purple; and the protective variant is indicated in green

The biological function of LRRK2 within cells remains unclear, largely because this protein has been linked to a multitude of different cell biological processes and a clear front runner has yet to emerge from the pack. For example, LRRK2 has been linked to effects on cell signalling, microtubule and other cytoskeletal dynamics, ciliogenesis, autophagy, endocytosis, synaptic vesicle trafficking and the biology of numerous cellular organelles such as mitochondria, the trans-Golgi network and lysosomes (reviewed by Berwick et al., 2019). Complicating things further, there is no real agreement over the type of cells in which LRRK2 dysfunction is most important for PD, with published data supporting many neural cell types and also immune cells (Berwick et al., 2019). In principle, therefore, LRRK2 dysfunction could cause either degeneration of dopaminergic neurons via a cell autonomous process or via a noncell autonomous neuroinflammatory mechanism—or potentially via both.

Nonetheless, within the context of this review, the uncertainty regarding LRRK2 function is not of great importance. This is because when it comes to the effects of *LRRK2* pathogenic mutations on LRRK2 itself, there is clear consensus on two key points:

1. *All pathogenic LRRK2 mutations increase both LRRK2 autophosphorylation and the LRRK2-mediated phosphorylation of certain Rab small GTPases in cells.* Whether all these mutations technically increase LRRK2 kinase activity is debatable, because only the common G2019S kinase domain mutation reproducibly increases kinase activity in biochemical assays performed *in vitro* (Greggio & Cookson, 2009). Furthermore, the G2385R risk variant is actually reported to reduce kinase activity in these experiments (Rudenko et al., 2012). However, this argument is ultimately a semantic one—the effect of all PD-causing or PD risk mutations in *LRRK2* is to increase substrate phosphorylation *in vivo* (Berwick et al., 2019; Kluss et al., 2019). To most intents and purposes, therefore, they can be considered to increase kinase activity; at the very least, they are gains of kinase function.

2. *All pathogenic LRRK2 mutations within the Roc or COR domains increase the fraction of GTP-bound LRRK2 relative to GDP-bound LRRK2.* This phenomenon is achieved either via increased binding of the LRRK2 Roc domain to GTP, or via a decrease in the catalytic activity of this domain (i.e. there is less hydrolysis of GTP to GDP), or via a combination of both mechanisms (Nixon-Abell, Berwick, & Harvey, 2016). Supporting the idea that GTP-bound LRRK2 is a more pathogenic species, the protective *LRRK2* risk variant, R1398H, displays decreased GTP-binding and increased GTPase activity and can therefore be expected to exist in a more GDP-bound state (Nixon-Abell, Berwick, Granno, et al., 2016).

Thus, there are clear and largely unambiguous correlations between increased LRRK2 kinase function *in vivo* and PD and between the increased association of LRRK2 with GTP and PD. How these mechanisms relate to each other is not yet fully understood, with biochemical evidence indicating that the two enzymatic activities of LRRK2 are to some extent interdependent. However, the fact that all pathogenic mutations promote substrate phosphorylation under physiological conditions indicates that increased kinase function is the pathogenic 'output' of LRRK2, even if the increased kinase function is not always increased kinase activity per se. Underscoring this idea, increased LRRK2 autophosphorylation and increased phosphorylation of the LRRK2 substrate Rab10 have also been observed in PD and animal models of PD that do not involve pathogenic *LRRK2* mutations (Di Maio et al., 2018). Importantly, these include post-mortem brains from human patients with sporadic PD, as well as brains from rats that overexpress α-synuclein or have been treated with the environmental toxin rotenone (Di Maio et al., 2018). As such, it can be assumed that multiple causes of PD—genetic and environmental—can give rise to increased LRRK2 kinase function and thus PD.

Although it has taken some time for the experimental evidence to convincingly support this idea, largely due the time taken for researchers to identify **Rab proteins** as the first widely agreed bona

fide LRRK2 substrates, autosomal dominant mutations in a kinase gene can logically be expected to increase the enzymatic activity of that kinase. Thus, from the perspective of the pharmaceutical industry, the identification of *LRRK2* as an autosomal dominant cause of hereditary PD was sufficient rationale to begin developing compounds that reduce LRRK2 kinase activity, irrespective of how well the assumed increased kinase function had actually been demonstrated empirically. As a result, the quest to develop LRRK2 inhibitors as candidate therapeutic treatments for PD began very early, with multiple companies involved, as well as considerable backing from the charitable sector. In fact, the pursuit of a clinically usable LRRK2 inhibitor has progressed to such an extent that clinical trials of one such compound in sporadic PD patients were announced only a matter of weeks after the first evidence that LRRK2 kinase function is actually increased in this cohort (Denali Therapeutics, 2018; Di Maio et al., 2018). In short, the progress of LRRK2 drug development relative to basic research into this protein is quite remarkable.

In the subsequent three sections of this article, we summarise the development of conventional LRRK2 kinase inhibitors, while also identifying potential risks and challenges in the road ahead. For more detailed descriptions of the chemistry of these molecules, we point the reader to the following more specialised reviews (Domingos et al., 2019; Hatcher et al., 2017). Later in the manuscript, we branch out to alternative pharmacological strategies to reduce the function of this protein, which together create a rich palette of potential therapeutic options.

3 | ATP-COMPETITIVE LRRK2 KINASE INHIBITORS

The majority of molecules targeting LRRK2 have been conventional or 'Type I' kinase inhibitors, which compete with ATP for binding to the ATP-binding pocket within the kinase domain (Hatcher et al., 2017; Lu et al., 2020). This story inevitably began with compounds that were a long way from candidate drugs ready to enter clinical trials, but over the years, these molecules have evolved to have superior pharmacological profiles and one compound is now in clinical trials.

The first compounds reported to inhibit LRRK2 kinase activity were either broad-range kinase inhibitors able to inhibit LRRK2 with high potency but very low selectivity, for example, **staurosporine**, which has IC_{50} values for LRRK2 of 1–2 nM (Anand et al., 2009; Covy & Giasson, 2009), or a series of Rho kinase inhibitors that were found to inhibit LRRK2 and Rho kinases with similar high nanomolar potencies (Nichols et al., 2009). Fortunately, these compounds were swiftly superseded by two more selective molecules, **LRRK2-IN-1** (Deng et al., 2011) and CZC-25146 (Ramsden et al., 2011), which were identified specifically as LRRK2 inhibitors via kinase inhibitor library screens. Both these compounds are reported to inhibit LRRK2 with IC_{50}s in the low nanomolar range and exhibit decent selectivity; for example, LRRK2-IN-1 was found to display greater than 90% inhibition towards only 12 out of 440 kinases using an Ambit KINOMEscan

that measures the binding of drugs to a panel of kinases as a proxy for kinase inhibition (Deng et al., 2011; Karaman et al., 2008).

The chemical structures of LRRK2-IN-1 and CZC-25146 and other selective LRRK2 inhibitors that are specifically mentioned within this article are shown in Figure 2.

In addition to LRRK2-IN-1 and CZC-25146, the first generation of LRRK2 inhibitors also included another high MW diaminopyrimidine named **TAE684**, a repurposed anaplastic lymphoma kinase (ALK) inhibitor with a similar potency but a slightly inferior selectivity (Zhang et al., 2012). Aminopyrimidines were not the only structural category represented however, with GSK2578215A, a potent ($IC_{50} = 9$ nM) and selective (90% inhibition towards only three out of 449 kinases) arylbenzamide, also being reported in 2012 (Reith et al., 2012). These four compounds have been integral to a great deal of basic research carried out over subsequent years, aided in part by the Michael J. Fox Foundation making them easily available to scientists worldwide. Nonetheless, as drugs for the clinic, all four compounds perished early in the pipeline. LRRK2-IN-1 and CZC-25146 had the critical failing of being unable to cross the blood–brain barrier, which is clearly a fundamental requirement of any pharmaceutical aimed at treating neurodegenerative disease. Somewhat surprisingly, the structurally similar but larger compound TAE684 did enter the brain in rodents, but it was found to be ineffective as a LRRK2 inhibitor in this organ. GSK2578215A, which is appreciably smaller than all three aminopyrimidine molecules, also crossed the rodent blood–brain barrier with little effect on LRRK2.

Given that LRRK2-IN-1, CZC-25146 and TAE684 are all similar in structure, it is unsurprising that a great many of the next generation of LRRK2 kinase inhibitors were smaller aminopyrimidine molecules that were developed with the hope of retaining potency and selectivity for LRRK2, while facilitating access to the brain. A number of such compounds were published in the next few years including **HG-10-102-01** (Chen et al., 2012; Choi et al., 2012) and a series of compounds—G1023, **GNE-7915**, **GNE-0877** and **GNE-9605**—made by Genentech (Estrada et al., 2012, 2014). All these compounds have potencies in the low (or sub-molar) nanomolar range and excellent selectivity and are able to enter the brains of mice and inhibit LRRK2 *in vivo*. The Genentech compounds were also tested in rats and cynomolgus monkeys, where they were found to have good pharmacokinetic and drug metabolism profiles (Estrada et al., 2012, 2014). Thus, LRRK2 kinase inhibitors moved from purely *in vitro* tools to compounds that could be used to study LRRK2 in the brains of animal models.

Over the next few years, a number of other structurally distinct LRRK2 kinase inhibitors (i.e. neither aminopyridines nor arylbenzamides) have been reported in the scientific literature, as these compounds continue to evolve and improve. Structural categories include pyrrolopyrimidines such as Pfizer's **PF-06447475** (Henderson et al., 2015), indazoles such as Merck's MLi-2 (Fell et al., 2015) and thiophenes, also from Merck (Greshock et al., 2016). In addition, further types of organic molecules that have not been described in the scientific literature are the subject of patents for use as LRRK2 inhibitors, such as macrocyclic derivatives from
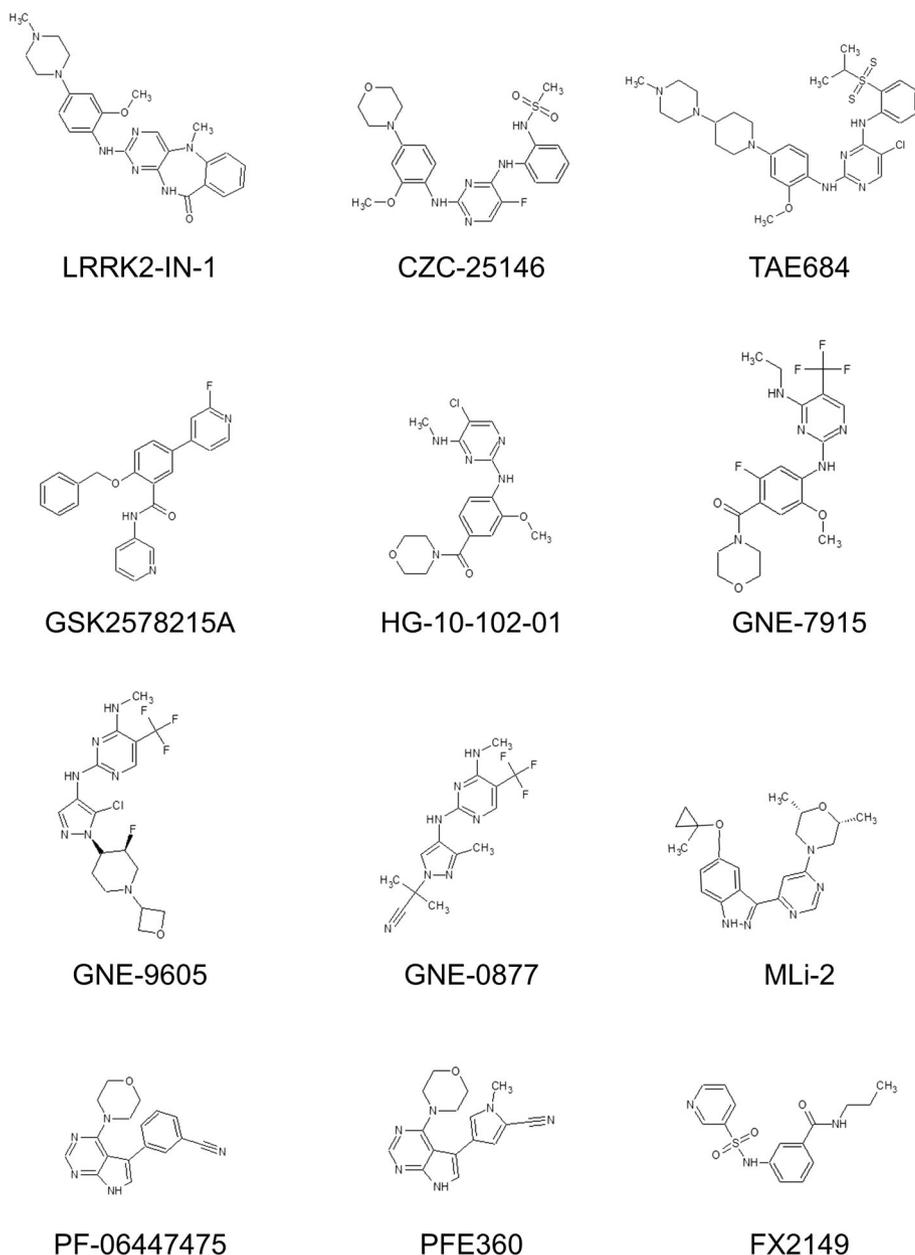
FIGURE 2 Chemical structures of the leucine rich repeat kinase 2 (LRRK2) inhibitors described in this review. The chemical structures of the selective LRRK2 inhibitors mentioned by name in this article are shown. Structures were generated using ACD/ChemSketch (ACD Labs) with structures cleaned and most likely tautomeric forms selected

GlaxoSmithKline and Ipsen Pharma (Ding & Ren, 2020). For a detailed review of the chemistry, we refer the reader to existing work in this area (Domingos et al., 2019; Hatcher et al., 2017).

One recent development that is worthy of mention, however, is the discovery of ATP-competitive LRRK2 inhibitors that exhibit selectivity for the pathogenic G2019S LRRK2 variant over wild-type LRRK2 (Garofalo et al., 2020). Via compound library screening followed by in silico docking analysis and systematic chemical modifications, Garofalo et al. (2020) identified a number of indazole molecules displaying selectivity for G2019S. The most selective compound was reported to have a >300× greater potency in cellular assays and >200× greater potency in vitro. As the authors themselves rightly observe, this 'is remarkable, given that G2019S-LRRK2 differs from WT-LRRK2 only by a single amino acid' (Garofalo et al., 2020).

Unfortunately, this molecule is unlikely to be developed further as it is unable to cross the blood–brain barrier, but we note that another G2019S-selective indazole compound identified in the same work has since been shown to inhibit LRRK2 kinase activity in peripheral blood mononuclear cells from homozygous G2019S LRRK2 patients, but not in cells from wild-type controls (Bright et al., 2021). Thus, even though these molecules may only be suitable for G2019S LRRK2 PD patients, there is clearly a lot of potential in this project and we await further developments.

Taking this section as a whole, it is evident that there are a multitude of promising ATP-competitive LRRK2 kinase inhibitors in the pipeline. As therapeutics for the treatment of PD, most of these compounds will inevitably fall by the wayside, but with a diversity of chemical structures available, there are good grounds for optimism. A

variety of chemical structures means a greater range of pharmacological properties and a better chance of finding a drug that is potent, selective and free from adverse effects. Later in this article, we will focus on LRRK2 inhibitors that are or have been used in human clinical trials, but before we get to that point, we will first comment on certain areas of concern that have been raised in preclinical studies.

4 | AREAS OF CONCERN FOR THE LRRK2 KINASE INHIBITOR DRUG PIPELINE

As mentioned, LRRK2 kinase inhibitors have been in development for well over a decade as a potential therapeutic strategy for PD. Nonetheless, there have long been concerns about this strategy that go beyond the now resolved difficulty in demonstrating elevated LRRK2 kinase function for any variant other than G2019S. For example, LRRK2 kinase inhibitors have been shown to display similar effects to pathogenic mutants in some cellular assays, for example, their effects on canonical Wnt signalling (Berwick et al., 2017; Berwick & Harvey, 2012) and binding of LRRK2 to filamentous microtubule structures (Blanca Ramirez et al., 2017; Schmidt et al., 2019). Thus, there is a potential that LRRK2 inhibition may increase certain PD symptoms. In addition, there is evidence that prolonged inhibition can lead to loss of protein stability, thereby compromising all functions of LRRK2, not just those that are kinase dependent (West, 2017). Although this can in principle be addressed by keeping the therapeutic dose within a tight window, this is potentially a serious problem, as LRRK2 has hundreds of reported interacting partners and is believed also to act as a scaffolding protein, not just as a kinase (Berwick et al., 2019; Lewis & Manzoni, 2012).

The greatest worry perhaps inevitably comes from *in vivo* studies of *Lrrk2* knockout mice and rats. Mice that are double knockout for both *Lrrk2* and its paralog *Lrrk1* display neurodegeneration (Giaime et al., 2017), yet because animals that only lack *Lrrk2* have no overt neurological phenotypes (for a detailed review of LRRK2 animal models, see Seegobin et al., 2020), this concern does not relate to the brain. Instead, *Lrrk2* knockout mice and rats display an unusual vacuolated phenotype in their kidneys, liver and lung (Baptista et al., 2013; Ness et al., 2013; Seegobin et al., 2020; Tong et al., 2010). None of these alterations appear to have any significant impacts on the health of these laboratory animals, but the lung phenotype is a particular concern given that respiratory problems are very common in PD (van de Wetering-van Dongen et al., 2020), so any further compromise to lung function could ultimately lead to a worsened clinical outcome. This concern was intensified following an investigation into the effect of two of the Genentech aminopyridine inhibitors, GNE-7915 and GNE-0877, in cynomolgus monkeys (Fuji et al., 2015). Worryingly, treatment of these animals with either compound caused vacuolated Type II pneumocytes, which was very consistent in appearance with the lung phenotype of *Lrrk2* knockout mice and rats. Subsequently, a similar effect was observed in mice treated with the indazole LRRK2 inhibitor MLi-2 developed by Merck (Fell et al., 2015). However, a recent dedicated toxicological study using GNE-7915, MLi-2 and the

Pfizer LRRK2 inhibitor PFE-360 in cynomolgus has indicated that these fears may be exaggerated (Baptista et al., 2020). Although each compound induced the same vacuolated Type II pneumocyte phenotype, thereby confirming that this is an on-target effect of LRRK2 kinase inhibition, lung function studies on the GNE-7915 and MLi-2-treated animals did not reveal any apparent limitations (the PFE-360-treated cohort was not examined). Importantly, other experiments indicate that the phenotype is reversible following withdrawal from treatment and that the PFE-360 compound, in particular, might be able to operate at a no-effect dose, where brain LRRK2 kinase activity is inhibited, but the lung phenotype is not observed (Baptista et al., 2020). As such, the worst case scenario seems to be that the lung phenotype can be avoided with adequate dosing and, if it does occur, the phenotype can be treated and is unlikely to have much of an impact anyway. Supporting this further are recent studies of humans carrying heterozygous loss-of-function *LRRK2* variants. It had already been reported that individuals with low expression of LRRK2 have no increased risk of PD (Blauwendraat et al., 2018), but the work of Whiffin et al. (2020) has extended this to a wide range of clinical outcomes. Using health records of individuals heterozygous for *LRRK2* variants containing premature stop codons, this group reported no increased frequency of 'lung, liver, kidney, cardiovascular system, nervous system, immunity or cancer' phenotypes (Whiffin et al., 2020). However, these results are to some extent contradicted by a more recent report associating LRRK2 loss-of-function with a type of lung cancer that appears to be Type II pneumocyte in origin (Lebovitz et al., 2021). This group had previously reported lowered LRRK2 expression in lung adenocarcinoma (Lebovitz et al., 2015) but have since looked at the connection in greater detail. They report that lower levels of LRRK2 are associated with decreased patient survival and that *Lrrk2* KO mice are more prone to developing lung adenomas when exposed to urethane, a cigarette smoke carcinogen (Lebovitz et al., 2021). These results are concerning, but it is probably pertinent to observe that lower LRRK2 expression is most strongly correlated with lung cancer in patients who are smokers and that there is currently little evidence to suggest decreased LRRK2 expression is *sufficient* to cause lung cancer (Lebovitz et al., 2021). Thus, LRRK2 inhibitors may not be suitable for current or recent smokers, but otherwise, the risk of lung cancer appears small.

A further complication is how to reliably measure the extent of LRRK2 kinase inhibition within the brains of patients, such that clinicians can establish an optimal dose and monitor treatment progression in their patients. Existing biomarkers, such as Rab protein phosphorylation (Eyers, 2018; Steger et al., 2016) or LRRK2 autophosphorylation on serine-1,292 (Sheng et al., 2012), which can be measured with phospho-specific antibodies, appear to be faithful proxies for LRRK2 kinase function in brain tissue from experimental animals, but these assays cannot easily be replicated on humans. Less invasive procedures will be needed, for example, proteins or metabolites present in patient blood or urine samples, although correlating these readouts with LRRK2 kinase inhibition in brain tissue is going to be a tough ask. With this in mind, we note that certain bis(monoacylglycerol)phosphates (BMP), phospholipids that are

considered specific to lysosomes, have been reported to be increased in the urine of *LRRK2* PD patients (Alcalay et al., 2020) and also decreased in the urine of both cynomolgus monkeys treated with LRRK2 kinase inhibitors and *Lrrk2* knockout mice (Fuji et al., 2015). As such, urine BMP levels may be an ideal non-invasive biomarker. It should be noted that sporadic PD patients do not appear to have elevated urine BMP levels compared with healthy controls (Alcalay et al., 2020). However, the same study found that in PD patients urine BMP levels appear to be inversely correlated with cognitive performance irrespective of *LRRK2* status. Thus, the overall difference in urine BMP levels between sporadic PD and control cohorts is likely a consequence of heterogeneity within each group and that at an individual level at least, urine BMP levels may be a faithful biomarker of disease progression (Alcalay et al., 2020). The full potential of BMP remains to be established, but unsurprisingly, this biomarker is already being used in clinical trials of LRRK2 inhibitors (Denali Therapeutics, 2020).

5 | LRRK2 KINASE INHIBITORS IN CLINICAL TRIALS

Although the field is very crowded, to date just two LRRK2 kinase inhibitors have been used in clinical trials: compounds DNL-201 and DNL-151, which are manufactured by Denali Therapeutics. Neither of these compounds have been described in the scientific literature, although it has been observed that this company published four patents that cover the use of an array of aminopyrimidine compounds in 2017 and 2018 (Ding & Ren, 2020), around the time trials began. In Phase 1a trials, both compounds were well tolerated in healthy volunteers at doses that 'achieved high levels of CSF exposure, robust target engagement as measured by two blood-based biomarkers of LRRK2 activity, and effects on biomarkers of lysosomal function' (Denali Therapeutics, 2018). It can be assumed that the blood biomarkers of LRRK2 activity were measurements of LRRK2 autophosphorylation or Rab protein phosphorylation or, alternatively, phosphorylation of LRRK2 at one or more of a series of serine residues within the LRR domain that are dependent on, but not directly mediated by, LRRK2 kinase activity (Kelly & West, 2020). Indeed, phosphorylation of Rab10 and LRRK2 phosphorylation on the serine-935 LRR domain site in peripheral blood mononuclear cells and whole blood are mentioned as secondary outcome measures within the Phase 1b clinical trial listings for the DNL-201 and DNL-151 (NCT03710707 and NCT04056689, respectively; clinicaltrials.gov). The assay of lysosomal function is not described in the clinical trial listings but is likely to be urine BMP levels, as mentioned in a subsequent announcement (Denali Therapeutics, 2020).

The trial has been expanded to PD patients in Phase 1b trials, with both compounds reporting good results. In light of the lung phenotype and the particular risk of respiratory impairment in PD, this is good news. Nonetheless, Denali have announced their intention to focus only on DNL-151 as Phase 1b trials are extended to cover a wider range of doses, as this compound has 'pharmacokinetic properties that provide additional dosing regimen flexibility' (Denali Therapeutics, 2020).

So what is the situation with other LRRK2 kinase inhibitors? At present, with Denali over 2 years into testing, it can be assumed that other companies are waiting for the full results of DNL-151's Phase 1 and 2 trials, before deciding whether to press ahead with their own compounds. Watching keenly will be Cerevel, who have a LRRK2 inhibitor listed as one of their pipeline drugs on their website (Cerevel, 2021) and have acquired the rights to Pfizer's neurological disease compounds. Presumably, this includes the pyrrolopyrimidine compound PFE-360, which, as mentioned above, was found to have a no-effect dose at which it inhibited LRRK2 in monkey brains but did not induce any lung phenotype (Baptista et al., 2020). Also paying close attention will be Merck, whose MLi-2 compound also showed signs of a no-effect dose, albeit within a much narrower window than was observed for PFE-360 (Baptista et al., 2020). Perhaps importantly, this drug has an especially high potency, with an IC_{50} in the high picomolar range (Fell et al., 2015). Clearly, Cerevel and Merck will not be the only interested parties. Thus, although the world plays wait and see, we can at least do so safe in the knowledge that if DNL-151 fails, there are plenty of alternative options.

6 | OTHER THERAPEUTIC APPROACHES TO REDUCE LRRK2 FUNCTION

Although there are plenty of alternative LRRK2 kinase inhibitors to DNL-151, there are also plenty of therapeutic strategies for reducing the function of LRRK2 that do not involve classical ATP-competitive kinase inhibition (Figure 3). This smorgasbord of approaches broadens the targets of potential therapeutic drugs far beyond the kinase domain and includes molecules that affect LRRK2 conformation, protein–protein interactions, GTPase activity and even *LRRK2* mRNA. We describe these molecules in the following sections.

7 | LRRK2 GTP-BINDING INHIBITORS

In addition to the LRRK2 kinase domain, the second enzymatic activity of LRRK2 represents another interesting drug target. Because there are only four ROCO GTPases in humans—LRRK1, LRRK2, **death-associated protein kinase (DAPK)** 1 and malignant fibrous histiocytoma-amplified sequence with leucine-rich tandem repeats 1 (MASL1) (Tomkins et al., 2018)—molecules targeting LRRK2 GTP binding have the potential to be especially selective. Moreover, there is also sound rationale for such compounds, as decreasing the proportion of pathogenic LRRK2 existing in a GTP-bound state can be expected to reduce LRRK2 kinase activity and would likely be neuroprotective. Therefore, selective and blood–brain barrier-permeable molecules that block LRRK2 GTP binding represent an alternative option to explore beyond kinase inhibition.

Surprisingly though, research on LRRK2 GTPase inhibitors has not been prolific and very few research groups in industry or
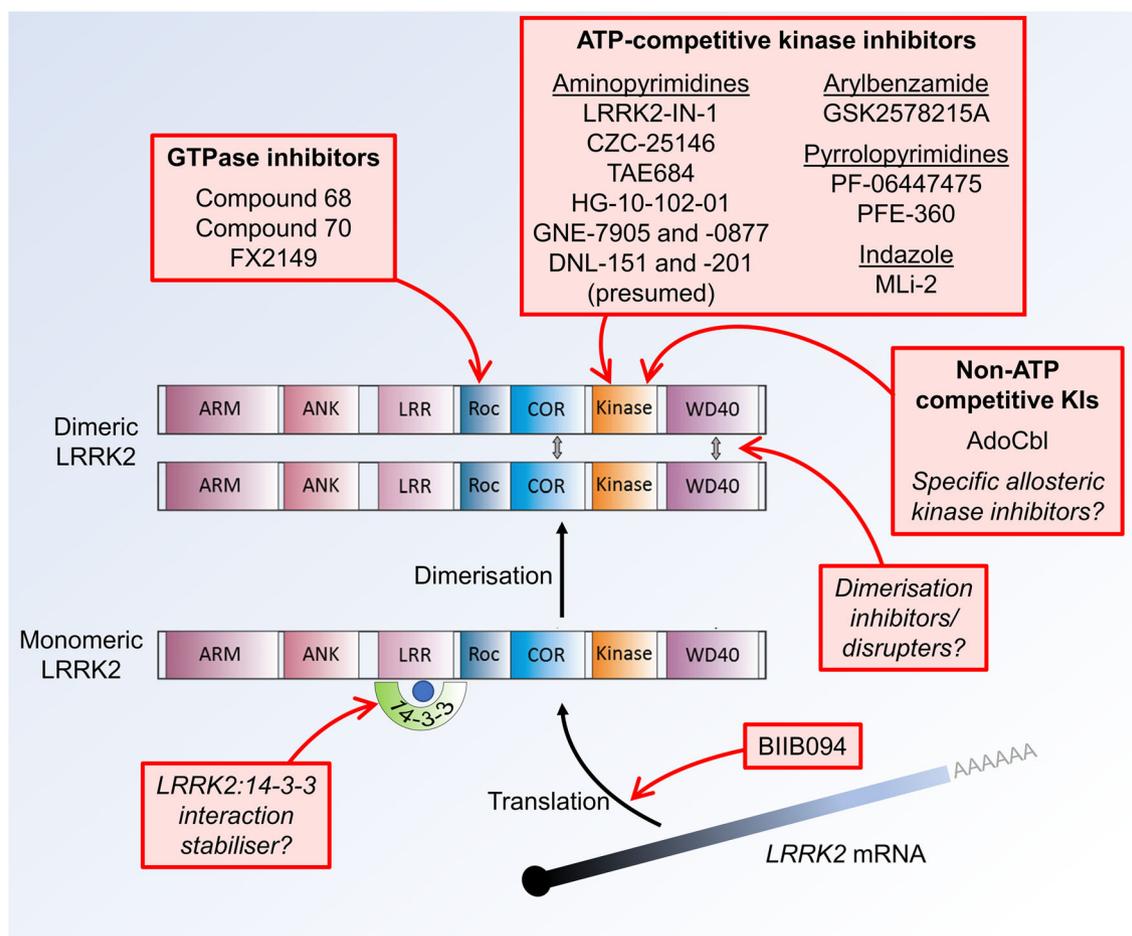
FIGURE 3 Sites of action of the leucine rich repeat kinase 2 (LRRK2) inhibitors described in this review. The various types of therapeutic strategy that are under development or have been proposed for targeting LRRK2 are summarised in the figure. Proposed strategies are written in italics. For simplicity, non-selective kinase inhibitors (e.g. staurosporine and ponatinib) are omitted. ATP-competitive kinase inhibitors are listed by chemical class; note that the categorisation of DNL-151 and DNL-201 is presumed on the basis of patents held by the manufacturer

academia have actively taken this route. To date, only Wanli Smith's group has reported LRRK2 GTP-binding inhibitors, the first of which, compounds 68 and 70, were identified via a computer-aided drug discovery screen (Li et al., 2014). In addition to blocking LRRK2 GTP binding in cells, these compounds were also able to reduce LRRK2 autophosphorylation, thereby confirming the secondary effect of inhibiting LRRK2 kinase activity (Li et al., 2014). Demonstrating their potential, both compounds promoted the survival of cultured neurons, whereas compound 68 also reduced **LPS**-induced neuroinflammation in a mouse model (Li et al., 2014). In subsequent work, this group reported an optimised analogue of compound 68, FX2149, which is more brain penetrant and correspondingly more potent in the same mouse model (Li et al., 2015). To the best of our knowledge, only one other group has published with these compounds. Fascinatingly, Blanca Ramirez et al. (2017) reported that compounds 68 and 70 reverse the increased binding of the pathogenic R1441C LRRK2 variant to filamentous microtubule structures. This is in contrast to LRRK2 kinase inhibitors, which, as mentioned above, were found to behave like PD-causing mutations in these assays (Blanca Ramirez et al., 2017). Although these compounds are under patent, we are

unaware of any moves towards entering LRRK2 GTP-binding inhibitors in clinical trials. Nonetheless, the published data indicate that they have potential.

8 | LRRK2 KNOCKDOWN

Although DNL-151 is the only chemical inhibitor of LRRK2 currently in clinical trials, it is not the only molecule seeking to reduce LRRK function being examined in humans. As part of a collaboration between Biogen and Ionis Pharmaceuticals, intrathecal injection of a molecule called BIIB094 is currently being studied in a Phase 1 clinical trial (NCT03976349; http://clinicaltrials.gov). As Biogen state on their website, BIIB094 is an antisense oligonucleotide (ASO) (Biogen, 2021). Antisense oligonucleotides are single-stranded DNA molecules of varying lengths that are designed to bind specifically to an mRNA molecule of interest via base pair complementarity, typically causing protein synthesis from that mRNA to be blocked (Chery, 2016). The nucleotides within antisense oligonucleotides are typically chemically modified to prevent degradation by nucleases, while also facilitating uptake across

the plasma membrane and enhancing binding to RNA (Silva et al., 2020). Importantly, because the mode of delivery used by Biogen and Ionis is intrathecal injection, that is, directly into the CSF, this strategy obviates the need for BIIB094 to cross the blood–brain barrier. The use of antisense oligonucleotides to target LRRK2 still carries many of the same concerns as use of LRRK2 kinase inhibitors and intrathecal delivery is a fairly invasive means of drug delivery, so there are evidently downsides to this approach. Nonetheless, it is important to note that the blood–brain barrier works in both directions, so intrathecal delivery will greatly reduce the chance of adverse effects in the periphery, not least the now familiar lung phenotype.

Fascinatingly, antisense oligonucleotides are already being used in the clinic for the treatment of spinal muscular atrophy, a rare neurodegenerative disease of motor neurons (Kolb & Kissel, 2015). In this case, the antisense oligonucleotide, which is referred to as nusinersen, is not acting to repress its target mRNA but to prevent the binding of a splicing factor, thereby promoting alternative splicing (Chiriboga et al., 2016). More conventional antisense oligonucleotides that repress gene expression have not yet progressed so far through the drug development pipeline for use in neurodegenerative diseases but show promise. Most notably, these molecules have been shown to decrease mutant gene expression and correspondingly improve the clinical symptoms in preclinical models with a range of different polyglutamine repeat disorders. These include Huntington's disease, spinal and bulbar muscular atrophy and a number of types of spinocerebellar ataxia (Silva et al., 2020). Treatments have only reached the clinical trial stage for Huntington's disease, but results have been impressive. In particular, ISIS3139 (also known as RO7234292), an antisense oligonucleotide also produced by Ionic Pharmaceuticals but this time working with Hoffman-La Roche, was found to produce no serious adverse effects while also lowering expression of mutant huntingtin in the CSF in a combined Phase 1 and 2 clinical trial (NCT02519036; clinicaltrials.gov) (Tabrizi et al., 2019). ISIS3139 has since progressed to a Phase 3 trial (NCT03761849; http://clinicaltrials.gov), with results expected in late 2022.

It is also worth observing that other antisense oligonucleotides are under investigation for use in Huntington's disease, which, unlike ISIS3139, specifically target mRNA produced by the disease-causing allele and not the wild type (Silva et al., 2020). This is potentially significant, because this strategy should in principle avoid reducing all huntingtin expression, thereby protecting the essential functions of this protein. Theoretically, PD patients with *LRRK2* mutations (although not sporadic PD patients) could be treated in a similar way. However, the ability to select between mutant and wild type that differ only by a single base change is going to be considerably more challenging than mutations like the expanded CAG repeats causing Huntington's disease.

9 | ALLOSTERIC INHIBITORS OF LRRK2 KINASE ACTIVITY

Allosteric kinase inhibitors can be defined as compounds that inhibit the enzymatic activity of a kinase by binding the kinase outside its

ATP-binding site and thus reducing kinase activity in an ATP non-competitive manner (Lu et al., 2020). In principle, the GTPase inhibitors described above, which bind the LRRK2 Roc domain and indirectly reduce LRRK2 kinase activity, meet this definition, but we have chosen to classify them separately, because kinase inhibition is not their primary objective. In practice, allosteric inhibitors are more likely to bind the kinase domain. There are advantages and disadvantages with any therapeutic strategy, but allosteric compounds have the potential to increase selectivity, by targeting regions that are less conserved than the ATP-binding pocket, although designing such molecules is more challenging.

Recently, Schaffner et al. (2019) used a high-throughput screen of Food and Drug Administration (FDA)-approved compounds to identify a physiological form of vitamin B_{12} (50-deoxyadenosylcobalamin or AdoCbl) as a non-ATP-competitive, 'mixed-type' allosteric inhibitor of LRRK2 kinase activity. Because vitamin B_{12} levels are inversely correlated with PD progression (Christine et al., 2018), these observations suggest an intriguing, if speculative, hypothesis that endogenous vitamin B_{12} levels may be affecting PD progression via modulation of LRRK2 and that AdoCbl may be a bona fide endogenous inhibitor of LRRK2 activity. In any case, with a reported IC_{50} of ~1 μM *in vitro*, AdoCbl is considerably less potent than conventional LRRK2 inhibitors, although the authors suggest that AdoCbl is likely to be highly selective for LRRK2 as this molecule is structurally dissimilar to any other known kinase inhibitor. This last point clearly needs to be tested empirically. In any case, this molecule was able inhibit LRRK2 kinase activity and neurotoxicity in both *in vitro* and *in vivo* models carrying pathogenic LRRK2 variants (Schaffner et al., 2019). The exact location within LRRK2 to which AdoCbl binds was not identified but based on enzymological data can be assumed to be distinct site(s) within the LRRK2 kinase domain but outside the ATP-binding pocket. The authors also report that AdoCbl disrupts LRRK2 dimerisation and promotes the proteolytic degradation of this protein. How these observations fit together mechanistically is not yet known, but because LRRK2 dimers have greater kinase activity than LRRK2 monomers, it is tempting to speculate that AdoCbl may be inhibiting LRRK2 kinase by preferentially binding to dimeric LRRK2 and inducing monomerisation. This story is clearly at its infancy and therapeutic potential of AdoCbl is unclear, but its identification as a new allosteric inhibitor of LRRK2 may at least open the door to the discovery of other similar molecules.

One strategy for the development of allosteric kinase inhibitors of LRRK2 that may be slightly less useful is the development of 'Type II' kinase inhibitors that target the DFGψ motif (sometimes abbreviated to DFG) of the kinase domain activation loop (Lu et al., 2020; Schmidt et al., 2019). (Type II allosteric inhibitors in general bind to kinase domain activation loops; DFGψ is a conserved sequence of amino acids with the activation loop, where D, F and G are aspartic acid, phenylalanine and glycine and ψ represents a hydrophobic residue.) Importantly, Type II kinase inhibitors are only able to bind kinase domain activation loops that are in an inactive 'DFG-out' conformation. In doing so, these compounds prevent the conformational change to an active 'DFG-in' conformation that is required to allow

access of ATP to the adjacent ATP-binding pocket (Schmidt et al., 2019). As such, Type II kinase inhibitors block kinase activity by gating access to ATP, rather than by competing with ATP. The limitation of developing Type II kinase inhibitors that target the DFGψ motif for LRRK2 is that this is the location of the amino acid substitutions encoded by two pathogenic mutations, G2019S and I2020T. Significantly, both G2019S and I2020T stabilise the LRRK2 activation loop in the active DFG-in conformation (Liu et al., 2013; Ray et al., 2014). Given this, Type II kinase inhibitors that target the DFGψ motif of LRRK2 can be expected to be less effective against these pathogenic variants than against wild-type LRRK2.

This expectation is supported by experimental data. To date, several Type II inhibitors have been reported, including four compounds identified by Liu et al. using molecular docking of previously described Type II allosteric inhibitors of other kinases to a structural model of the LRRK2 kinase domain (Liu et al., 2013; Ray & Liu, 2012). Two of these inhibitors target the activation loop hinge region, rather than the DFGψ motif, and these compounds displayed little difference in potency when tested against wild-type LRRK2 and the G2019S and I2020T variants (Ray & Liu, 2012). By contrast, the two DFGψ motif inhibitors showed clear preferences for wild-type LRRK2, including the most potent compound, **ponatinib** (O'Hare et al., 2009), which has an *in vitro* IC$_{50}$ for wild-type LRRK2 of 31 nM but only 200 and 600 nM for G2019S and I2020T, respectively (Ray & Liu, 2012). Taken together, these observations suggest that Type II kinase inhibitors that target the DFGψ motif will not be suitable for patients with G2019S or I2020T *LRRK2* mutations, although there is no a priori reason why they may not be a valid therapeutic strategy for patients with mutations outside the LRRK2 kinase domain or for idiopathic PD. There is also no reason to doubt the potential of Type II kinase inhibitors that target other parts of the activation loop. In addition to the hinge region, Ray and Liu (2012) observed that a series of cysteine residues located near the ATP-binding site might be a fruitful site for developing covalent LRRK2 inhibitors.

10 | EVIDENCE OF NEUROPROTECTION PROVIDED BY LRRK2 INHIBITORS OR LRRK2 ANTISENSE OLIGONUCLEOTIDES IN PD-RELATED ANIMAL MODELS

Preclinical data—in particular, more physiologically relevant studies performed in animal models—may provide some pointers towards the results we might reasonably expect in patients. In this section, therefore, we briefly summarise experiments assessing the ability of LRRK2 kinase inhibitors and antisense oligonucleotides to promote cell survival in PD-relevant animal models.

Because LRRK2 transgenic animals do not have a robust neurological phenotype (Daniel & Moore, 2015; Seegobin et al., 2020), *in vivo* investigations into neuroprotection offered by LRRK2 kinase inhibitors have tended to use viral expression systems to transduce animal brains with high levels of a pathogenic protein. In studies using overexpression of the pathogenic G2019S LRRK2 variant, it seems

fairly clear that LRRK2 kinase inhibitors can effectively reduce the resulting neurodegeneration in both mice (Lee et al., 2010) and rats (Daher et al., 2015; Nguyen et al., 2020). These data are supported by observations of an equivalent effect of introducing an artificial kinase inactivating mutation into the overexpressed LRRK2 protein (Lee et al., 2010; Nguyen et al., 2020; Tsika et al., 2015). Of note, mutations that perturb LRRK2 GTPase function, by either blocking binding to GTP or elevating the rate of GTP hydrolysis, cause a similar reduction in neurodegeneration, thus supporting the use of LRRK2 GTPase inhibitors as an alternative strategy (Nguyen et al., 2020).

The above studies clearly demonstrate that LRRK2 kinase inhibition can be neuroprotective in vivo. However, there remains a question as to how accurately the neurodegeneration caused by viral overexpression of a pathogenic LRRK2 variant represents the normal dopaminergic cell death in PD, where LRRK2 is not overexpressed. So, what about other models of neurodegeneration? An alternative experimental system is viral transduction of α-synuclein. Unfortunately, results using these technologies are mixed. In the first study, Daher et al. (2015) found that PF-06447475 reduced α-synuclein-induced neurodegeneration in rats. By contrast, Andersen et al. (2018) were unable to show neuroprotection or rescue of motor symptoms using PFE-360, although they did report a reversal of an aberrant subthalamic firing pattern that the authors describe as resembling alterations seen in PD. However, in a follow-up report describing the effects of longer term PFE-360 treatment, the same group found no improvement in subthalamic nucleus firing, although a mild improvement in motor function was observed (Andersen et al., 2019). Taking their observations together, the authors conclude that their findings 'do not strongly support LRRK2 inhibition for the treatment of PD' (Andersen et al., 2019).

There are many potential reasons for the disagreement, such as the different viral systems used, the degree of α-synuclein expression achieved and the treatment times used. Although structurally very similar (Figure 2), the LRRK2 inhibitors used could also be a factor. It is also worth observing that even if the results were in complete agreement, they would still need to be treated with caution, because the rate at which α-synuclein accumulates in the brains of PD patients cannot be anything like as fast as that achieved by viral transduction.

With this in mind, recent observations made using the pesticide rotenone to induce dopaminergic neurodegeneration in mice may be insightful (Rocha et al., 2020). This study measured both rotenone-induced dopaminergic cell death and the preceding changes to endolysosomal and autophagic systems that mirror defects seen in the surviving neurons from patients with idiopathic PD (Rocha et al., 2020). Remarkably, the Type I LRRK2 kinase inhibitor PFE-360 reduced the effects of rotenone on all these measures. It must be stressed that these data are only directly transferable to humans who develop PD following acute rotenone exposure, but these results are nonetheless encouraging. Chronic exposure to pesticides including rotenone is an established environmental cause of increased PD risk and the endolysosomal and autophagic defects that were rescued are consistent with idiopathic PD. As such, these data point to LRRK2 inhibitors having use in idiopathic PD and *LRRK2* PD.

Despite the widespread use of the neurotoxins 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to induce dopaminergic damage in rodent PD models (Schober, 2004), we are aware of only one report of an attempt to rescue the phenotype of animals treated with these compounds using LRRK2 inhibition. This study used a low dose of MPTP that was not toxic in wild-type animals but induced significant impairments in transgenic mice that overexpress either wild-type LRRK2 or G2019S-LRRK2, with a greater effect seen in the G2019S animals (Arbez et al., 2020). Remarkably, treatment with LRRK2-IN-1 dramatically reduced dopaminergic cell death and also rescued motor deficits to a similar extent to L-DOPA (Arbez et al., 2020). There are clearly some caveats with these data, not least the choice of LRRK2 inhibitor—more selective compounds are available—but these data are nonetheless encouraging. Perhaps most informative is the fact that an ordinarily subtoxic dose of MPTP was used, which would have caused less dopaminergic damage than standard doses. Perhaps consistent with this, the rotenone study mentioned above used a dosing regimen optimised to cause the least amount of neurodegeneration required to guarantee a reproducible motor deficit (Cannon et al., 2009). As such, the studies of Rocha et al. and Arbez et al. most likely point to a situation where LRRK2 inhibitors may have great potential to maintain motor function in PD patients, but only if administered while there are sufficient dopaminergic neurons remaining.

Research into the therapeutic potential of LRRK2 antisense oligonucleotides in preclinical models is also thin on the ground, although the use of LRRK2 antisense oligonucleotides is to some extent informed by the preclinical work using LRRK2 inhibitors described above (and vice versa). The first study using LRRK2 antisense oligonucleotides is that of Zhao et al. (2017), who investigated the effect of intracerebral ventricular injection of Lrrk2 antisense oligonucleotides in mice injected with synthetic α-synuclein fibrils. Somewhat surprisingly, given that the two different antisense oligonucleotides used only achieved a ~50% reduction in Lrrk2 expression, both oligonucleotides markedly reduced α-synuclein aggregation and dopaminergic cell death and almost entirely rescued motor defects (Zhao et al., 2017). In the absence of independent replication, these observations must be treated with caution, but the idea that a modest knockdown of LRRK2 expression is sufficient to elicit a significant biological effect in the mouse brain is supported by the only other paper using antisense oligonucleotides targeting LRRK2 in animal models. In this report (Korecka et al., 2020), Korecka et al. (2019) used an antisense oligonucleotide that they have previously shown to perturb the normal splicing of human LRRK2 mRNA by causing exon 41 to be skipped causing no functional LRRK2 protein produced. When injected into the cerebral ventricles of transgenic mice expressing human wild-type or G2019S LRRK2, this antisense oligonucleotide elicited significant decreases in phosphorylation of the LRRK2 substrate Rab10 and also affected autophagic flux, despite causing exon 41 to be skipped in no more than 30% of transcripts (Korecka et al., 2020).

Taken together, these studies provide evidence that both strategies may prevent neurodegeneration and thus delay or halt the progression of PD. Furthermore, because modest effects on LRRK2 expression appear sufficient to elicit promising biological effects, the data suggest that LRRK2 antisense oligonucleotides may be the more effective strategy. In principle, this may suggest the existence of additional pathological mechanisms that are independent of LRRK2 kinase activity. However, the lack of publications and issues with reproducibility make these predictions extremely speculative. Perhaps more convincing is the suggestion that the degree of neurodegeneration present at time of treatment will be a decisive factor in determining whether inhibiting or knocking down LRRK2 can be effective. Where sufficient dopaminergic neurons remain, these strategies may maintain motor function; but if the disease is too far advanced, targeting LRRK2 may be to no avail.

11 | FINAL POINTS OF DISCUSSION

There are clearly a wide range of therapeutic strategies aiming to treat both LRRK2 and idiopathic PD by reducing the function of this enigmatic protein. These include both ATP-competitive and non-ATP-competitive kinase inhibitors, pharmacological inhibitors of LRRK2 GTPase activity and knockdown of LRRK2 mRNA. The kinase inhibitor DNL151 and the antisense RNA molecule BIIB094 are currently in clinical trials with further results eagerly awaited. There is therefore optimism within the field, as well as a plethora of fallback options should DNL151 and BIIB094 fail.

Nonetheless, there are other aspects of LRRK2 biology that may in future become viable therapeutic targets. As mentioned, LRRK2 dimers are believed to have greater kinase activity that LRRK2 monomers, and the cycling of LRRK2 between these states is has been suggested to play a key role in the normal regulation of this protein (Berwick et al., 2019). Indeed, work performed in zebrafish models indicates that the deletion of the C-terminal WD40 domain is sufficient to prevent dimerisation, thereby reducing autophosphorylation and reducing the neurotoxicity of LRRK2 (Jorgensen et al., 2009). Thus, disruption of LRRK2 dimerisation can be expected to, in turn, impair LRRK2 kinase activity, creating a mechanism that could be harnessed for the development of selective inhibitors of LRRK2 kinase. Such approaches have already been used successfully to identify compounds that prevent dimerisation of other proteins, for example, ST2825, a heptapeptide that blocks dimerisation of myeloid differentiation primary response 88 (MyD88) (Loiarro et al., 2007). However, this may be a trickier task for LRRK2, because LRRK2 dimerisation appears to have several intramolecular interactions involved, with the WD40 and COR domains implicated in particular (Greggio et al., 2008; Guaitoli et al., 2016; Jorgensen et al., 2009). As such, efficient disruption of LRRK2 dimers may require a cocktail of molecules, each targeting a different protein interaction interface.

On a similar note, in addition to roles as a kinase and GTPase, LRRK2 has long been suggested to have a function as a scaffold protein, interacting with numerous other proteins to form dynamic signalling complexes at specific cellular localisations (Berwick et al., 2019; Lewis & Manzoni, 2012). As such, disruption of protein–protein interactions required for the pathogenic effects of LRRK2 may be fertile

ground for drug discovery. Nonetheless, although many hundreds of LRRK2 interaction partners have been reported, when the expression levels of these proteins have been modulated in cellular models, very few appear to exert a robust influence on measures relevant to pathogenicity of PD-causing LRRK2 variants. An important exception, however, may be 14-3-3 proteins. 14-3-3 proteins bind LRRK2 in a manner dependent on the phosphorylation of LRRK2. Phosphorylation sites to which 14-3-3 proteins associate include Ser910 and Ser935, in the LRR domain (Nichols et al., 2010), Ser1444 in the Roc domain (Muda et al., 2014) and Thr2524 at the very C-terminus (Manschwetus et al., 2020). Although the interplay between these 14-3-3 binding events is complex and not yet fully understood, the functional consequences of LRRK2 interacting with these proteins are clear. Most notably, binding of 14-3-3 proteins to LRR domain residues stabilises LRRK2 in a monomeric and cytoplasmic form that can be assumed to have decreased kinase activity (depicted in Figure 3). Consistently, pathogenic LRRK2 mutations have been reported to disrupt LRRK2–14-3-3 interactions (Lavalley et al., 2016; Nichols et al., 2010). Based on these and other observations, Soliman et al. (2020) have speculated that small molecule stabilisers of 14-3-3 protein–protein interactions, some of which have recently been described in the literature (Ballone et al., 2018), could be used to produce molecules that stabilise LRRK2-14-3-3 complexes. We await the next instalment of this story.

Finally, it is important to mention the impact two very recent breakthroughs in LRRK2 structural biology that have already provided data relevant to LRRK2 inhibitor development. In the first work, Watanabe et al. (2020) used a combination of cryo-electron tomography and integrative modelling to produce a 14-Å resolution in situ model for the entire LRRK2 protein bound to microtubules (PDB:6XR4). This model recapitulated the filamentous LRRK2 phenotype observed by others, where LRRK2 filament formation is enhanced by both most LRRK2 pathogenic mutants and conventional LRRK2 kinase inhibitors (Blanca Ramirez et al., 2017; Schmidt et al., 2019), and found that LRRK2 filaments form via homotypic interactions between COR and WD40 domains (Watanabe et al., 2020). Fascinatingly, Deniston et al. (2020) integrated the 6XR4 structure with their own high-resolution (3.5 Å) cryo-electron microscopy structure of amino acids 1327–2527 of LRRK2, containing Roc, COR, kinase and WD40 domains, to reveal further insights. Most notably, this group confirmed the prediction made by Watanabe et al. that LRRK2 would be more likely to form filaments when in a closed kinase confirmation. This provides a rationale for why conventional LRRK2 kinase inhibitors (i.e. ATP-competitive or Type I), which lock LRRK2 in a closed kinase confirmation, promote filament formation. In support of this, the Type II kinase inhibitors ponatinib (O'Hare et al., 2009) and **GZD-824** (Ren et al., 2013), which would be expected to lock LRRK2 in an open kinase confirmation, were found to prevent filament formation (Deniston et al., 2020). Interestingly, this latter observation mirrors those made for the LRRK2 GTPase inhibitors, compounds 68 and 60 (Blanca Ramirez et al., 2017).

In conclusion, therefore, with so many routes to achieving LRRK2 inhibition being taken, the prospects for developing new PD medications based around targeting LRRK2 are good. Where lead molecules and different strategies fall by the wayside, there are plenty of alternatives to take over. Nonetheless, with two molecules in clinical trials, the extent to which these treatments bring clinical benefits will undoubtedly be the hot topic over the next few years.

11.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to PHARMACOLOGY http://www.guidetopharmacology.org and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander et al., 2019).

REFERENCES
Agalliu, I., San Luciano, M., Mirelman, A., Giladi, N., Waro, B., Aasly, J., Inzelberg, R., Hassin-Baer, S., Friedman, E., Ruiz-Martinez, J., & Marti-Masso, J. F. (2015). Higher frequency of certain cancers in *LRRK2* G2019S mutation carriers with Parkinson disease: A pooled analysis. *JAMA Neurology*, *72*, 58–65.

Alcalay, R. N., Hsieh, F., Tengstrand, E., Padmanabhan, S., Baptista, M., Kehoe, C., Narayan, S., Boehme, A. K., & Merchant, K. (2020). Higher urine bis(monoacylglycerol)phosphate levels in LRRK2 G2019S mutation carriers: Implications for therapeutic development. *Movement Disorders*, *35*, 134–141. https://doi.org/10.1002/mds.27818

AlDakheel, A., Kalia, L. V., & Lang, A. E. (2014). Pathogenesis-targeted, disease-modifying therapies in Parkinson disease. *Neurotherapeutics*, *11*, 6–23.

Alexander, S. P. H., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Pawson, A. J., Sharman, J. L., & Southan, C. (2019). The Concise Guide to PHARMACOLOGY 2019/20: Introduction and other protein targets. *British Journal of Pharmacology*, *176*, S1–S20.

Allegra, R., Tunesi, S., Cilia, R., Pezzoli, G., & Goldwurm, S. (2014). *LRRK2*-G2019S mutation is not associated with an increased cancer risk: A kin-cohort study. *Movement Disorders*, *29*, 1325–1326.

Anand, V. S., Reichling, L. J., Lipinski, K., Stochaj, W., Duan, W., Kelleher, K., Pungaliya, P., Brown, E. L., Reinhart, P. H., Somberg, R., &

Hirst, W. (2009). Investigation of leucine-rich repeat kinase 2: Enzymological properties and novel assays. *The FEBS Journal*, 276, 466–478.

Andersen, M. A., Christensen, K. V., Badolo, L., Smith, G. P., Jeggo, R., Jensen, P. H., Andersen, K. J., & Sotty, F. (2018). Parkinson's disease-like burst firing activity in subthalamic nucleus induced by AAV-α-synuclein is normalized by LRRK2 modulation. *Neurobiology of Disease*, 116, 13–27.

Andersen, M. A., Sotty, F., Jensen, P. H., Badolo, L., Jeggo, R., Smith, G. P., & Christensen, K. V. (2019). Long-term exposure to PFE-360 in the AAV-α-synuclein rat model: Findings and implications. *ENeuro*, 6, ENEURO.0453-18.2019. https://doi.org/10.1523/ENEURO.0453-18.2019

Arbez, N., He, X., Huang, Y., Ren, M., Liang, Y., Nucifora, F. C., Wang, X., Pei, Z., Tessarolo, L., Smith, W. W., & Ross, C. A. (2020). G2019S-LRRK2 mutation enhances MPTP-linked Parkinsonism in mice. *Human Molecular Genetics*, 29, 580–590. https://doi.org/10.1093/hmg/ddz271

Armstrong, M. J., & Okun, M. S. (2020). Diagnosis and treatment of Parkinson disease: A review. *Journal of American Medical Association*, 323, 548–560. https://doi.org/10.1001/jama.2019.22360

Ballone, A., Centorrino, F., & Ottmann, C. (2018). 14-3-3: A case study in PPI modulation. *Molecules*, 23, 1386. https://doi.org/10.3390/molecules23061386

Baptista, M. A., Dave, K. D., Frasier, M. A., Sherer, T. B., Greeley, M., Beck, M. J., Varsho, J. S., Parker, G. A., Moore, C., Churchill, M. J., & Meshul, C. K. (2013). Loss of leucine-rich repeat kinase 2 (LRRK2) in rats leads to progressive abnormal phenotypes in peripheral organs. *PLoS ONE*, 8, e80705. https://doi.org/10.1371/journal.pone.0080705

Baptista, M. A. S., Merchant, K., Barrett, T., Bhargava, S., Bryce, D. K., Ellis, J. M., Estrada, A. A., Fell, M. J., Fiske, B. K., Fuji, R. N., & Galatsis, P. (2020). LRRK2 inhibitors induce reversible changes in non-human primate lungs without measurable pulmonary deficits. *Science Translational Medicine*, 12, eaav0820. https://doi.org/10.1126/scitranslmed.aav0820

Barrett, J. C., Hansoul, S., Nicolae, D. L., Cho, J. H., Duerr, R. H., Rioux, J. D., Brant, S. R., Silverberg, M. S., Taylor, K. D., Barmada, M. M., & Bitton, A. (2008). Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nature Genetics*, 40, 955–962. https://doi.org/10.1038/ng.175

Berger, Z., Smith, K. A., & Lavoie, M. J. (2010). Membrane localization of LRRK2 is associated with increased formation of the highly active LRRK2 dimer and changes in its phosphorylation. *Biochemistry*, 49, 5511–5523. https://doi.org/10.1021/bi100157u

Berwick, D. C., & Harvey, K. (2012). LRRK2 functions as a Wnt signaling scaffold, bridging cytosolic proteins and membrane-localized LRP6. *Human Molecular Genetics*, 21, 4966–4979.

Berwick, D. C., Heaton, G. R., Azeggagh, S., & Harvey, K. (2019). LRRK2 biology from structure to dysfunction: Research progresses, but the themes remain the same. *Molecular Neurodegeneration*, 14, 49. https://doi.org/10.1186/s13024-019-0344-2

Berwick, D. C., Javaheri, B., Wetzel, A., Hopkinson, M., Nixon-Abell, J., Grannò, S., Pitsillides, A. A., & Harvey, K. (2017). Pathogenic *LRRK2* variants are gain-of-function mutations that enhance LRRK2-mediated repression of β-catenin signaling. *Molecular Neurodegeneration*, 12, 9–9. https://doi.org/10.1186/s13024-017-0153-4

Biogen. (2021). Pipeline. Biogen. https://www.biogen.com/en_us/pipeline.html

Blanca Ramirez, M., Lara Ordonez, A. J., Fdez, E., Madero-Perez, J., Gonnelli, A., Drouyer, M., Chartier-Harlin, M. C., Taymans, J. M., Bubacco, L., Greggio, E., & Hilfiker, S. (2017). GTP binding regulates cellular localization of Parkinson's disease-associated LRRK2. *Human Molecular Genetics*, 26, 2747–2767.

Blauwendraat, C., Reed, X., Kia, D. A., Gan-Or, Z., Lesage, S., Pihlstrøm, L., Guerreiro, R., Gibbs, J. R., Sabir, M., Ahmed, S., & Ding, J. (2018). Frequency of loss of function variants in *LRRK2* in Parkinson disease. *JAMA Neurology*, 75, 1416–1422.

Bright, J. M., Carlisle, H. J., Toda, A. M. A., Murphy, M., Molitor, T. P., Wren, P., Andruska, K. M., Liu, E., & Barlow, C. (2021). Differential inhibition of LRRK2 in Parkinson's disease patient blood by a G2019S selective LRRK2 inhibitor. *Movement Disorders*. https://doi.org/10.1002/mds.28490

Cannon, J. R., Tapias, V. M., Na, H. M., Honick, A. S., Drolet, R. E., & Greenamyre, J. T. (2009). A highly reproducible rotenone model of Parkinson's disease. *Neurobiology of Disease*, 34, 279–290.

Carlsson, A., Lindqvist, M., & Magnusson, T. (1957). 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. *Nature*, 180, 1200–1200. https://doi.org/10.1038/1801200a0

Cerevel. (2021). Striving to develop new therapies for neuroscience diseases. Cerevel. https://www.cerevel.com/rd/#pipeline

Chaudhuri, K. R., Healy, D. G., Schapira, A. H. V., & National Institute for Clinical Excellence. (2006). Non-motor symptoms of Parkinson's disease: Diagnosis and management. *Lancet Neurology*, 5, 235–245.

Chaudhuri, K. R., & Schapira, A. H. (2009). Non-motor symptoms of Parkinson's disease: Dopaminergic pathophysiology and treatment. *Lancet Neurology*, 8, 464–474. https://doi.org/10.1016/S1474-4422(09)70068-7

Chen, H., Chan, B. K., Drummond, J., Estrada, A. A., Gunzner-Toste, J., Liu, X., Moffat, J., Shore, D., Sweeney, Z. K., & Tran, T. (2012). Discovery of selective LRRK2 inhibitors guided by computational analysis and molecular modeling. *Journal of Medicinal Chemistry*, 55, 5536–5545.

Chery, J. (2016). RNA therapeutics: RNAi and antisense mechanisms and clinical applications. *Postdoc Journal: A Journal of Postdoctoral Research and Postdoctoral Affairs*, 4, 35–50.

Chiriboga, C. A., Swoboda, K. J., Darras, B. T., Iannaccone, S. T., Montes, J., Vivo, D. C. D., De Vivo, D. C., Norris, D. A., Bennett, C. F., & Bishop, K. M. (2016). Results from a phase 1 study of nusinersen (ISIS-SMNRx) in children with spinal muscular atrophy. *Neurology*, 86, 890–897. https://doi.org/10.1212/WNL.0000000000002445

Choi, H. G., Zhang, J., Deng, X., Hatcher, J. M., Patricelli, M. P., Zhao, Z., Alessi, D. R., & Gray, N. S. (2012). Brain penetrant LRRK2 inhibitor. *ACS Medicinal Chemistry Letters*, 3, 658–662.

Christine, C. W., Auinger, P., Joslin, A., Yelpaala, Y., & Green, R. (2018). Vitamin B12 and homocysteine levels predict different outcomes in early Parkinson's disease. *Movement Disorders*, 33, 762–770.

Cookson, M. R. (2015). LRRK2 pathways leading to neurodegeneration. *Current Neurology and Neuroscience Reports*, 15, 42. https://doi.org/10.1007/s11910-015-0564-y

Cotzias, G. C., Van Woert, M. H., & Schiffer, L. M. (1967). *Aromatic amino acids and modification of parkinsonism*. Massachusetts Medical Society.

Covy, J. P., & Giasson, B. I. (2009). Identification of compounds that inhibit the kinase activity of leucine-rich repeat kinase 2. *Biochemical and Biophysical Research Communications*, 378, 473–477.

Daher, J. P., Abdelmotilib, H. A., Hu, X., Volpicelli-Daley, L. A., Moehle, M. S., Fraser, K. B., Needle, E., Chen, Y., Steyn, S. J., Galatsis, P., & Hirst, W. D. (2015). Leucine-rich repeat kinase 2 (LRRK2) pharmacological inhibition abates α-synuclein gene-induced neurodegeneration. *The Journal of Biological Chemistry*, 290, 19433–19444. https://doi.org/10.1074/jbc.M115.660001

Daniel, G., & Moore, D. J. (2015). Modeling LRRK2 pathobiology in Parkinson's disease: From yeast to rodents. *Current Topics in Behavioral Neurosciences*, 22, 331–368.

Denali Therapeutics. (2018). Denali Therapeutics announces positive clinical results from LRRK2 inhibitor program for Parkinson's disease. Denali. https://www.globenewswire.com/news-release/2018/08/01/1545852/0/en/Denali-Therapeutics-Announces-Positive-Clinical-Results-From-LRRK2-Inhibitor-Program-for-Parkinson-s-Disease.html

Denali Therapeutics. (2020). Denali Therapeutics announces decision to advance DNL151 into late stage clinical studies in Parkinson's patients. Denali.

Deng, X., Dzamko, N., Prescott, A., Davies, P., Liu, Q., Yang, Q., Lee, J. D., Patricelli, M. P., Nomanbhoy, T. K., Alessi, D. R., & Gray, N. S. (2011). Characterization of a selective inhibitor of the Parkinson's disease kinase LRRK2. *Nature Chemical Biology*, *7*, 203–205. https://doi.org/10.1038/nchembio.538

Deniston, C., Salogiannis, J., Mathea, S., Snead, D., Lahiri, I., Matyszewski, M., Donosa, O., Watanabe, R., Böhning, J., Shiau, A. K., & Knapp, S. (2020). Structure of LRRK2 in Parkinson's disease and model for microtubule interaction. *Nature*, *588*, 344–349. https://doi.org/10.1038/s41586-020-2673-2

Di Maio, R., Hoffman, E. K., Rocha, E. M., Keeney, M. T., Sanders, L. H., De Miranda, B. R., Zharikov, A., Van Laar, A., Stepan, A. F., Lanz, T. A., & Kofler, J. K. (2018). LRRK2 activation in idiopathic Parkinson's disease (PLA). *Science Translational Medicine*, *10*, eaar5429.

Ding, X., & Ren, F. (2020). Leucine-rich repeat kinase 2 inhibitors: A patent review (2014–present). *Expert Opinion on Therapeutic Patents*, *30*, 275–286.

Do, C. B., Tung, J. Y., Dorfman, E., Kiefer, A. K., Drabant, E. M., Francke, U., Mountain, J. L., Goldman, S. M., Tanner, C. M., Langston, J. W., Wojcicki, A., & Eriksson, N. (2011). Web-based genome-wide association study identifies two novel loci and a substantial genetic component for Parkinson's disease. *PLoS Genetics*, *7*, e1002141. https://doi.org/10.1371/journal.pgen.1002141

Domingos, S., Duarte, T., Saraiva, L., Guedes, R. C., & Moreira, R. (2019). Targeting leucine-rich repeat kinase 2 (LRRK2) for the treatment of Parkinson's disease. *Future Medicinal Chemistry*, *11*, 1953–1977. https://doi.org/10.4155/fmc-2018-0484

Duty, S., & Jenner, P. (2011). Animal models of Parkinson's disease: A source of novel treatments and clues to the cause of the disease. *British Journal of Pharmacology*, *164*, 1357–1391. https://doi.org/10.1111/j.1476-5381.2011.01426.x

Elbaz, A., Bower, J. H., Maraganore, D. M., McDonnell, S. K., Peterson, B. J., Ahlskog, J. E., Schaid, D. J., & Rocca, W. A. (2002). Risk tables for parkinsonism and Parkinson's disease. *Journal of Clinical Epidemiology*, *55*, 25–31.

Estrada, A. A., Chan, B. K., Baker-Glenn, C., Beresford, A., Burdick, D. J., Chambers, M., Chen, H., Dominguez, S. L., Dotson, J., Drummond, J., & Flagella, M. (2014). Discovery of highly potent, selective, and brain-penetrant aminopyrazole leucine-rich repeat kinase 2 (LRRK2) small molecule inhibitors. *Journal of Medicinal Chemistry*, *57*, 921–936.

Estrada, A. A., Liu, X., Baker-Glenn, C., Beresford, A., Burdick, D. J., Chambers, M., Chen, H., Dominguez, S. L., Dotson, J., Drummond, J., & Flagella, M. (2012). Discovery of highly potent, selective, and brain-penetrable leucine-rich repeat kinase 2 (LRRK2) small molecule inhibitors. *Journal of Medicinal Chemistry*, *55*, 9416–9433.

Eyers, P. A. (2018). Back to the future: New target-validated Rab antibodies for evaluating LRRK2 signalling in cell biology and Parkinson's disease. *The Biochemical Journal*, *475*, 185–189. https://doi.org/10.1042/BCJ20170870

Farrer, M. J. (2006). Genetics of Parkinson disease: Paradigm shifts and future prospects. *Nature Reviews. Genetics*, *7*, 306–318.

Fell, M. J., Mirescu, C., Basu, K., Cheewatrakoolpong, B., DeMong, D. E., Ellis, J. M., Hyde, L. A., Lin, Y., Markgraf, C. G., Mei, H., Miller, M., Poulet, F. M., Scott, J. D., Smith, M. D., Yin, Z., Zhou, X., Parker, E. M., Kennedy, M. E., & Morrow, J. A. (2015). MLi-2, a potent, selective, and centrally active compound for exploring the therapeutic potential and safety of LRRK2 kinase inhibition. *The Journal of Pharmacology and Experimental Therapeutics*, *355*, 397–409. https://doi.org/10.1124/jpet.115.227587

Franke, A., McGovern, D. P. B., Barrett, J. C., Wang, K., Radford-Smith, G. L., Ahmad, T., Lees, C. W., Balschun, T., Lee, J., Roberts, R., Anderson, C. A., Bis, J. C., Bumpstead, S., Ellinghaus, D., Festen, E. M., Georges, M., Green, T., Haritunians, T., Jostins, L., … Parkes, M. (2010). Meta-analysis increases to 71 the tally of confirmed Crohn's disease susceptibility loci. *Nature Genetics*, *42*, 1118–1125. https://doi.org/10.1038/ng.717

Fuji, R. N., Flagella, M., Baca, M., Baptista, M. A., Brodbeck, J., Chan, B. K., Fiske, B. K., Honigberg, L., Jubb, A. M., Katavolos, P., & Lee, D. W. (2015). Effect of selective LRRK2 kinase inhibition on nonhuman primate lung. *Science Translational Medicine*, *7*, 273ra15.

Gaig, C., Marti, M. J., Ezquerra, M., Rey, M. J., Cardozo, A., & Tolosa, E. (2007). G2019S *LRRK2* mutation causing Parkinson's disease without Lewy bodies. *Journal of Neurology, Neurosurgery, and Psychiatry*, *78*, 626–628.

Gan-Or, Z., Dion, P. A., & Rouleau, G. A. (2015). Genetic perspective on the role of the autophagy-lysosome pathway in Parkinson disease. *Autophagy*, *11*, 1443–1457.

Garofalo, A. W., Bright, J., De Lombaert, S., Toda, A. M. A., Zobel, K., Andreotti, D., Beato, C., Bernardi, S., Budassi, F., Caberlotto, L., & Gao, P. (2020). Selective inhibitors of G2019S-LRRK2 kinase activity. *Journal of Medicinal Chemistry*, *63*, 14821–14839. https://doi.org/10.1021/acs.jmedchem.0c01243

Giaime, E., Tong, Y., Wagner, L. K., Yuan, Y., Huang, G., & Shen, J. (2017). Age-dependent dopaminergic neurodegeneration and impairment of the autophagy-lysosomal pathway in LRRK-deficient mice. *Neuron*, *96*, 796–807. e6

Gotthardt, K., Weyand, M., Kortholt, A., Van Haastert, P. J., & Wittinghofer, A. (2008). Structure of the Roc–COR domain tandem of *C. tepidum*, a prokaryotic homologue of the human LRRK2 Parkinson kinase. *The EMBO Journal*, *27*, 2239–2249.

Greggio, E., & Cookson, M. R. (2009). Leucine-rich repeat kinase 2 mutations and Parkinson's disease: Three questions. *ASN Neuro*, *1*, AN20090007. https://doi.org/10.1042/AN20090007

Greggio, E., Zambrano, I., Kaganovich, A., Beilina, A., Taymans, J. M., Daniels, V., Lewis, P., Jain, S., Ding, J., Syed, A., & Thomas, K. J. (2008). The Parkinson disease-associated leucine-rich repeat kinase 2 (LRRK2) is a dimer that undergoes intramolecular autophosphorylation. *The Journal of Biological Chemistry*, *283*, 16906–16914.

Greshock, T. J., Sanders, J. M., Drolet, R. E., Rajapakse, H. A., Chang, R. K., Kim, B., Rada, V. L., Tiscia, H. E., Su, H., Lai, M. T., Sur, S. M., Sanchez, R. I., Bilodeau, M. T., Renger, J. J., Kern, J. T., & McCauley, J. A. (2016). Potent, selective and orally bioavailable leucine-rich repeat kinase 2 (LRRK2) inhibitors. *Bioorganic & Medicinal Chemistry Letters*, *26*, 2631–2635. https://doi.org/10.1016/j.bmcl.2016.04.021

Guaitoli, G., Raimondi, F., Gilsbach, B. K., Gomez-Llorente, Y., Deyaert, E., Renzi, F., Li, X., Schaffner, A., Jagtap, P. K., Boldt, K., & von Zweydorf, F. (2016). Structural model of the dimeric Parkinson's protein LRRK2 reveals a compact architecture involving distant interdomain contacts. *Proceedings of the National Academy of Sciences*, *113*, E4357–E4366. https://doi.org/10.1073/pnas.1523708113

Hartlova, A., Herbst, S., Peltier, J., Rodgers, A., Bilkei-Gorzo, O., Fearns, A., Dill, B. D., Lee, H., Flynn, R., Cowley, S. A., & Davies, P. (2018). LRRK2 is a negative regulator of *Mycobacterium tuberculosis* phagosome maturation in macrophages. *The EMBO Journal*, *37*, e98694.

Hatcher, J. M., Choi, H. G., Alessi, D. R., & Gray, N. S. (2017). Small-molecule inhibitors of LRRK2. In *Leucine-rich repeat kinase 2 (LRRK2)* (pp. 241–264).

Henderson, J. L., Kormos, B. L., Hayward, M. M., Coffman, K. J., Jasti, J., Kurumbail, R. G., Wager, T. T., Verhoest, P. R., Noell, G. S., Chen, Y., & Needle, E. (2015). Discovery and preclinical profiling of 3-[4-(morpholin-4-yl)-7*H*-pyrrolo[2,3-d]pyrimidin-5-yl]benzonitrile (PF-06447475), a highly potent, selective, brain penetrant, and in vivo active LRRK2 kinase inhibitor. *Journal of Medicinal Chemistry*, *58*, 419–432.

Ho, D. H., Je, A. R., Lee, H., Son, I., Kweon, H.-S., Kim, H.-G., & Seol, W. (2018). LRRK2 kinase activity induces mitochondrial fission in

microglia via Drp1 and modulates neuroinflammation. *Experimental Neurobiology*, 27, 171–171, 180. https://doi.org/10.5607/en.2018.27.3.171

Hui, K. Y., Fernandez-Hernandez, H., Hu, J., Schaffner, A., Pankratz, N., Hsu, N. Y., Chuang, L. S., Carmi, S., Villaverde, N., Li, X., & Rivas, M. (2018). Functional variants in the *LRRK2* gene confer shared effects on risk for Crohn's disease and Parkinson's disease. *Science Translational Medicine*, 10, eaai7795. https://doi.org/10.1126/scitranslmed.aai7795

Inzelberg, R., Cohen, O. S., Aharon-Peretz, J., Schlesinger, I., Gershoni-Baruch, R., Djaldetti, R., Nitsan, Z., Ephraty, L., Tunkel, O., Kozlova, E., & Inzelberg, L. (2012). The *LRRK2* G2019S mutation is associated with Parkinson disease and concomitant non-skin cancers. *Neurology*, 78, 781–786.

Iqbal, K., Liu, F., Gong, C.-X., & Grundke-Iqbal, I. (2010). Tau in Alzheimer disease and related tauopathies. *Current Alzheimer Research*, 7, 656–664.

Jankovic, J. (2008). Parkinson's disease: Clinical features and diagnosis. *Journal of Neurology, Neurosurgery, and Psychiatry*, 79, 368–376. https://doi.org/10.1136/jnnp.2007.131045

Jorgensen, N. D., Peng, Y., Ho, C. C., Rideout, H. J., Petrey, D., Liu, P., & Dauer, W. T. (2009). The WD40 domain is required for LRRK2 neurotoxicity. *PLoS ONE*, 4, e8463.

Kalia, L. V., Lang, A. E., Hazrati, L. N., Fujioka, S., Wszolek, Z. K., Dickson, D. W., Ross, O. A., Van Deerlin, V. M., Trojanowski, J. Q., Hurtig, H. I., & Alcalay, R. N. (2015). Clinical correlations with Lewy body pathology in LRRK2-related Parkinson disease. *JAMA Neurology*, 72, 100–105.

Karaman, M. W., Herrgard, S., Treiber, D. K., Gallant, P., Atteridge, C. E., Campbell, B. T., Chan, K. W., Ciceri, P., Davis, M. I., Edeen, P. T., Faraoni, R., Floyd, M., Hunt, J. P., Lockhart, D. J., Milanov, Z. V., Morrison, M. J., Pallares, G., Patel, H. K., Pritchard, S., ... Zarrinkar, P. P. (2008). A quantitative analysis of kinase inhibitor selectivity. *Nature Biotechnology*, 26, 127–132. https://doi.org/10.1038/nbt1358

Karuppagounder, S. S., Brahmachari, S., Lee, Y., Dawson, V. L., Dawson, T. M., & Ko, H. S. (2014). The c-Abl inhibitor, nilotinib, protects dopaminergic neurons in a preclinical animal model of Parkinson's disease. *Scientific Reports*, 4, 4874. https://doi.org/10.1038/srep04874

Kelly, K., & West, A. B. (2020). Pharmacodynamic biomarkers for emerging LRRK2 therapeutics. *Frontiers in Neuroscience*, 14, 807. https://doi.org/10.3389/fnins.2020.00807

Kluss, J. H., Mamais, A., & Cookson, M. R. (2019). LRRK2 links genetic and sporadic Parkinson's disease. *Biochemical Society Transactions*, 47, 651–661. https://doi.org/10.1042/BST20180462

Kolb, S. J., & Kissel, J. T. (2015). Spinal muscular atrophy. *Neurologic Clinics*, 33, 831–846.

Korecka, J. A., Thomas, R., Christensen, D. P., Hinrich, A. J., Ferrari, E. J., Levy, S. A., Hastings, M. L., Hallett, P. J., & Isacson, O. (2019). Mitochondrial clearance and maturation of autophagosomes are compromised in LRRK2 G2019S familial Parkinson's disease patient fibroblasts. *Human Molecular Genetics*, 28, 3232–3243.

Korecka, J. A., Thomas, R., Hinrich, A. J., Moskites, A. M., Macbain, Z. K., Hallett, P. J., Isacson, O., & Hastings, M. (2020). Splice-switching antisense oligonucleotides reduce LRRK2 kinase activity in human LRRK2 transgenic mice. *Molecular Therapy--Nucleic Acids*, 21, 623–635.

Lavalley, N. J., Slone, S. R., Ding, H., West, A. B., & Yacoubian, T. A. (2016). 14-3-3 proteins regulate mutant LRRK2 kinase activity and neurite shortening. *Human Molecular Genetics*, 25, 109–122.

Lebovitz, C., Wretham, N., Osooly, M., Milne, K., Dash, T., Thornton, S., Tessier-Cloutier, B., Sathiyaseelan, P., Bortnik, S., Go, N. E., & Halvorsen, E. (2021). Loss of Parkinson's susceptibility gene LRRK2 promotes carcinogen-induced lung tumorigenesis. *Scientific Reports*, 11, 2097.

Lebovitz, C. B., Robertson, A. G., Goya, R., Jones, S. J., Morin, R. D., Marra, M. A., & Gorski, S. M. (2015). Cross-cancer profiling of molecular alterations within the human autophagy interaction network. *Autophagy*, 11, 1668–1687.

Lee, B. D., Shin, J. H., VanKampen, J., Petrucelli, L., West, A. B., Ko, H. S., Lee, Y. I., Maguire-Zeiss, K. A., Bowers, W. J., Federoff, H. J., & Dawson, V. L. (2010). Inhibitors of leucine-rich repeat kinase-2 protect against models of Parkinson's disease. *Nature Medicine*, 16, 998–1000.

Lewis, P. A., & Manzoni, C. (2012). LRRK2 and human disease: A complicated question or a question of complexes? *Science Signaling*, 5, pe2. https://doi.org/10.1126/scisignal.2002680

Li, T., He, X., Thomas, J. M., Yang, D., Zhong, S., Xue, F., & Smith, W. W. (2015). A novel GTP-binding inhibitor, FX2149, attenuates LRRK2 toxicity in Parkinson's disease models. *PLoS ONE*, 10, 1–15.

Li, T., Yang, D., Zhong, S., Thomas, J. M., Xue, F., Liu, J., Kong, L., Voulalas, P., Hassan, H. E., Park, J. S., & MacKerell, A. D. Jr. (2014). Novel LRRK2 GTP-binding inhibitors reduced degeneration in Parkinson's disease cell and mouse models. *Human Molecular Genetics*, 23, 6212–6222.

Lill, C. M., Roehr, J. T., McQueen, M. B., Kavvoura, F. K., Bagade, S., Schjeide, B. M., Schjeide, L. M., Meissner, E., Zauft, U., Allen, N. C., & Liu, T. (2012). Comprehensive research synopsis and systematic meta-analyses in Parkinson's disease genetics: The PDGene database. *PLoS Genetics*, 8, e1002548. https://doi.org/10.1371/journal.pgen.1002548

Ling, H., Kara, E., Bandopadhyay, R., Hardy, J., Holton, J., Xiromerisiou, G., Lees, A., Houlden, H., & Revesz, T. (2013). TDP-43 pathology in a patient carrying G2019S *LRRK2* mutation and a novel p.Q124E *MAPT*. *Neurobiology of Aging*, 34, 2889. e5–9

Liu, M., Bender, S. A., Cuny, G. D., Sherman, W., Glicksman, M., & Ray, S. S. (2013). Type II kinase inhibitors show an unexpected inhibition mode against Parkinson's disease-linked LRRK2 mutant G2019S. *Biochemistry*, 52, 1725–1736.

Liu, Z., Bryant, N., Kumaran, R., Beilina, A., Abeliovich, A., Cookson, M. R., & West, A. B. (2018). LRRK2 phosphorylates membrane-bound Rabs and is activated by GTP-bound Rab7L1 to promote recruitment to the trans-Golgi network. *Human Molecular Genetics*, 27, 385–395. https://doi.org/10.1093/hmg/ddx410

Loiarro, M., Capolunghi, F., Fantò, N., Gallo, G., Campo, S., Arseni, B., Carsetti, R., Carminati, P., de Santis, R., Ruggiero, V., & Sette, C. (2007). Pivotal advance: Inhibition of MyD88 dimerization and recruitment of IRAK1 and IRAK4 by a novel peptidomimetic compound. *Journal of Leukocyte Biology*, 82, 801–810. https://doi.org/10.1189/jlb.1206746

Lu, X., Smaill, J. B., & Ding, K. (2020). New promise and opportunities for allosteric kinase inhibitors. *Angewandte Chemie (International Ed. in English)*, 59, 13764–13776. https://doi.org/10.1002/anie.201914525

Maegawa, G. H. B., Tropak, M. B., Buttner, J. D., Rigat, B. A., Fuller, M., Pandit, D., Tang, L., Kornhaber, G. J., Hamuro, Y., Clarke, J. T., & Mahuran, D. J. (2009). Identification and characterization of ambroxol as an enzyme enhancement agent for Gaucher disease*. *The Journal of Biological Chemistry*, 284, 23502–23516.

Manschwetus, J. T., Wallbott, M., Fachinger, A., Obergruber, C., Pautz, S., Bertinetti, D., Schmidt, S. H., & Herberg, F. W. (2020). Binding of the human 14-3-3 isoforms to distinct sites in the leucine-rich repeat kinase 2. *Frontiers in Neuroscience*, 14, 302. https://doi.org/10.3389/fnins.2020.00302

Migdalska-Richards, A., Ko, W. K. D., Li, Q., Bezard, E., & Schapira, A. H. V. (2017). Oral ambroxol increases brain glucocerebrosidase activity in a nonhuman primate. *Synapse*, 71, e21967.

Muda, K., Bertinetti, D., Gesellchen, F., Hermann, J. S., von Zweydorf, F., Geerlof, A., Jacob, A., Ueffing, M., Gloeckner, C. J., & Herberg, F. W. (2014). Parkinson-related LRRK2 mutation R1441C/G/H impairs PKA phosphorylation of LRRK2 and disrupts its interaction with 14-3-3. *Proceedings of the National Academy of Sciences*, 111, E34–E43.

Nalls, M. A., Pankratz, N., Lill, C. M., Do, C. B., Hernandez, D. G., Saad, M., DeStefano, Kara, E., Bras, J., Sharma, M., & Schulte, C. (2014).

Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. *Nature Genetics*, *46*, 989–993. https://doi.org/10.1038/ng.3043

Ness, D., Ren, Z., Gardai, S., Sharpnack, D., Johnson, V. J., Brennan, R. J., Brigham, E. F., & Olaharski, A. J. (2013). Leucine-rich repeat kinase 2 (LRRK2)-deficient rats exhibit renal tubule injury and perturbations in metabolic and immunological homeostasis. *PLoS ONE*, *8*, e66164.

Nguyen, A. P. T., Tsika, E., Kelly, K., Levine, N., Chen, X., West, A. B., Boularand, S., Barneoud, P., & Moore, D. J. (2020). Dopaminergic neurodegeneration induced by Parkinson's disease-linked G2019S LRRK2 is dependent on kinase and GTPase activity. *Proceedings of the National Academy of Sciences of the United States of America*, *117*, 17296–17307.

Nichols, R. J., Dzamko, N., Hutti, J. E., Cantley, L. C., Deak, M., Moran, J., Bamborough, P., Reith, A. D., & Alessi, D. R. (2009). Substrate specificity and inhibitors of LRRK2, a protein kinase mutated in Parkinson's disease. *The Biochemical Journal*, *424*, 47–60.

Nichols, R. J., Dzamko, N., Morrice, N. A., Campbell, D. G., Deak, M., Ordureau, A., Macartney, T., Tong, Y., Shen, J., Prescott, A. R., & Alessi, D. R. (2010). 14-3-3 binding to LRRK2 is disrupted by multiple Parkinson's disease-associated mutations and regulates cytoplasmic localization. *The Biochemical Journal*, *430*, 393–404.

Nixon-Abell, J., Berwick, D. C., Granno, S., Spain, V. A., Blackstone, C., & Harvey, K. (2016). Protective LRRK2 R1398H variant enhances GTPase and Wnt signaling activity. *Frontiers in Molecular Neuroscience*, *9*, 18.

Nixon-Abell, J., Berwick, D. C., & Harvey, K. (2016). L'RRK de Triomphe: A solution for LRRK2 GTPase activity? *Biochemical Society Transactions*, *44*, 1625–1634.

O'Hare, T., Shakespeare, W. C., Zhu, X., Eide, C. A., Rivera, V. M., Wang, F., Adrian, L. T., Zhou, T., Huang, W. S., Xu, Q., & Metcalf, C. A. III (2009). AP24534, a pan-BCR-ABL inhibitor for chronic myeloid leukemia, potently inhibits the T315I mutant and overcomes mutation-based resistance. *Cancer Cell*, *16*, 401–412.

Paisan-Ruiz, C., Jain, S., Evans, E. W., Gilks, W. P., Simon, J., van der Brug, M., De Munain, A. L., Aparicio, S., Gil, A. M., Khan, N., & Johnson, J. (2004). Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron*, *44*, 595–600.

Parkinson, J. (1817). *An essay on the shaking palsy*. London: Sherwood, Neely and Jones.

Pfeiffer, R. F. (2016). Non-motor symptoms in Parkinson's disease. *Parkinsonism & Related Disorders*, *22*(Suppl 1), S119–S122. https://doi.org/10.1016/j.parkreldis.2015.09.004

Postuma, R. B., Berg, D., Stern, M., Poewe, W., Olanow, C. W., Oertel, W., Obeso, J., Marek, K., Litvan, I., Lang, A. E., & Halliday, G. (2015). MDS clinical diagnostic criteria for Parkinson's disease. *Movement Disorders*, *30*, 1591–1601.

Purlyte, E., Dhekne, H. S., Sarhan, A. R., Gomez, R., Lis, P., Wightman, M., Martinez, T. N., Tonelli, F., Pfeffer, S. R., & Alessi, D. R. (2019). Rab29 activation of the Parkinson's disease-associated LRRK 2 kinase. *The EMBO Journal*, *38*, 101237–101237.

Ramsden, N., Perrin, J., Ren, Z., Lee, B. D., Zinn, N., Dawson, V. L., Tam, D., Bova, M., Lang, M., Drewes, G., Bantscheff, M., Bard, F., Dawson, T. M., & Hopf, C. (2011). Chemoproteomics-based design of potent LRRK2-selective lead compounds that attenuate Parkinson's disease-related toxicity in human neurons. *ACS Chemical Biology*, *6*, 1021–1028. https://doi.org/10.1021/cb2002413

Ray, S., Bender, S., Kang, S., Lin, R., Glicksman, M. A., & Liu, M. (2014). The Parkinson disease-linked LRRK2 protein mutation I2020T stabilizes an active state conformation leading to increased kinase activity. *The Journal of Biological Chemistry*, *289*, 13042–13053.

Ray, S., & Liu, M. (2012). Current understanding of LRRK2 in Parkinson's disease: Biochemical and structural features and inhibitor design. *Future Medicinal Chemistry*, *4*, 1701–1713.

Reeve, A., Simcox, E., & Turnbull, D. (2014). Ageing and Parkinson's disease: Why is advancing age the biggest risk factor? *Ageing Research Reviews*, *14*, 19–30.

Reith, A. D., Bamborough, P., Jandu, K., Andreotti, D., Mensah, L., Dossang, P., Choi, H. G., Deng, X., Zhang, J., Alessi, D. R., & Gray, N. S. (2012). GSK2578215A; A potent and highly selective 2-arylmethyloxy-5-substitutent-*N*-arylbenzamide LRRK2 kinase inhibitor. *Bioorganic & Medicinal Chemistry Letters*, *22*, 5625–5629.

Ren, X., Pan, X., Zhang, Z., Wang, D., Lu, X., Li, Y., Wen, D., Long, H., Luo, J., Feng, Y., & Zhuang, X. (2013). Identification of GZD824 as an orally bioavailable inhibitor that targets phosphorylated and non-phosphorylated breakpoint cluster region–Abelson (Bcr-Abl) kinase and overcomes clinically acquired mutation-induced resistance against imatinib. *Journal of Medicinal Chemistry*, *56*, 879–894.

Rocca, W. A., McDonnell, S. K., Strain, K. J., Bower, J. H., Ahlskog, J. E., Elbaz, A., Schaid, D. J., & Maraganore, D. M. (2004). Familial aggregation of Parkinson's disease: The Mayo Clinic family study. *Annals of Neurology*, *56*, 495–502.

Rocha, E. M., De Miranda, B. R., Castro, S., Drolet, R., Hatcher, N. G., Yao, L., Smith, S. M., Keeney, M. T., Di Maio, R., Kofler, J., & Hastings, T. G. (2020). LRRK2 inhibition prevents endolysosomal deficits seen in human Parkinson's disease. *Neurobiology of Disease*, *134*, 104626.

Ross, O. A., Soto-Ortolaza, A. I., Heckman, M. G., Aasly, J. O., Abahuni, N., Annesi, G., Bacon, J. A., Bardien, S., Bozi, M., Brice, A., Brighina, L., van Broeckhoven, C., Carr, J., Chartier-Harlin, M. C., Dardiotis, E., Dickson, D. W., Diehl, N. N., Elbaz, A., Ferrarese, C., ... Genetic Epidemiology Of Parkinson's Disease (GEO-PD) Consortium. (2011). Association of *LRRK2* exonic variants with susceptibility to Parkinson's disease: A case–control study. *Lancet Neurology*, *10*, 898–908. https://doi.org/10.1016/S1474-4422(11)70175-2

Rudenko, I. N., Kaganovich, A., Hauser, D. N., Beylina, A., Chia, R., Ding, J., Maric, D., Jaffe, H., & Cookson, M. R. (2012). The G2385R variant of leucine-rich repeat kinase 2 associated with Parkinson's disease is a partial loss-of-function mutation. *The Biochemical Journal*, *446*, 99–111. https://doi.org/10.1042/BJ20120637

Ruiz-Martinez, J., de la Riva, P., Rodriguez-Oroz, M. C., Mondragon Rezola, E., Bergareche, A., Gorostidi, A., Gago, B., Estanga, A., Larranaga, N., Sarasqueta, C., & Lopez de Munain, A. (2014). Prevalence of cancer in Parkinson's disease related to R1441G and G2019S mutations in *LRRK2*. *Movement Disorders*, *29*, 750–755.

Satake, W., Nakabayashi, Y., Mizuta, I., Hirota, Y., Ito, C., Kubo, M., Kawaguchi, T., Tsunoda, T., Watanabe, M., Takeda, A., Tomiyama, H., Nakashima, K., Hasegawa, K., Obata, F., Yoshikawa, T., Kawakami, H., Sakoda, S., Yamamoto, M., Hattori, N., ... Toda, T. (2009). Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. *Nature Genetics*, *41*, 1303–1307. https://doi.org/10.1038/ng.485

Saunders-Pullman, R., Barrett, M. J., Stanley, K. M., Luciano, M. S., Shanker, V., Severt, L., Hunt, A., Raymond, D., Ozelius, L. J., & Bressman, S. B. (2010). *LRRK2* G2019S mutations are associated with an increased cancer risk in Parkinson disease. *Movement Disorders*, *25*, 2536–2541.

Schaffner, A., Li, X., Gomez-Llorente, Y., Leandrou, E., Memou, A., Clemente, N., Yao, C., Afsari, F., Zhi, L., Pan, N., & Morohashi, K. (2019). Vitamin B$_{12}$ modulates Parkinson's disease LRRK2 kinase activity through allosteric regulation and confers neuroprotection. *Cell Research*, *29*, 313–329.

Schmidt, S. H., Knape, M. J., Boassa, D., Mumdey, N., Kornev, A. P., Ellisman, M. H., Taylor, S. S., & Herberg, F. W. (2019). The dynamic switch mechanism that leads to activation of LRRK2 is embedded in the DFGψ motif in the kinase domain. *Proceedings of the National Academy of Sciences of the United States of America*, *116*, 14979–14988. https://doi.org/10.1073/pnas.1900289116

Schober, A. (2004). Classic toxin-induced animal models of Parkinson's disease: 6-OHDA and MPTP. *Cell and Tissue Research*, *318*, 215–224.

Seegobin, S. P., Heaton, G. R., Liang, D., Choi, I., Blanca Ramirez, M., Tang, B., & Yue, Z. (2020). Progress in *LRRK2*-associated Parkinson's disease animal models. *Frontiers in Neuroscience*, *14*, 674. https://doi.org/10.3389/fnins.2020.00674

Sheng, Z., Zhang, S., Bustos, D., Kleinheinz, T., Le Pichon, C. E., Dominguez, S. L., Solanoy, H. O., Drummond, J., Zhang, X., Ding, X., & Cai, F. (2012). Ser1292 autophosphorylation is an indicator of LRRK2 kinase activity and contributes to the cellular effects of PD mutations. *Science Translational Medicine*, *4*, 164ra161.

Silva, A. C., Lobo, D. D., Martins, I. M., Lopes, S. M., Henriques, C., Duarte, S. P., Dodart, J. C., Nobre, R. J., & Pereira de Almeida, L. (2020). Antisense oligonucleotide therapeutics in neurodegenerative diseases: The case of polyglutamine disorders. *Brain*, *143*, 407–429.

Simon-Sanchez, J., Schulte, C., Bras, J. M., Sharma, M., Gibbs, J. R., Berg, D., Paisan-Ruiz, C., Lichtner, P., Scholz, S. W., Hernandez, D. G., & Krüger, R. (2009). Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nature Genetics*, *41*, 1308–1312.

Soliman, A., Cankara, F. N., & Kortholt, A. (2020). Allosteric inhibition of LRRK2, where are we now. *Biochemical Society Transactions*, *48*, 2185–2194. https://doi.org/10.1042/BST20200424

Steger, M., Tonelli, F., Ito, G., Davies, P., Trost, M., Vetter, M., Wachter, S., Lorentzen, E., Duddy, G., Wilson, S., & Baptista, M. A. (2016). Phosphoproteomics reveals that Parkinson's disease kinase LRRK2 regulates a subset of Rab GTPases. *eLife*, *5*, e12813. https://doi.org/10.7554/eLife.12813

Sveinbjörnsdottir, S., Hicks, A. A., Jonsson, T., Pétursson, H., Guǧmundsson, G., Frigge, M. L., Kong, A., Gulcher, J. R., & Stefansson, K. (2000). Familial aggregation of Parkinson's disease in Iceland. *The New England Journal of Medicine*, *343*, 1765–1770.

Tabrizi, S. J., Leavitt, B. R., Landwehrmeyer, G. B., Wild, E. J., Saft, C., Barker, R. A., Blair, N. F., Craufurd, D., Priller, J., Rickards, H., & Rosser, A. (2019). Targeting huntingtin expression in patients with Huntington's disease. *The New England Journal of Medicine*, *380*, 2307–2316.

Takanashi, M., Funayama, M., Matsuura, E., Yoshino, H., Li, Y., Tsuyama, S., Takashima, H., Nishioka, K., & Hattori, N. (2018). Isolated nigral degeneration without pathological protein aggregation in autopsied brains with *LRRK2* p.R1441H homozygous and heterozygous mutations. *Acta Neuropathologica Communications*, *6*, 105.

Tan, E. K., Peng, R., Teo, Y. Y., Tan, L. C., Angeles, D., Ho, P., Chen, M. L., Lin, C. H., Mao, X. Y., Chang, X. L., & Prakash, K. M. (2010). Multiple *LRRK2* variants modulate risk of Parkinson disease: A Chinese multicenter study. *Human Mutation*, *31*, 561–568.

Tomkins, J. E., Dihanich, S., Beilina, A., Ferrari, R., Ilacqua, N., Cookson, M. R., Lewis, P. A., & Manzoni, C. (2018). Comparative protein interaction network analysis identifies shared and distinct functions for the human ROCO proteins. *Proteomics*, *18*, 1–37.

Tong, Y., Yamaguchi, H., Giaime, E., Boyle, S., Kopan, R., Kelleher, R. J., & Shen, J. (2010). Loss of leucine-rich repeat kinase 2 causes impairment of protein degradation pathways, accumulation of α-synuclein, and apoptotic cell death in aged mice. *Proceedings of the National Academy of Sciences*, *107*, 9879–9884. https://doi.org/10.1073/pnas.1004676107

Tran, T. N., Vo, T. N. N., Frei, K., & Truong, D. D. (2018). Levodopa-induced dyskinesia: Clinical features, incidence, and risk factors. *Journal of Neural Transmission*, *125*, 1109–1117.

Tsika, E., Nguyen, A. P., Dusonchet, J., Colin, P., Schneider, B. L., & Moore, D. J. (2015). Adenoviral-mediated expression of G2019S LRRK2 induces striatal pathology in a kinase-dependent manner in a rat model of Parkinson's disease. *Neurobiology of Disease*, *77*, 49–61.

van de Wetering-van Dongen, V. A., Kalf, J. G., van der Wees, P. J., Bloem, B. R., & Nijkrake, M. J. (2020). The effects of respiratory training in Parkinson's disease: A systematic review. *Journal of Parkinson's Disease*, *10*, 1315–1333.

Wagner, J., Ryazanov, S., Leonov, A., Levin, J., Shi, S., Schmidt, F., Prix, C., Pan-Montojo, F., Bertsch, U., Mitteregger-Kretzschmar, G., & Geissen, M. (2013). Anle138b: A novel oligomer modulator for disease-modifying therapy of neurodegenerative diseases such as prion and Parkinson's disease. *Acta Neuropathologica*, *125*, 795–813.

Wang, D., Xu, L., Lv, L., Su, L. Y., Fan, Y., Zhang, D. F., Bi, R., Yu, D., Zhang, W., Li, X. A., & Li, Y. Y. (2015). Association of the *LRRK2* genetic polymorphisms with leprosy in Han Chinese from Southwest China. *Genes and Immunity*, *16*, 112–119.

Watanabe, R., Buschauer, R., Böhning, J., Audagnotto, M., Lasker, K., Lu, T.-W., Boassa, D., Taylor, S., & Villa, E. (2020). The in situ structure of Parkinson's disease-linked LRRK2. *Cell*, *182*, 1508–1518. e16

West, A. B. (2017). Achieving neuroprotection with LRRK2 kinase inhibitors in Parkinson disease. *Experimental Neurology*, *298*, 236–245.

Whiffin, N., Armean, I. M., Kleinman, A., Marshall, J. L., Minikel, E. V., Goodrich, J. K., Quaife, N. M., Cole, J. B., Wang, Q., Karczewski, K. J., & Cummings, B. B. (2020). The effect of LRRK2 loss-of-function variants in humans. *Nature Medicine*, *26*, 869–877.

Wider, C., Dickson, D. W., & Wszolek, Z. K. (2010). Leucine-rich repeat kinase 2 gene-associated disease: Redefining genotype-phenotype correlation. *Neurodegenerative Diseases*, *7*, 175–179. https://doi.org/10.1159/000289232

Zhang, F. R., Huang, W., Chen, S. M., Sun, L. D., Liu, H., Li, Y., Cui, Y., Yan, X. X., Yang, H. T., Rong-de Yang, Chu, T. S., Zhang, C., Zhang, L., Han, J. W., Yu, G. Q., Quan, C., Yu, Y. X., Zhang, Z., Shi, B. Q., … Liu, J. J. (2009). Genomewide association study of leprosy. *The New England Journal of Medicine*, *361*, 2609–2618. https://doi.org/10.1056/NEJMoa0903753

Zhang, J., Deng, X., Choi, H. G., Alessi, D. R., & Gray, N. S. (2012). Characterization of TAE684 as a potent LRRK2 kinase inhibitor. *Bioorganic & Medicinal Chemistry Letters*, *22*, 1864–1869.

Zhao, H. T., John, N., Delic, V., Ikeda-Lee, K., Kim, A., Weihofen, A., Swayze, E. E., Kordasiewicz, H. B., West, A. B., & Volpicelli-Daley, L. A. (2017). LRRK2 antisense oligonucleotides ameliorate α-synuclein inclusion formation in a Parkinson's disease mouse model. *Molecular Therapy-Nucleic Acids*, *8*, 508–519.

Zimprich, A., Biskup, S., Leitner, P., Lichtner, P., Farrer, M., Lincoln, S., Kachergus, J., Hulihan, M., Uitti, R. J., Calne, D. B., & Stoessl, A. J. (2004). Mutations in *LRRK2* cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron*, *44*, 601–607.