The development of inhibitors of LRRK2 as a therapeutic strategy for Parkinson's disease: the current state of play

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List of Abbreviations

6-OHDA	6-hydroxydopamine
AdoCbl	50-deoxyadenosylcobalamin
ALK	Anaplastic lymphoma kinase
ASO	Antisense oligonucleotide
BMP	Bis(monoacylglycerol)phosphates
CD	Crohn's disease
COR	c-terminal of Roc
CSF	Cerebrospinal fluid
DAPK	Death-associated protein kinase
GCase	β-glucocerebrospinocidase
L-DOPA	L-dehydroxyphenylalanine
LRR	Leucine-rich repeat
LRRK1	LRR kinase 1
LRRK2	LRR kinase 2
MASL1	Malignant fibrous histiocytoma-amplified sequence with leucine-rich tandem repeats 1
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MyD88	Myeloid differentiation primary response 88
PBMC	Peripheral blood mononuclear cells
PD	Parkinson's disease
Roc	Ras of complex proteins
TB	Tuberculosis
TDP-43	Transactive response DNA binding protein 43 kDa

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ABSTRACT

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 LRRK2. Gain-of-function mutations in *LRRK2* account for a notable proportion of familial PD cases, and significantly, elevated LRRK2 kinase activity is reported in idiopathic PD. Here, we describe progress in finding therapeutically effective LRRK2 inhibitors, summarising studies that range from in vitro experiments through 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, but 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.

Accepted Article

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Parkinson's disease

Parkinson's disease (PD) is 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 (Sveinbjörnsdottir et al., 2000; Rocca et al., 2004). 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 to 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). PD is not limited to motor dysfunction however (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 non-motor symptoms include anosmia, depression, and constipation. Since these non-motor 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 non-motor symptoms, which typically do not involve loss of dopaminergic neurones (Chaudhuri et al., 2006; Postuma et al., 2015). PD patients will require additional medications to treat these symptoms, such as selective 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.

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The need for new therapeutic strategies for PD is clear. Moreover, since 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 etiology offer great potential. In principle, such approaches may not only arrest PD progression, but 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 as an important risk factor contributing towards the more common sporadic form of the condition (Paisan-Ruiz et al., 2004; Zimprich et al., 2004; Nalls et al., 2014; Kluss et al., 2019). 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 other therapies targeting other genes involved in PD aetiology. For example, clinical trials are currently underway aiming to restore the function of the lysosomal enzyme β-glucocerebrocidase (GCase; the 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 GCase 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; 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.

Leucine-rich repeat kinase 2 (LRRK2)

Leucine-rich repeat kinase 2 protein (LRRK2) 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 (Nalls et al., 2014; Kluss et al., 2019). 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 (GWAS), 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, TDP-43 pathology, more usually associated with amyotrophic lateral sclerosis has been reported in a small number of *LRRK2* brains (Wider et al., 2010; Ling et al., 2013), as have post-mortem brains with no apparent proteinopathies (Gaig et al., 2007; Takanashi et al., 2018).

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Importantly, GWAS have also shown that *LRRK2* has a major influence on an individual's risk of developing sporadic PD (Satake et al., 2009; Simon-Sanchez et al., 2009; Do et al., 2011; Lill et al., 2012). Thus, with gene variants of different severity either causing familial PD or contributing risk of sporadic PD, *LRRK2* 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, GWAS have also implicated *LRRK2* as a factor in the genetic risk of Crohn's disease (CD) (Barrett et al., 2008; Franke et al., 2010) and leprosy (Zhang et al., 2009; Wang et al., 2015), whilst other studies have linked LRRK2 to tuberculosis (TB) (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 (Saunders-Pullman et al., 2010; Inzelberg et al., 2012; Agalliu et al., 2015), 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 CD, TB and leprosy are curious, since 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 CD and PD (Tan et al., 2010; Ross et al., 2011; Hui et al., 2018). 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 (Paisan-Ruiz et al., 2004; Zimprich et al., 2004; Gotthardt et al., 2008). 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 RocCOR 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 kinases 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 dimerization suggested to regulate LRRK2 subcellular location and function (Ho et al., 2018). Fascinatingly, monomers are generally cytosolic, whilst dimers are predominantly located on organelle membranes (Nichols et al., 2010; Berwick and Harvey, 2012), and membrane-bound LRRK2 has been reported to possess a higher kinase activity (Berger et al., 2010). Thus, LRRK2 dimerization and membrane recruitment has 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).

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

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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 either cause degeneration of dopaminergic neurons via a cell autonomous process or via a non-cell 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, since only the common G2019S kinase domain mutation reproducibly increases kinase activity in biochemical assays performed *in vitro* (Greggio and 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, there 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 et al., 2016b). 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 et al., 2016a).

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 isn't always increased kinase activity *per se*. Underscoring this idea, increased LRRK2 autophosphorylation and increased phosphorylation of the LRRK2 substrate Rab10 has 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 over-express 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

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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 (Hatcher et al., 2017; Domingos et al., 2019). 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.

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 withing 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, e.g. staurosporine, which has IC50 values for LRRK2 of 1-2 nM (Anand et al., 2009; Covy and 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), that were identified specifically as LRRK2 inhibitors via kinase inhibitor library screens. Both these compounds are reported to inhibit LRRK2 with IC50s 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 (Karaman et al., 2008; Deng et al., 2011).

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 molecular weight diaminopyrimidine named TAE684, a repurposed 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 (IC50 = 9 nM) and selective (90% inhibition towards only 3 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

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

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 and colleagues identified a number of indazole molecules displaying selectivity for G2019S (Garofalo et al., 2020). The most selective compound was reported to have a >300x greater potency in cellular assays and >200x 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 (PBMCs) 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,

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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 pre-clinical studies.

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 and Harvey, 2012; Berwick et al., 2017) 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). Whilst 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 (Lewis and Manzoni, 2012; Berwick et al., 2019).

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), but since animals that only lack *Lrrk2* have no overt neurological phenotypes (reviewed by Seegobin et al., 2020), these concerns do not relate to the brain. Instead, *Lrrk2* knockout mice and rats display an unusual vacuolated phenotype in their kidneys, liver and lung (Tong et al., 2010; Baptista et al., 2013; Ness et al., 2013; Seegobin et al., 2020). 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 were 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 and colleagues has extended this to a wide range of clinical outcomes

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(Whiffin et al., 2020). 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 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 (Steger et al., 2016; Eyers, 2018) or LRRK2 autophosphorylation on serine-1292 (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 (BMPs), 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 to 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 this biomarker remains to be established, but unsurprisingly, this biomarker is already being used in clinical trials of LRRK2 inhibitors (Denali Therapeutics, 2020).

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 and 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 cerebrospinal fluid (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,

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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 and West, 2020). Indeed, phosphorylation of Rab10 in PBMCs and LRRK2 phosphorylation on the serine-935 LRR domain site in PBMCs 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 two 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 IC50 in the high picomolar range (Fell et al., 2015). Clearly, Cerevel and Merck will not be the only interested parties; thus, while 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.

Other therapeutic approaches to reduce LRRK2 function

Whilst 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.

LRRK2 GTP-binding inhibitors

In addition to the LRRK2 kinase domain, the second enzymatic activity of LRRK2 represents another interesting drug target. Since there are only four ROCO GTPases in humans – LRRK1, LRRK2, DAPK1 and 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-

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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 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, while compound 68 also reduced lipopolysaccharide-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 have published with these compounds. Fascinatingly, Blanca Ramirez and colleagues reported that compounds 68 and 70 reverse the increased binding of the pathogenic R1441C LRRK2 variant to filamentous microtubule structures (Blanca Ramirez et al., 2017). 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 toward entering LRRK2-GTP binding inhibitors in clinical trials. Nonetheless, the published data indicate that they have potential.

LRRK2 Knockdown

Whilst DNL-151 is the only chemical inhibitor of LRRK2 currently in clinical trials, it is not the only molecule seeking to reduce LRRK2 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; clinicaltrials.gov). As Biogen state on their website, BIIB094 is an antisense oligonucleotide (Biogen, 2021). Antisense oligonucleotides (ASOs) 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 ASOs 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, since the mode of delivery used by Biogen and Ionis is intrathecal injection, i.e. directly into the CSF, this strategy obviates the need for BIIB094 to cross the blood-brain barrier. The use of ASOs 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, ASOs are already being used in the clinic for the treatment of spinal muscular atrophy, a rare neurodegenerative disease of motor neurons (Kolb and Kissel, 2015). In this case, the ASO, 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 ASOs 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

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molecules have been shown to decrease mutant gene expression and correspondingly improve the clinical symptoms in pre-clinical 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 ASO 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; clinicaltrials.gov), with results expected in late 2022.

It is also worth observing that other ASOs are under investigation for use in Huntington's disease, which, unlike ISIS3139, specifically target mRNA produced by the disease-causing allele and not the wildtype (Silva et al., 2020). This is potentially significant, since 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 wildtype 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.

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, since 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 and colleagues used a high-throughput screen of FDA-approved compounds to identify a physiological form of vitamin B12 (50-deoxyadenosylcobalamin, or AdoCbl) as a non-ATP competitive, "mixed-type" allosteric inhibitor of LRRK2 kinase activity (Schaffner et al., 2019). Since vitamin B12 levels are inversely correlated with PD progression (Christine et al., 2018), these observations suggest an intriguing, if speculative, hypothesis that endogenous vitamin B12 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 IC50 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(s) 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 dimerization and promotes the proteolytic degradation of this protein. How these observations fit together mechanistically is not yet known, but since 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

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monomerization. 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 (Schmidt et al., 2019; Lu et al., 2020). (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 wildtype LRRK2.

This expectation is supported by experimental data. To date, several Type II inhibitors have been reported, including four compounds identified by Liu and colleagues using molecular docking of previously described Type II allosteric inhibitors of other kinases to a structural model of the LRRK2 kinase domain (Ray and Liu, 2012; Liu et al., 2013). 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 wildtype LRRK2 and the G2019S and I2020T variants (Ray and Liu, 2012). By contrast, the two DFGψ motif inhibitors showed clear preferences for wildtype LRRK2, including the most potent compound, ponatinib (O'Hare et al., 2009), which has an *in vitro* IC50 for wildtype LRRK2 of 31 nM, but only 200 nM and 600 nM for G2019S and I2020T respectively (Ray and 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 observed that a series of cysteine residues located near the ATP binding site might be a fruitful site for developing covalent LRRK2 inhibitors (Ray and Liu, 2012).

Evidence of neuroprotection provided by LRRK2 inhibitors or LRRK2 ASOs in PD-related animal models Pre-clinical 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 ASOs to promote cell survival in PD-relevant animal models.

Since LRRK2 transgenic animals do not have a robust neurological phenotype (Daniel and 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 over-expression 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).

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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; Tsika et al., 2015; Nguyen et al., 2020). Of note, mutations that perturb LRRK2 GTPase function, either by 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 over-expression of a pathogenic LRRK2 variant represents the normal dopaminergic cell death in PD, where LRRK2 is not over-expressed. 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 and colleagues found that PF-06447475 reduced α-synuclein-induced neurodegeneration in rats (Daher et al., 2015). By contrast, Andersen and colleagues 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 (Andersen et al., 2018). 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, since 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 are 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 wildtype animals, but induced significant impairments in transgenic mice that over-express 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

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compounds are available – but these data are nonetheless encouraging. Perhaps most informative is the fact that an ordinarily sub-toxic 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 prevent maintain motor function in PD patients, but only if administered while there are sufficient dopaminergic neurons remaining.

Research into the therapeutic potential of LRRK2 ASOs in pre-clinical models is also thin on the ground, although the use of LRRK2 ASOs is to some extent informed by the pre-clinical work using LRRK2 inhibitors described above (and *vice versa*). The first study using LRRK2 ASOs is that of Zhao and colleagues, who investigated the effect of intracerebral ventricular injection of Lrrk2 ASOs on mice injected with synthetic α-synuclein fibrils (Zhao et al., 2017). Somewhat surprisingly, given that the two different ASOs 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 ASOs targeting LRRK2 in animal models. In this this report (Korecka et al., 2020), Korecka and colleagues used an ASO 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 (Korecka et al., 2019). When injected into the cerebral ventricles of transgenic mice expressing human wildtype or G2019S LRRK2, this ASO 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, since modest effects on LRRK2 expression appear sufficient to elicit promising biological effects, the data suggest that LRRK2 ASOs 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.

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 fall-back 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

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the normal regulation of this protein (Berwick et al., 2019). Indeed, work performed in zebrafish models indicates the deletion of the C-terminal WD40 domain is sufficient to prevent dimerization, thereby reducing autophosphorylation and reducing the neurotoxicity of LRRK2 (Jorgensen et al., 2009). Thus, disruption of LRRK2 dimerization 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 dimerization of other proteins, for example ST2825, a heptapeptide that blocks dimerization of MyD88 (Loiarro et al., 2007). However, this may be a trickier task for LRRK2, since LRRK2 dimerization appears to have several intramolecular interactions involved, with the WD40 and COR domains implicated in particular (Greggio et al., 2008, 2008; Jorgensen et al., 2009; Guaitoli et al., 2016). 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 (Lewis and Manzoni, 2012; Berwick et al., 2019). As such, disruption of protein-protein interactions required for the pathogenic effects of LRRK2 may be fertile ground for drug discovery. Nonetheless, whilst 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 a bind LRRK2 in a manner dependent on the phosphorylation of LRRK2. Phosphorylation sites to which 14-3-3 proteins associate include Ser910, 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). Whilst 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 (Nichols et al., 2010; Lavalley et al., 2016). Based on these and other observations, Soliman and colleagues 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 stabilize LRRK2-14-3-3 complexes (Soliman et al., 2020). 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 and colleagues used a combination of cryo-electron tomography and integrative modelling to produce a 14Å resolution *in situ* model for the entire LRRK2 protein bound to microtubules (PDB:6XR4) (Watanabe et al., 2020). 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 and colleagues integrated the 6XR4 structure with their own high resolution (3.5Å) cryo-electron microscopy structure of amino acids 1327-2527 of LRRK2, containing Roc, COR, kinase and WD40 domains, to reveal further insights (Deniston et al., 2020). 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, which provides a rationale for why conventional LRRK2 kinase inhibitors (i.e. ATP-competitive or Type I),

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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 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.

Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander et al., 2019).

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Competing Interests Statement

None

Author Contributions

SA wrote first drafts of the sections on Parkinson's disease, LRRK2, allosteric inhibition, LRRK2 dimerisation, 14-3-3 interactions and provided Table 1. DCB planned the article, wrote all other sections and revised the manuscript. Both authors contributed to Figures 1 and 3; DCB created Figure 2.

Data Availability Statement

Data sharing is not applicable to this article because no new data were created or analysed in this study.

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Reference List

Agalliu, I., San Luciano, M., Mirelman, A., Giladi, N., Waro, B., Aasly, J., et al. (2015). Higher frequency of certain cancers in LRRK2 G2019S mutation carriers with Parkinson disease: a pooled analysis. JAMA Neurol *72*: 58–65.

Alcalay, R.N., Hsieh, F., Tengstrand, E., Padmanabhan, S., Baptista, M., Kehoe, C., et al. (2020). Higher Urine bis(Monoacylglycerol)Phosphate Levels in LRRK2 G2019S Mutation Carriers: Implications for Therapeutic Development. Mov. Disord. *35*: 134–141.

AlDakheel, A., Kalia, L.V., and Lang, A.E. (2014). Pathogenesis-targeted, disease-modifying therapies in Parkinson disease. Neurother. J. Am. Soc. Exp. Neurother. *11*: 6–23.

Alexander, S.P.H., Kelly, E., Mathie, A., Peters, J.A., Veale, E.L., Armstrong, J.F., et al. (2019). THE CONCISE GUIDE TO PHARMACOLOGY 2019/20: Introduction and Other Protein Targets. Br. J. Pharmacol. *176*: S1–S20.

Allegra, R., Tunesi, S., Cilia, R., Pezzoli, G., and Goldwurm, S. (2014). LRRK2-G2019S mutation is not associated with an increased cancer risk: a kin-cohort study. Mov Disord *29*: 1325–6.

Anand, V.S., Reichling, L.J., Lipinski, K., Stochaj, W., Duan, W., Kelleher, K., et al. (2009). Investigation of leucine-rich repeat kinase 2 : enzymological properties and novel assays. FEBS J *276*: 466–78.

Andersen, M.A., Christensen, K.V., Badolo, L., Smith, G.P., Jeggo, R., Jensen, P.H., et al. (2018). Parkinson's disease-like burst firing activity in subthalamic nucleus induced by AAV-α-synuclein is normalized by LRRK2 modulation. Neurobiol. Dis. *116*: 13–27.

Andersen, M.A., Sotty, F., Jensen, P.H., Badolo, L., Jeggo, R., Smith, G.P., et al. (2019). Long-Term Exposure to PFE-360 in the AAV-α-Synuclein Rat Model: Findings and Implications. ENeuro *6*:.

Arbez, N., He, X., Huang, Y., Ren, M., Liang, Y., Nucifora, F.C., et al. (2020). G2019S-LRRK2 mutation enhances MPTP-linked Parkinsonism in mice. Hum. Mol. Genet. *29*: 580–590.

Armstrong, M.J., and Okun, M.S. (2020). Diagnosis and Treatment of Parkinson Disease: A Review. JAMA - J. Am. Med. Assoc. *323*: 548–560.

Ballone, A., Centorrino, F., and Ottmann, C. (2018). 14-3-3: A Case Study in PPI Modulation. Molecules *23*: 1386.

Baptista, M.A., Dave, K.D., Frasier, M.A., Sherer, T.B., Greeley, M., Beck, M.J., et al. (2013). Loss of leucine-rich repeat kinase 2 (LRRK2) in rats leads to progressive abnormal phenotypes in peripheral organs. PLoS One *8*: e80705.

Baptista, M.A.S., Merchant, K., Barrett, T., Bhargava, S., Bryce, D.K., Ellis, J.M., et al. (2020). LRRK2 inhibitors induce reversible changes in nonhuman primate lungs without measurable pulmonary deficits. Sci. Transl. Med. *12*:.

Barrett, J.C., Hansoul, S., Nicolae, D.L., Cho, J.H., Duerr, R.H., Rioux, J.D., et al. (2008). Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. Nat Genet *40*: 955–62.

Berger, Z., Smith, K.A., and 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*: 5511–23.

This article is protected by copyright. All rights reserved.

Berwick, D.C., and Harvey, K. (2012). LRRK2 functions as a Wnt signaling scaffold, bridging cytosolic proteins and membrane-localized LRP6. Hum Mol Genet *21*: 4966–79.

Berwick, D.C., Heaton, G.R., Azeggagh, S., and Harvey, K. (2019). LRRK2 Biology from structure to dysfunction: research progresses, but the themes remain the same. Mol. Neurodegener. *14*: 49.

Berwick, D.C., Javaheri, B., Wetzel, A., Hopkinson, M., Nixon-Abell, J., Grannò, S., et al. (2017). Pathogenic LRRK2 variants are gain-of-function mutations that enhance LRRK2-mediated repression of β-catenin signaling. Mol. Neurodegener. *12*: 9–9.

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., et al. (2017). GTP binding regulates cellular localization of Parkinson's disease-associated LRRK2. Hum Mol Genet *26*: 2747–2767.

Blauwendraat, C., Reed, X., Kia, D.A., Gan-Or, Z., Lesage, S., Pihlstrøm, L., et al. (2018). Frequency of Loss of Function Variants in LRRK2 in Parkinson Disease. JAMA Neurol. *75*: 1416–1422.

Bright, J.M., Carlisle, H.J., Toda, A.M.A., Murphy, M., Molitor, T.P., Wren, P., et al. (2021). Differential Inhibition of LRRK2 in Parkinson's Disease Patient Blood by a G2019S Selective LRRK2 Inhibitor. Mov. Disord. Off. J. Mov. Disord. Soc.

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

Carlsson, A., Lindqvist, M., and Magnusson, T. (1957). 3,4-Dihydroxyphenylalanine and 5-Hydroxytryptophan as Reserpine Antagonists. Nature *180*: 1200–1200.

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., and National Institute for Clinical Excellence (2006). Non-motor symptoms of Parkinson's disease: diagnosis and management. Lancet Neurol. *5*: 235–245.

Chaudhuri, K.R., and Schapira, A.H. (2009). Non-motor symptoms of Parkinson's disease: dopaminergic pathophysiology and treatment. Lancet Neurol. *8*: 464–474.

Chen, H., Chan, B.K., Drummond, J., Estrada, A.A., Gunzner-Toste, J., Liu, X., et al. (2012). Discovery of selective LRRK2 inhibitors guided by computational analysis and molecular modeling. J Med Chem *55*: 5536–45.

Chery, J. (2016). RNA therapeutics: RNAi and antisense mechanisms and clinical applications. Postdoc J. J. Postdr. Res. Postdr. Aff. *4*: 35–50.

Chiriboga, C.A., Swoboda, K.J., Darras, B.T., Iannaccone, S.T., Montes, J., Vivo, D.C.D., et al. (2016). Results from a phase 1 study of nusinersen (ISIS-SMNRx) in children with spinal muscular atrophy. Neurology *86*: 890–897.

Choi, H.G., Zhang, J., Deng, X., Hatcher, J.M., Patricelli, M.P., Zhao, Z., et al. (2012). Brain Penetrant LRRK2 Inhibitor. ACS Med Chem Lett *3*: 658–662.

This article is protected by copyright. All rights reserved.

Christine, C.W., Auinger, P., Joslin, A., Yelpaala, Y., and Green, R. (2018). Vitamin B12 and Homocysteine Levels Predict Different Outcomes in Early Parkinson's Disease. Mov. Disord. *33*: 762–770.

Cookson, M.R. (2015). LRRK2 Pathways Leading to Neurodegeneration. Curr. Neurol. Neurosci. Rep. *15*: 42.

Cotzias, G.C., Van Woert, M.H., and Schiffer, L.M. (1967). Aromatic Amino Acids and Modification of Parkinsonism (Massachusetts Medical Society).

Covy, J.P., and Giasson, B.I. (2009). Identification of compounds that inhibit the kinase activity of leucine-rich repeat kinase 2. Biochem Biophys Res Commun *378*: 473–7.

Daher, J.P., Abdelmotilib, H.A., Hu, X., Volpicelli-Daley, L.A., Moehle, M.S., Fraser, K.B., et al. (2015). Leucine-rich Repeat Kinase 2 (LRRK2) Pharmacological Inhibition Abates alpha-Synuclein Gene-induced Neurodegeneration. J Biol Chem *290*: 19433–44.

Daniel, G., and Moore, D.J. (2015). Modeling LRRK2 Pathobiology in Parkinson's Disease: From Yeast to Rodents. Curr Top Behav Neurosci *22*: 331–68.

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., et al. (2011). Characterization of a selective inhibitor of the Parkinson's disease kinase LRRK2. Nat Chem Biol *7*: 203–5.

Deniston, C., Salogiannis, J., Mathea, S., Snead, D., Lahiri, I., Matyszewski, M., et al. (2020). Structure of LRRK2 in Parkinson's disease and model for microtubule interaction. Nature *588*: 344–349.

Di Maio, R., Hoffman, E.K., Rocha, E.M., Keeney, M.T., Sanders, L.H., De Miranda, B.R., et al. (2018). LRRK2 activation in idiopathic Parkinson's disease (PLA). Sci. Transl. Med. *10*: eaar5429–eaar5429.

Ding, X., and Ren, F. (2020). Leucine-rich repeat kinase 2 inhibitors: a patent review (2014-present). Expert Opin. Ther. Pat. *30*: 275–286.

Do, C.B., Tung, J.Y., Dorfman, E., Kiefer, A.K., Drabant, E.M., Francke, U., et al. (2011). Web-based genome-wide association study identifies two novel loci and a substantial genetic component for Parkinson's disease. PLoS Genet *7*: e1002141.

Domingos, S., Duarte, T., Saraiva, L., Guedes, R.C., and Moreira, R. (2019). Targeting leucine-rich repeat kinase 2 (LRRK2) for the treatment of Parkinson's disease. Future Med. Chem. *11*: 1953–1977.

Duty, S., and Jenner, P. (2011). Animal models of Parkinson's disease: A source of novel treatments and clues to the cause of the disease. Br. J. Pharmacol. *164*: 1357–1391.

Elbaz, A., Bower, J.H., Maraganore, D.M., McDonnell, S.K., Peterson, B.J., Ahlskog, J.E., et al. (2002). Risk tables for parkinsonism and Parkinson's disease. J Clin Epidemiol *55*: 25–31.

This article is protected by copyright. All rights reserved.

Estrada, A.A., Chan, B.K., Baker-Glenn, C., Beresford, A., Burdick, D.J., Chambers, M., et al. (2014). Discovery of highly potent, selective, and brain-penetrant aminopyrazole leucine-rich repeat kinase 2 (LRRK2) small molecule inhibitors. J Med Chem *57*: 921–36.

Estrada, A.A., Liu, X., Baker-Glenn, C., Beresford, A., Burdick, D.J., Chambers, M., et al. (2012). Discovery of highly potent, selective, and brain-penetrable leucine-rich repeat kinase 2 (LRRK2) small molecule inhibitors. J Med Chem *55*: 9416–33.

Eyers, P.A. (2018). Back to the future: new target-validated Rab antibodies for evaluating LRRK2 signalling in cell biology and Parkinson's disease. Biochem. J. *475*: 185–189.

Farrer, M.J. (2006). Genetics of Parkinson disease: paradigm shifts and future prospects. Nat. Rev. Genet. *7*: 306–318.

Fell, M.J., Mirescu, C., Basu, K., Cheewatrakoolpong, B., DeMong, D.E., Ellis, J.M., et al. (2015). MLi-2, a Potent, Selective, and Centrally Active Compound for Exploring the Therapeutic Potential and Safety of LRRK2 Kinase Inhibition. J Pharmacol Exp Ther *355*: 397–409.

Franke, A., McGovern, D.P.B., Barrett, J.C., Wang, K., Radford-Smith, G.L., Ahmad, T., et al. (2010). Meta-Analysis Increases to 71 the Tally of Confirmed Crohn's Disease Susceptibility Loci. Nat. Genet. *42*: 1118–1125.

Fuji, R.N., Flagella, M., Baca, M., Baptista, M.A., Brodbeck, J., Chan, B.K., et al. (2015). Effect of selective LRRK2 kinase inhibition on nonhuman primate lung. Sci Transl Med *7*: 273ra15.

Gaig, C., Marti, M.J., Ezquerra, M., Rey, M.J., Cardozo, A., and Tolosa, E. (2007). G2019S LRRK2 mutation causing Parkinson's disease without Lewy bodies. J Neurol Neurosurg Psychiatry *78*: 626–8.

Gan-Or, Z., Dion, P.A., and Rouleau, G.A. (2015). Genetic perspective on the role of the autophagy-lysosome pathway in Parkinson disease. Autophagy *11*: 1443–57.

Garofalo, A.W., Bright, J., De Lombaert, S., Toda, A.M.A., Zobel, K., Andreotti, D., et al. (2020). Selective Inhibitors of G2019S-LRRK2 Kinase Activity. J. Med. Chem. *63*: 14821–14839.

Giaime, E., Tong, Y., Wagner, L.K., Yuan, Y., Huang, G., and 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., and Wittinghofer, A. (2008). Structure of the Roc-COR domain tandem of C. tepidum, a prokaryotic homologue of the human LRRK2 Parkinson kinase. EMBO J *27*: 2239–49.

Greggio, E., and Cookson, M.R. (2009). Leucine-rich repeat kinase 2 mutations and Parkinson's disease: three questions. ASN Neuro *1*:.

Greggio, E., Zambrano, I., Kaganovich, A., Beilina, A., Taymans, J.M., Daniels, V., et al. (2008). The Parkinson disease-associated leucine-rich repeat kinase 2 (LRRK2) is a dimer that undergoes intramolecular autophosphorylation. J Biol Chem *283*: 16906–14.

Greshock, T.J., Sanders, J.M., Drolet, R.E., Rajapakse, H.A., Chang, R.K., Kim, B., et al. (2016). Potent, selective and orally bioavailable leucine-rich repeat kinase 2 (LRRK2) inhibitors. Bioorg Med Chem Lett *26*: 2631–5.

This article is protected by copyright. All rights reserved.

Guaitoli, G., Raimondi, F., Gilsbach, B.K., Gomez-Llorente, Y., Deyaert, E., Renzi, F., et al. (2016). Structural model of the dimeric Parkinson's protein LRRK2 reveals a compact architecture involving distant interdomain contacts. Proc Natl Acad Sci U A *113*: E4357-66.

Harding, S.D., Sharman, J.L., Faccenda, E., Southan, C., Pawson, A.J., Ireland, S., et al. (2018). The IUPHAR/BPS Guide to PHARMACOLOGY in 2018: updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. Nucleic Acids Res. *46*: D1091–D1106.

Hartlova, A., Herbst, S., Peltier, J., Rodgers, A., Bilkei-Gorzo, O., Fearns, A., et al. (2018). LRRK2 is a negative regulator of Mycobacterium tuberculosis phagosome maturation in macrophages. EMBO J *37*:.

Hatcher, J.M., Choi, H.G., Alessi, D.R., and Gray, N.S. (2017). Small-Molecule Inhibitors of LRRK2. pp 241–264.

Henderson, J.L., Kormos, B.L., Hayward, M.M., Coffman, K.J., Jasti, J., Kurumbail, R.G., et al. (2015). Discovery and preclinical profiling of 3-[4-(morpholin-4-yl)-7H-pyrrolo[2,3-d]pyrimidin-5-yl]benzonitrile (PF-06447475), a highly potent, selective, brain penetrant, and in vivo active LRRK2 kinase inhibitor. J Med Chem *58*: 419–32.

Ho, D.H., Je, A.R., Lee, H., Son, I., Kweon, H.-S., Kim, H.-G., et al. (2018). LRRK2 Kinase Activity Induces Mitochondrial Fission in Microglia via Drp1 and Modulates Neuroinflammation. Exp. Neurobiol. *27*: 171–171.

Hui, K.Y., Fernandez-Hernandez, H., Hu, J., Schaffner, A., Pankratz, N., Hsu, N.Y., et al. (2018). Functional variants in the LRRK2 gene confer shared effects on risk for Crohn's disease and Parkinson's disease. Sci Transl Med *10*:.

Inzelberg, R., Cohen, O.S., Aharon-Peretz, J., Schlesinger, I., Gershoni-Baruch, R., Djaldetti, R., et al. (2012). The LRRK2 G2019S mutation is associated with Parkinson disease and concomitant non-skin cancers. Neurology *78*: 781–6.

Iqbal, K., Liu, F., Gong, C.-X., and Grundke-Iqbal, I. (2010). Tau in Alzheimer Disease and Related Tauopathies. Curr. Alzheimer Res. *7*: 656–664.

Jankovic, J. (2008). Parkinson's disease: Clinical features and diagnosis. J. Neurol. Neurosurg. Psychiatry *79*: 368–376.

Jorgensen, N.D., Peng, Y., Ho, C.C., Rideout, H.J., Petrey, D., Liu, P., et al. (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., et al. (2015). Clinical correlations with Lewy body pathology in LRRK2-related Parkinson disease. JAMA Neurol *72*: 100–5.

Karaman, M.W., Herrgard, S., Treiber, D.K., Gallant, P., Atteridge, C.E., Campbell, B.T., et al. (2008). A quantitative analysis of kinase inhibitor selectivity. Nat. Biotechnol. *26*: 127–132.

Karuppagounder, S.S., Brahmachari, S., Lee, Y., Dawson, V.L., Dawson, T.M., and Ko, H.S. (2014). The c-Abl inhibitor, nilotinib, protects dopaminergic neurons in a preclinical animal model of Parkinson's disease. Sci. Rep. *4*: 4874.

Kelly, K., and West, A.B. (2020). Pharmacodynamic Biomarkers for Emerging LRRK2 Therapeutics. Front. Neurosci. *14*:.

This article is protected by copyright. All rights reserved.

Kluss, J.H., Mamais, A., and Cookson, M.R. (2019). LRRK2 links genetic and sporadic Parkinson's disease. Biochem. Soc. Trans. *47*: 651–661.

Kolb, S.J., and Kissel, J.T. (2015). Spinal Muscular Atrophy. Neurol. Clin. *33*: 831–846.

Korecka, J.A., Thomas, R., Christensen, D.P., Hinrich, A.J., Ferrari, E.J., Levy, S.A., et al. (2019). Mitochondrial clearance and maturation of autophagosomes are compromised in LRRK2 G2019S familial Parkinson's disease patient fibroblasts. Hum. Mol. Genet. *28*: 3232–3243.

Korecka, J.A., Thomas, R., Hinrich, A.J., Moskites, A.M., Macbain, Z.K., Hallett, P.J., et al. (2020). Splice-Switching Antisense Oligonucleotides Reduce LRRK2 Kinase Activity in Human LRRK2 Transgenic Mice. Mol. Ther. Nucleic Acids *21*: 623–635.

Lavalley, N.J., Slone, S.R., Ding, H., West, A.B., and Yacoubian, T.A. (2016). 14-3-3 Proteins regulate mutant LRRK2 kinase activity and neurite shortening. Hum Mol Genet *25*: 109–22.

Lebovitz, C., Wretham, N., Osooly, M., Milne, K., Dash, T., Thornton, S., et al. (2021). Loss of Parkinson's susceptibility gene LRRK2 promotes carcinogen-induced lung tumorigenesis. Sci. Rep. *11*: 2097.

Lebovitz, C.B., Robertson, A.G., Goya, R., Jones, S.J., Morin, R.D., Marra, M.A., et al. (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., et al. (2010). Inhibitors of leucine-rich repeat kinase-2 protect against models of Parkinson's disease. Nat Med *16*: 998–1000.

Lewis, P. a, and Manzoni, C. (2012). LRRK2 and human disease: a complicated question or a question of complexes? Sci. Signal. *5*: pe2–pe2.

Li, T., He, X., Thomas, J.M., Yang, D., Zhong, S., Xue, F., et al. (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., et al. (2014). Novel LRRK2 GTP-binding inhibitors reduced degeneration in Parkinson's disease cell and mouse models. Hum. Mol. Genet. *23*: 6212–6222.

Lill, C.M., Roehr, J.T., McQueen, M.B., Kavvoura, F.K., Bagade, S., Schjeide, B.M., et al. (2012). Comprehensive research synopsis and systematic meta-analyses in Parkinson's disease genetics: The PDGene database. PLoS Genet *8*: e1002548.

Ling, H., Kara, E., Bandopadhyay, R., Hardy, J., Holton, J., Xiromerisiou, G., et al. (2013). TDP-43 pathology in a patient carrying G2019S LRRK2 mutation and a novel p.Q124E MAPT. Neurobiol Aging *34*: 2889 e5–9.

Liu, M., Bender, S.A., Cuny, G.D., Sherman, W., Glicksman, M., and Ray, S.S. (2013). Type II kinase inhibitors show an unexpected inhibition mode against Parkinson's disease-linked LRRK2 mutant G2019S. Biochemistry *52*: 1725–36.

Liu, Z., Bryant, N., Kumaran, R., Beilina, A., Abeliovich, A., Cookson, M.R., et al. (2018). LRRK2 phosphorylates membrane-bound Rabs and is activated by GTP-bound Rab7L1 to promote recruitment to the trans-Golgi network. Hum. Mol. Genet. *27*: 385–395.

This article is protected by copyright. All rights reserved.

Loiarro, M., Capolunghi, F., Fantò, N., Gallo, G., Campo, S., Arseni, B., et al. (2007). Pivotal Advance: Inhibition of MyD88 dimerization and recruitment of IRAK1 and IRAK4 by a novel peptidomimetic compound. J. Leukoc. Biol. *82*: 801–810.

Lu, X., Smaill, J.B., and Ding, K. (2020). New Promise and Opportunities for Allosteric Kinase Inhibitors. Angew. Chem. Int. Ed Engl. *59*: 13764–13776.

Maegawa, G.H.B., Tropak, M.B., Buttner, J.D., Rigat, B.A., Fuller, M., Pandit, D., et al. (2009). Identification and Characterization of Ambroxol as an Enzyme Enhancement Agent for Gaucher Disease *. J. Biol. Chem. *284*: 23502–23516.

Manschwetus, J.T., Wallbott, M., Fachinger, A., Obergruber, C., Pautz, S., Bertinetti, D., et al. (2020). Binding of the Human 14-3-3 Isoforms to Distinct Sites in the Leucine-Rich Repeat Kinase 2. Front. Neurosci. *14*:.

Migdalska-Richards, A., Ko, W.K.D., Li, Q., Bezard, E., and 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., Zweydorf, F. von, Geerlof, A., et al. (2014). Parkinson-related LRRK2 mutation R1441C/G/H impairs PKA phosphorylation of LRRK2 and disrupts its interaction with 14-3-3. Proc Natl Acad Sci U A *111*: E34-43.

Nalls, M.A., Pankratz, N., Lill, C.M., Do, C.B., Hernandez, D.G., Saad, M., et al. (2014). Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. Nat. Genet. *46*: 989–993.

Ness, D., Ren, Z., Gardai, S., Sharpnack, D., Johnson, V.J., Brennan, R.J., et al. (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., et al. (2020). Dopaminergic neurodegeneration induced by Parkinson's disease-linked G2019S LRRK2 is dependent on kinase and GTPase activity. Proc. Natl. Acad. Sci. U. S. A. *117*: 17296–17307.

Nichols, R.J., Dzamko, N., Hutti, J.E., Cantley, L.C., Deak, M., Moran, J., et al. (2009). Substrate specificity and inhibitors of LRRK2, a protein kinase mutated in Parkinson's disease. Biochem J *424*: 47–60.

Nichols, R.J., Dzamko, N., Morrice, N.A., Campbell, D.G., Deak, M., Ordureau, A., et al. (2010). 14-3-3 binding to LRRK2 is disrupted by multiple Parkinson's disease-associated mutations and regulates cytoplasmic localization. Biochem J *430*: 393–404.

Nixon-Abell, J., Berwick, D.C., Granno, S., Spain, V.A., Blackstone, C., and Harvey, K. (2016a). Protective LRRK2 R1398H Variant Enhances GTPase and Wnt Signaling Activity. Front Mol Neurosci *9*: 18.

Nixon-Abell, J., Berwick, D.C., and Harvey, K. (2016b). L'RRK de Triomphe: a solution for LRRK2 GTPase activity? Biochem Soc Trans *44*: 1625–1634.

O'Hare, T., Shakespeare, W.C., Zhu, X., Eide, C.A., Rivera, V.M., Wang, F., et al. (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.

This article is protected by copyright. All rights reserved.

Paisan-Ruiz, C., Jain, S., Evans, E.W., Gilks, W.P., Simon, J., Brug, M. van der, et al. (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 Relat. Disord. *22 Suppl 1*: S119-122.

Postuma, R.B., Berg, D., Stern, M., Poewe, W., Olanow, C.W., Oertel, W., et al. (2015). MDS clinical diagnostic criteria for Parkinson's disease. Mov. Disord. Off. J. Mov. Disord. Soc. *30*: 1591–1601.

Purlyte, E., Dhekne, H.S., Sarhan, A.R., Gomez, R., Lis, P., Wightman, M., et al. (2019). Rab29 activation of the Parkinson's disease-associated LRRK 2 kinase. EMBO J. *38*: 101237–101237.

Ramsden, N., Perrin, J., Ren, Z., Lee, B.D., Zinn, N., Dawson, V.L., et al. (2011). Chemoproteomics-based design of potent LRRK2-selective lead compounds that attenuate Parkinson's disease-related toxicity in human neurons. ACS Chem Biol *6*: 1021–8.

Ray, S., Bender, S., Kang, S., Lin, R., Glicksman, M.A., and Liu, M. (2014). The Parkinson disease-linked LRRK2 protein mutation I2020T stabilizes an active state conformation leading to increased kinase activity. J Biol Chem *289*: 13042–53.

Ray, S., and Liu, M. (2012). Current understanding of LRRK2 in Parkinson's disease: biochemical and structural features and inhibitor design. Future Med Chem *4*: 1701–13.

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

Reith, A.D., Bamborough, P., Jandu, K., Andreotti, D., Mensah, L., Dossang, P., et al. (2012). GSK2578215A; a potent and highly selective 2-arylmethyloxy-5-substitutent-N-arylbenzamide LRRK2 kinase inhibitor. Bioorg Med Chem Lett *22*: 5625–9.

Ren, X., Pan, X., Zhang, Z., Wang, D., Lu, X., Li, Y., et al. (2013). Identification of GZD824 as an orally bioavailable inhibitor that targets phosphorylated and nonphosphorylated breakpoint cluster region-Abelson (Bcr-Abl) kinase and overcomes clinically acquired mutation-induced resistance against imatinib. J. Med. Chem. *56*: 879–894.

Rocca, W.A., McDonnell, S.K., Strain, K.J., Bower, J.H., Ahlskog, J.E., Elbaz, A., et al. (2004). Familial aggregation of Parkinson's disease: The Mayo Clinic family study. Ann. Neurol. *56*: 495–502.

Rocha, E.M., De Miranda, B.R., Castro, S., Drolet, R., Hatcher, N.G., Yao, L., et al. (2020). LRRK2 inhibition prevents endolysosomal deficits seen in human Parkinson's disease. Neurobiol. Dis. *134*: 104626.

Ross, O.A., Soto-Ortolaza, A.I., Heckman, M.G., Aasly, J.O., Abahuni, N., Annesi, G., et al. (2011). Association of LRRK2 exonic variants with susceptibility to Parkinson's disease: a case-control study. Lancet Neurol *10*: 898–908.

Rudenko, I.N., Kaganovich, A., Hauser, D.N., Beylina, A., Chia, R., Ding, J., et al. (2012). The G2385R variant of leucine-rich repeat kinase 2 associated with Parkinson's disease is a partial loss-of-function mutation. Biochem J *446*: 99–111.

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Ruiz-Martinez, J., Riva, P. de la, Rodriguez-Oroz, M.C., Mondragon Rezola, E., Bergareche, A., Gorostidi, A., et al. (2014). Prevalence of cancer in Parkinson's disease related to R1441G and G2019S mutations in LRRK2. Mov Disord *29*: 750–5.

Satake, W., Nakabayashi, Y., Mizuta, I., Hirota, Y., Ito, C., Kubo, M., et al. (2009). Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. Nat Genet *41*: 1303–7.

Saunders-Pullman, R., Barrett, M.J., Stanley, K.M., Luciano, M.S., Shanker, V., Severt, L., et al. (2010). LRRK2 G2019S mutations are associated with an increased cancer risk in Parkinson disease. Mov Disord *25*: 2536–41.

Schaffner, A., Li, X., Gomez-Llorente, Y., Leandrou, E., Memou, A., Clemente, N., et al. (2019). Vitamin B12 modulates Parkinson's disease LRRK2 kinase activity through allosteric regulation and confers neuroprotection. Cell Res *29*: 313–329.

Schmidt, S.H., Knape, M.J., Boassa, D., Mumdey, N., Kornev, A.P., Ellisman, M.H., et al. (2019). The dynamic switch mechanism that leads to activation of LRRK2 is embedded in the DFGψ motif in the kinase domain. Proc. Natl. Acad. Sci. U. S. A. *116*: 14979–14988.

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

Seegobin, S.P., Heaton, G.R., Liang, D., Choi, I., Blanca Ramirez, M., Tang, B., et al. (2020). Progress in LRRK2-Associated Parkinson's Disease Animal Models. Front. Neurosci. *14*:.

Sheng, Z., Zhang, S., Bustos, D., Kleinheinz, T., Le Pichon, C.E., Dominguez, S.L., et al. (2012). Ser1292 autophosphorylation is an indicator of LRRK2 kinase activity and contributes to the cellular effects of PD mutations. Sci Transl Med *4*: 164ra161.

Silva, A.C., Lobo, D.D., Martins, I.M., Lopes, S.M., Henriques, C., Duarte, S.P., et al. (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., et al. (2009). Genome-wide association study reveals genetic risk underlying Parkinson's disease. Nat Genet *41*: 1308–12.

Soliman, A., Cankara, F.N., and Kortholt, A. (2020). Allosteric inhibition of LRRK2, where are we now. Biochem. Soc. Trans. *48*: 2185–2194.

Steger, M., Tonelli, F., Ito, G., Davies, P., Trost, M., Vetter, M., et al. (2016). Phosphoproteomics reveals that Parkinson's disease kinase LRRK2 regulates a subset of Rab GTPases. Elife *5*:.

Sveinbjörnsdottir, S., Hicks, A.A., Jonsson, T., Pétursson, H., Guğmundsson, G., Frigge, M.L., et al. (2000). Familial aggregation of Parkinson's disease in Iceland. N. Engl. J. Med. *343*: 1765–1770.

Tabrizi, S.J., Leavitt, B.R., Landwehrmeyer, G.B., Wild, E.J., Saft, C., Barker, R.A., et al. (2019). Targeting Huntingtin Expression in Patients with Huntington's Disease. N. Engl. J. Med. *380*: 2307–2316.

Takanashi, M., Funayama, M., Matsuura, E., Yoshino, H., Li, Y., Tsuyama, S., et al. (2018). Isolated nigral degeneration without pathological protein aggregation in autopsied brains with LRRK2 p.R1441H homozygous and heterozygous mutations. Acta Neuropathol. Commun. *6*: 105.

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Tan, E.K., Peng, R., Teo, Y.Y., Tan, L.C., Angeles, D., Ho, P., et al. (2010). Multiple LRRK2 variants modulate risk of Parkinson disease: a Chinese multicenter study. Hum Mutat *31*: 561–8.

Tomkins, J.E., Dihanich, S., Beilina, A., Ferrari, R., Ilacqua, N., Cookson, M.R., et al. (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., et al. (2010). Loss of leucine-rich repeat kinase 2 causes impairment of protein degradation pathways, accumulation of alpha-synuclein, and apoptotic cell death in aged mice. Proc Natl Acad Sci U A *107*: 9879–84.

Tran, T.N., Vo, T.N.N., Frei, K., and Truong, D.D. (2018). Levodopa-induced dyskinesia: clinical features, incidence, and risk factors. J. Neural Transm. Vienna Austria 1996 *125*: 1109–1117.

Tsika, E., Nguyen, A.P., Dusonchet, J., Colin, P., Schneider, B.L., and 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. Neurobiol Dis *77*: 49–61.

Wagner, J., Ryazanov, S., Leonov, A., Levin, J., Shi, S., Schmidt, F., et al. (2013). Anle138b: a novel oligomer modulator for disease-modifying therapy of neurodegenerative diseases such as prion and Parkinson's disease. Acta Neuropathol. (Berl.) *125*: 795–813.

Wang, D., Xu, L., Lv, L., Su, L.Y., Fan, Y., Zhang, D.F., et al. (2015). Association of the LRRK2 genetic polymorphisms with leprosy in Han Chinese from Southwest China. Genes Immun *16*: 112–9.

Watanabe, R., Buschauer, R., Böhning, J., Audagnotto, M., Lasker, K., Lu, T.-W., et al. (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. Exp Neurol *298*: 236–245.

Wetering-van Dongen, V.A. van de, Kalf, J.G., Wees, P.J. van der, Bloem, B.R., and Nijkrake, M.J. (2020). The Effects of Respiratory Training in Parkinson's Disease: A Systematic Review. J. Park. Dis. *10*: 1315–1333.

Whiffin, N., Armean, I.M., Kleinman, A., Marshall, J.L., Minikel, E.V., Goodrich, J.K., et al. (2020). The effect of LRRK2 loss-of-function variants in humans. Nat. Med. *26*: 869–877.

Wider, C., Dickson, D.W., and Wszolek, Z.K. (2010). Leucine-rich repeat kinase 2 gene-associated disease: redefining genotype-phenotype correlation. Neurodegener Dis *7*: 175–9.

Zhang, F.R., Huang, W., Chen, S.M., Sun, L.D., Liu, H., Li, Y., et al. (2009). Genomewide association study of leprosy. N Engl J Med *361*: 2609–18.

Zhang, J., Deng, X., Choi, H.G., Alessi, D.R., and Gray, N.S. (2012). Characterization of TAE684 as a potent LRRK2 kinase inhibitor. Bioorg Med Chem Lett *22*: 1864–9.

Zhao, H.T., John, N., Delic, V., Ikeda-Lee, K., Kim, A., Weihofen, A., et al. (2017). LRRK2 Antisense Oligonucleotides Ameliorate α-Synuclein Inclusion Formation in a Parkinson's Disease Mouse Model. Mol. Ther. Nucleic Acids *8*: 508–519.

Zimprich, A., Biskup, S., Leitner, P., Lichtner, P., Farrer, M., Lincoln, S., et al. (2004). Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. Neuron *44*: 601–7.

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Table 1: Overview of the current treatments for Parkinson's disease.

This table summarises the most commonly used PD treatments that target motor dysfunction by re-establishing the physiological dopamine pathway. However, some non-motor symptoms also respond to the treatments (Jankovic, 2008; Chaudhuri and Schapira, 2009; Duty and Jenner, 2011; AlDakheel et al., 2014; Postuma et al., 2015; Pfeiffer, 2016; Armstrong and Okun, 2020).

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	Monoamine oxidase-B inhibitors (e.g., rasagiline), catecholamine-O-methyltransferase inhibitors (e.g., entacapone)
	Glutamatergic activity decrease (promote dopamine synthesis) and other neurotransmitters	Amantadine, NMDA Receptors antagonist, cholinesterase inhibitors
	Surgical Intervention	e.g., Deep Brain Stimulation
	Non-pharmacological approaches	Exercise, rehabilitative therapy, physiotherapy
Non-motor symptoms	Approaches used in the general population	Psychiatric symptoms (e.g., depression, anxiety, psychosis): dopamine-related (Quetiapine), antidepressants, serotonin and norepinephrine reuptake inhibitors, cholinesterase inhibitors
		Cognitive impairments: cholinesterase inhibitors, monoamine oxidase-B inhibitors

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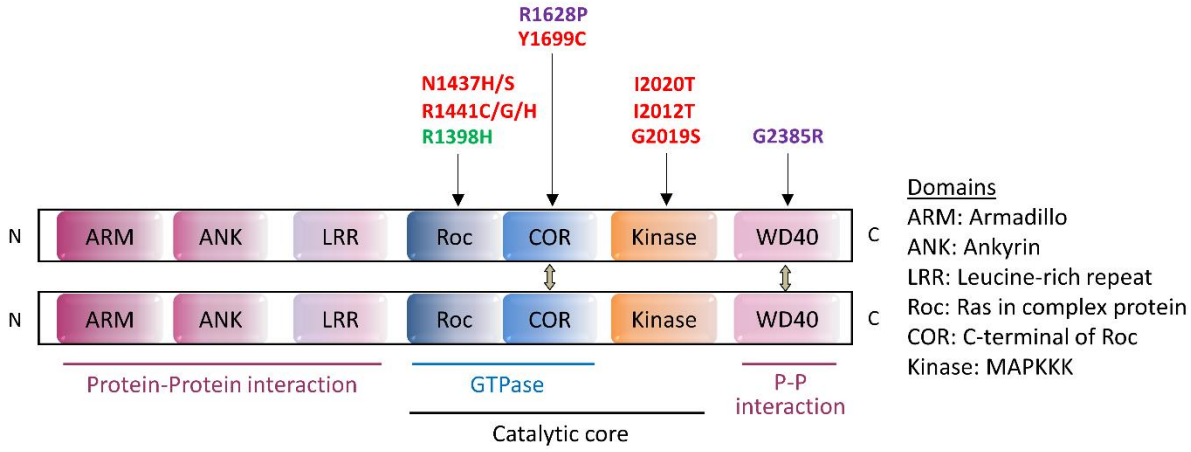

Figure 1: LRRK2 structure and mutations

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.

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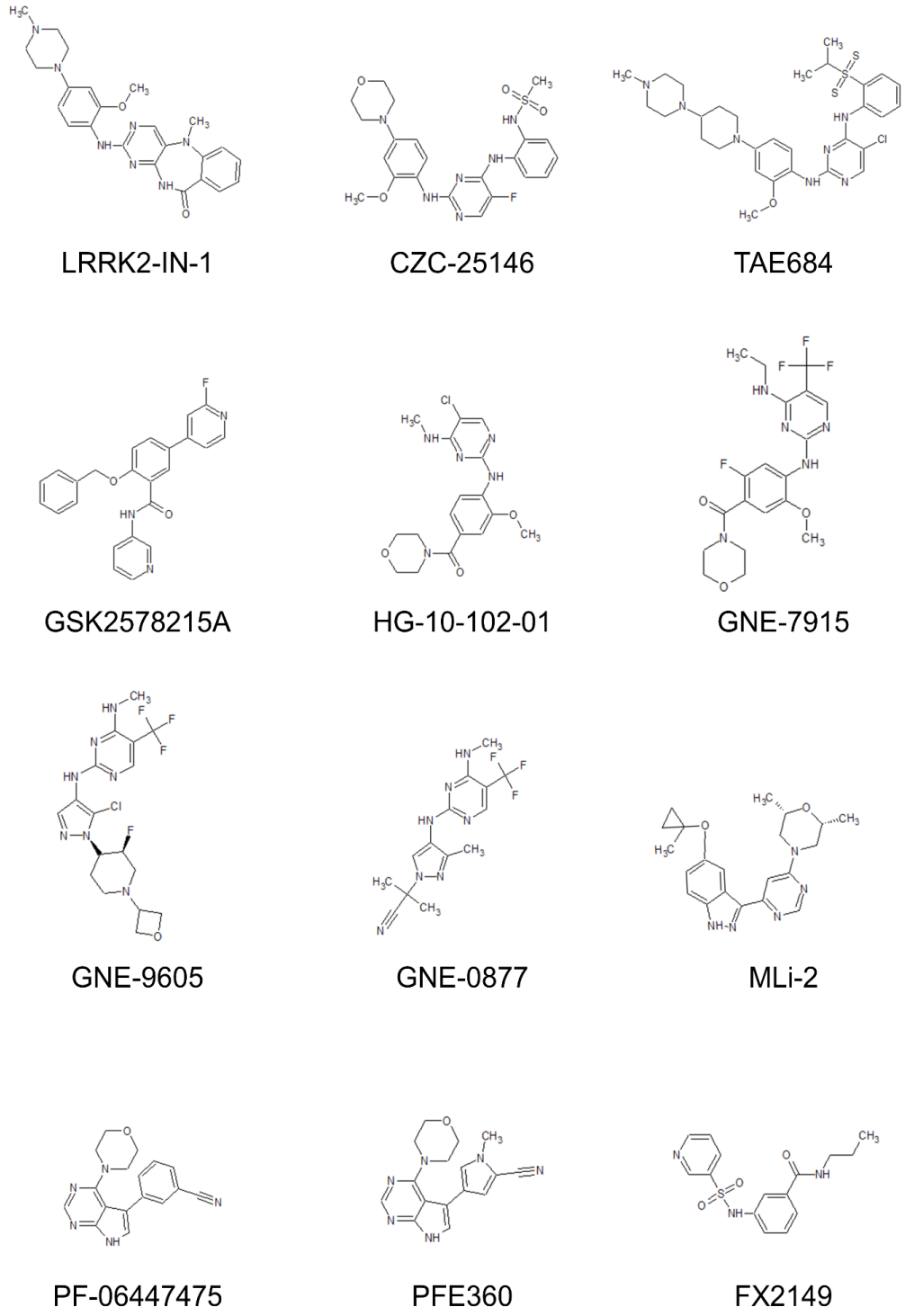

Figure 2: Chemical structures of the LRRK2 inhibitors described in this review

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.

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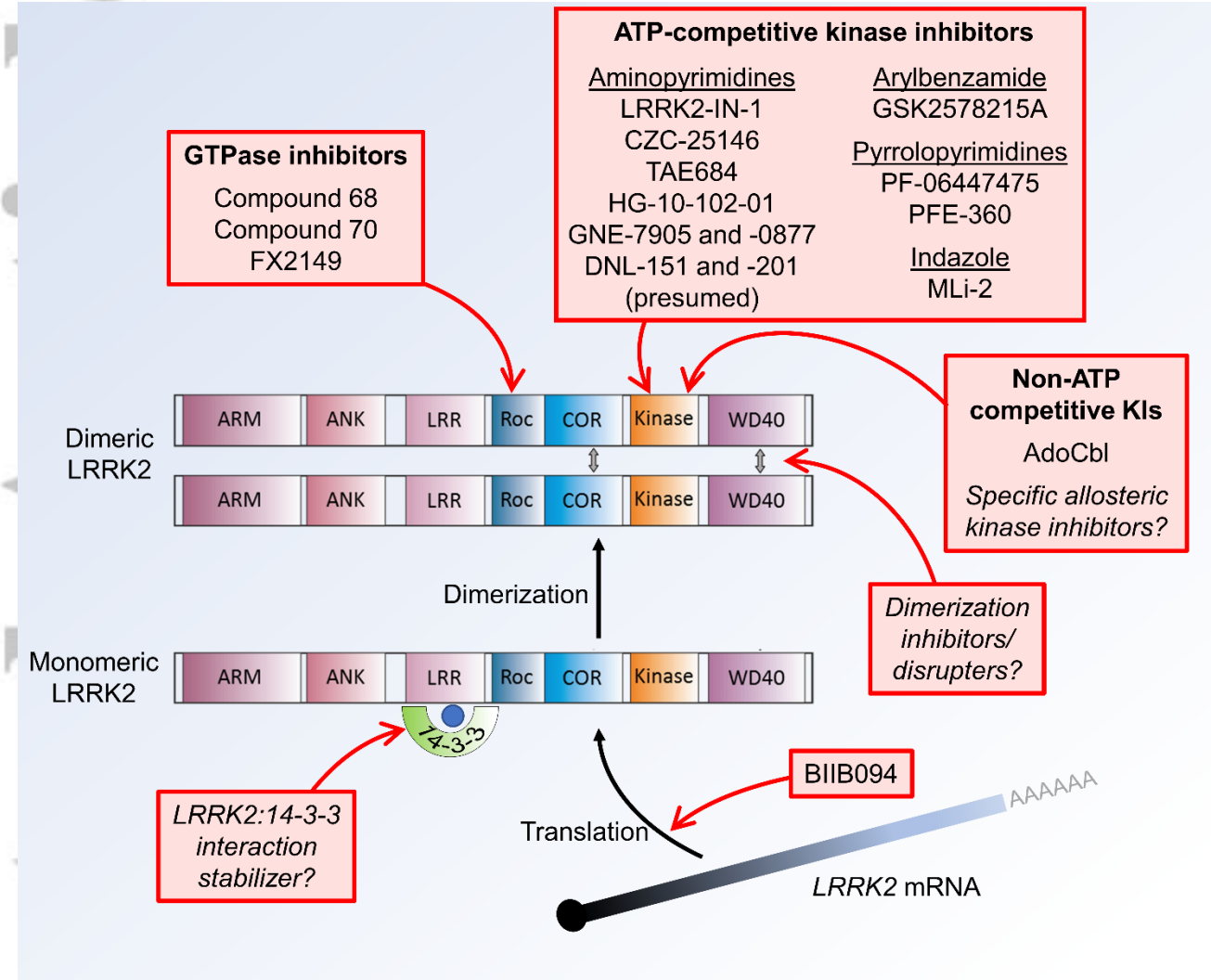

Figure 3: Sites of action of the LRRK2 inhibitors described in this review

The various types of therapeutic strategy that are under-development or have been proposed for targeting LRRK2 are summarized in the figure. Proposed strategies are written in italics. For simplicity, non-selective kinase inhibitors (e.g. staurosporine, ponatinib) are omitted. ATP-competitive kinase inhibitors are listed by chemical class; note that the categorization of DNL-151 and DNL-201 are presumed based on patents held by the manufacturer.

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