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LRRK2 Kinase Inhibition as a Therapeutic Strategy for Parkinson's Disease, Where Do We Stand?

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Abstract: One of the most promising therapeutic targets for potential disease-modifying treatment of Parkinson's disease (PD) is leucine-rich repeat kinase 2 (LRRK2). Specifically, targeting LRRK2's kinase function has generated a lot of interest from both industry and academia. This work has yielded several published studies showing the feasibility of developing potent, selective and brain permeable LRRK2 kinase inhibitors. The availability of these experimental drugs is contributing to filling in the gaps in our knowledge on the safety and efficacy of LRRK2 kinase inhibition. Recent studies of LRRK2 kinase inhibition in preclinical models point to potential undesired effects in peripheral tissues such as lung and kidney. Also, while strategies are now emerging to measure target engagement of LRRK2 inhibitors, there remains an important need to expand efficacy studies in preclinical models of progressive PD. Future work in the LRRK2 inhibition field must therefore be directed towards developing molecules and treatment regimens which demonstrate efficacy in mammalian models of disease in conditions where safety liabilities are reduced to a minimum.

Keywords: kinase inhibitor, LRRK2, Parkinson's disease, phenotypic assay.

INTRODUCTION

Parkinson's disease (PD) is a debilitating neurodegenerative movement disorder for which there is currently no cure. Neuropathologically, brains of PD patients show degeneration of dopaminergic neurons of the *substantia nigra* pars compacta, which gives rise to the motor deficits, including resting tremor, bradykinesia and postural instability. Symptomatic treatments have been available now for more than 5 decades, however their efficacy declines as the disease progresses. An important challenge in the field is therefore to develop disease-modifying therapies capable of stalling or even halting disease progression. Clues to address this challenge lay in the study of genes, called PARK genes, which are genetically linked to familial forms of PD. For example, mutations in Parkin (PARK2) and PTEN-induced putative kinase 1 (PINK1, PARK6) cause autosomal-recessive forms of PD, while mutations in α-synuclein (SNCA, PARK1/4) and mutations in leucine-rich repeat kinase type 2 (LRRK2, PARK8) are linked to autosomal-dominant forms of PD. Also, although microtubule associated protein tau (MAPT) protein deposition is a feature of Alzheimer's disease, MAPT gene mutations cause fronto temporal dementia with parkinsonism [1, 2]. Furthermore, genome-wide association studies have identified genomic variations as risk factors for sporadic PD, including at the glucosidase beta acid (GBA), SNCA, MAPT and LRRK2 genomic loci [3, 4]. Of the genes involved in PD, LRRK2 has emerged as one of the key players in PD pathogenesis.

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Leucine-rich repeat kinase 2 (LRRK2) is a complex, scaffolding protein containing ankyrin, leucine-rich and WD40 repeats, and a catalytic core with Ras-Of-Complex (ROC) GTPase and serine-threonine kinase activities [5]. LRRK2 belongs to the family of ROCOs, multidomain proteins identified in a wide range of species, from prokaryotes to eukaryotes including humans [6]. ROCO proteins possess a ROC domain invariably followed by a C-terminus Of ROC (COR) domain likely involved in protein dimerization [7, 8]. ROCO proteins, including LRRK2, have been implicated in a variety of fundamental biological processes converging in cytoskeletal and vesicle dynamics.

Interest in studying the biology of LRRK2 started in 2004 when missense mutations in the *LRRK2* gene were linked with inherited autosomal dominant Parkinson's disease (PD) [9-12]. In addition, genome-wide association studies have also revealed genomic variation a the LRRK2 locus as a risk factor for sporadic PD [3, 13, 14]. One mutation, the glycine to serine substitution in position 2019 within the activation loop of the kinase domain, was soon recognized as a common cause of PD across a number of populations [15]. Although overall prevalence of LRRK2 mutations is 2%, this can rise to up to 40% in certain population groups such as Ashkenazi Jews or Arab-Berber patients [16-18]. Finally, PD patients carrying the LRRK2 mutations show a clinical and neuropathological profile which is virtually indistinguishable from sporadic PD [19], indicating that LRRK2 contributes to a disease pathway common to both familial and sporadic PD.

The observations that this mutation confers increased kinase activity [20, 21] and that pathological kinase activity mediates cytotoxicity in cultured neurons (reviewed in [22]), attracted the interest of researchers, pharmaceutical companies

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and funding agencies to explore LRRK2 as therapeutic target for PD. More than 10 years after this discovery, considerable progress has been made toward the understanding of LRRK2 cellular function and dysfunction; however some challenges remain in the path towards further development of LRRK2 kinase inhibitors as PD therapeutics. For example, the detailed pathways that lead to PD in the presence of LRRK2 mutations are poorly understood, as is the precise role of LRRK2 kinase activity in the pathogenesis of the disease. Also, recent studies in rodents and non-human primates indicate that treatment with LRRK2 kinase inhibitor scan result in side effects in peripheral tissues [23-25], pointing to potential safety issues to be dealt with as the field moves forward.

In this review, we discuss the evidence implicating LRRK2 kinase activity in the pathogenesis of PD and the effects of LRRK2 pharmacological inhibition *in vitro* and *in vivo*. Furthermore, the potential side effects of a pharmacological therapy targeting LRRK2 kinase as well as the putative alternative strategies beyond LRRK2 inhibition for the treatment of PD are critically presented.

THE KINASE FUNCTION OF LRRK2

In 2005, West and colleagues reported for the first time that LRRK2 purified from mammalian cells undergoes autophosphorylation using *in vitro* kinase assays and that its activity is increased in the presence of LRRK2 pathogenic mutations [20]. Subsequent biochemical studies confirmed that mutant LRRK2 is hyperactive *in vitro*, but only the G2019S mutation within the activation loop of the kinase domain has been consistently reported as a gain of function mutation [26-28]. Furthermore, early reports provided one additional evidence: toxicity of LRRK2 disease mutants is dependent on its kinase activity in cultured neurons [21, 29] and *in vivo* [30]. Also, pharmacological inhibition of the kinase, with repurposed inhibitors that are moderately specific but reasonably potent, was reported to revert the toxic phenotype [30]. Based on the multiple indications implicating LRRK2 kinase activity in the pathogenesis of PD, several laboratories sought to identify LRRK2 cellular substrates. Ten years later, however, there is no unanimous consensus on what are LRRK2's heterologous substrates, although there is a consensus that LRRK2 is a substrate of itself. In fact, besides being capable of *in vitro* autophosphorylation at multiple sites with a major prevalence within the ROC domain [31, 32], LRRK2 autophosphorylation has also been confirmed *in vivo* at serine 1292 [33] (Fig. **1A**). Interestingly, in addition to G2019S, several pathogenic mutants including N1437H, R1441G/C and I2020T possess increased auto-phosphorylation when overexpressed in cells [33], strengthening the notion that mutant LRRK2 exerts its toxicity through a gain of function mechanism.

In terms of phosphorylation of heterologous proteins, a number of LRRK2 candidate substrates have been reported, although the majority of them still await for *in vivo* and/or independent validation. Nominated LRRK2 substrates are clustered within three major biological categories – (1) cytoskeleton dynamics, (2) vesicle-related processes and (3) protein transcription/translation - and there are robust functional indications linking LRRK2 with these processes. With regard to cytoskeleton dynamics, LRRK2 has been reported to phosphorylate a number of cytoskeletal-related proteins including tau [34, 35], the tau kinase MARK1 [36], tubulins [37], ARHGEF7 [38], ezrin-radixin-moesin (ERM) [39, 40] and the Drosophila microtubule (MT)-binding protein Futsch [41]. Although phosphorylation of these substrates was predominantly shown *in vitro*, additional evidence places LRRK2 at the cytoskeleton. LRRK2 physically interacts with F-actin [42] and microtubules [43] and mutant or knock-out LRRK2 cells exhibit cytoskeletal defects [42, 44, 45] and increased tubulin acetylation [46, 47]. Therefore, pathogenic LRRK2 kinase may alter the dynamics of microtubules and F-actin through abnormal phosphorylation of cytoskeletal proteins leading to pathological outcomes, which manifest as defective neurite outgrowth [48, 49] and/or axonal transport [47].

Besides being a kinase, LRRK2 can participate in intracellular signaling as scaffold by assembling heterologous complexes through its multiple protein-protein interaction domains. As intracellular organelles travel along the cytoskeleton, one interesting possibility is that LRRK2 bridges cytoskeletal and vesicular components. Accordingly, convincing evidence links LRRK2 with membrane trafficking. At the presynapse, LRRK2 was shown to control vesicle trafficking [50] *via* kinase activity [51]. At least three presynaptic LRRK2 substrates have been proposed:snapin, EndophilinA and Rab5b, all involved in synaptic vesicle exo-endocytosis [52-55]. Specifically, LRRK2 phosphorylates snapin, an adaptor protein interacting with the SNARE protein SNAP-25 [56]. Phosphorylation occurs at T117 resulting in decreased interaction with SNAP-25 and reduced exocytotic release [56]. Moreover, LRRK2 impacts synaptic endocytosis by phosphorylating Drosophila and mammalian EndophilinA at S75, a residue important for EndoA-dependent membrane tubulation [53, 54]. Of interest, phosphorylation of the Drosophila microtubule-associated protein 1B (MAP1B) homolog Futsch by LRRK2 was shown to occur at the presynapse [41]. Thus, LRRK2 may function as a hub coordinating synaptic vesicle traffic through the cytoskeleton *via* its kinase activity. Other evidence linking LRRK2 with vesicle biology is its well-described role in regulating the autophagy/lysosomal pathway. Pathogenic LRRK2 was shown to inhibit autophagy leading to accumulation of autophagic organelles whilst kinase inhibition appears to induce the autophagic flux, which could be beneficial in a therapeutic perspective [57-59].

A third biological process where LRRK2 kinase may play a role is protein translation. In 2008, Imai and collaborators reported the eukaryotic initiation factor 4E (eIF4E)-binding protein, 4E-BP1, as a potential substrate of LRRK2 kinase activity *in vitro* and in Drosophila with phosphorylation occurring at Thr37 and Thr46 [60]. The authors proposed a model where hyperphosphorylation of 4E-BP1 by mutant LRRK2 may result in excessive protein translation and consequent neurodegeneration, although the link is not obvious and certainly not direct. Of interest, a following study showed that 4E-BP functionally interacts with Drosophila LRRK to control postsynaptic protein synthesis [41], further suggesting that the major site of LRRK2 action may be the synaptic compartment. However, caution needs to be taken before translating findings made in

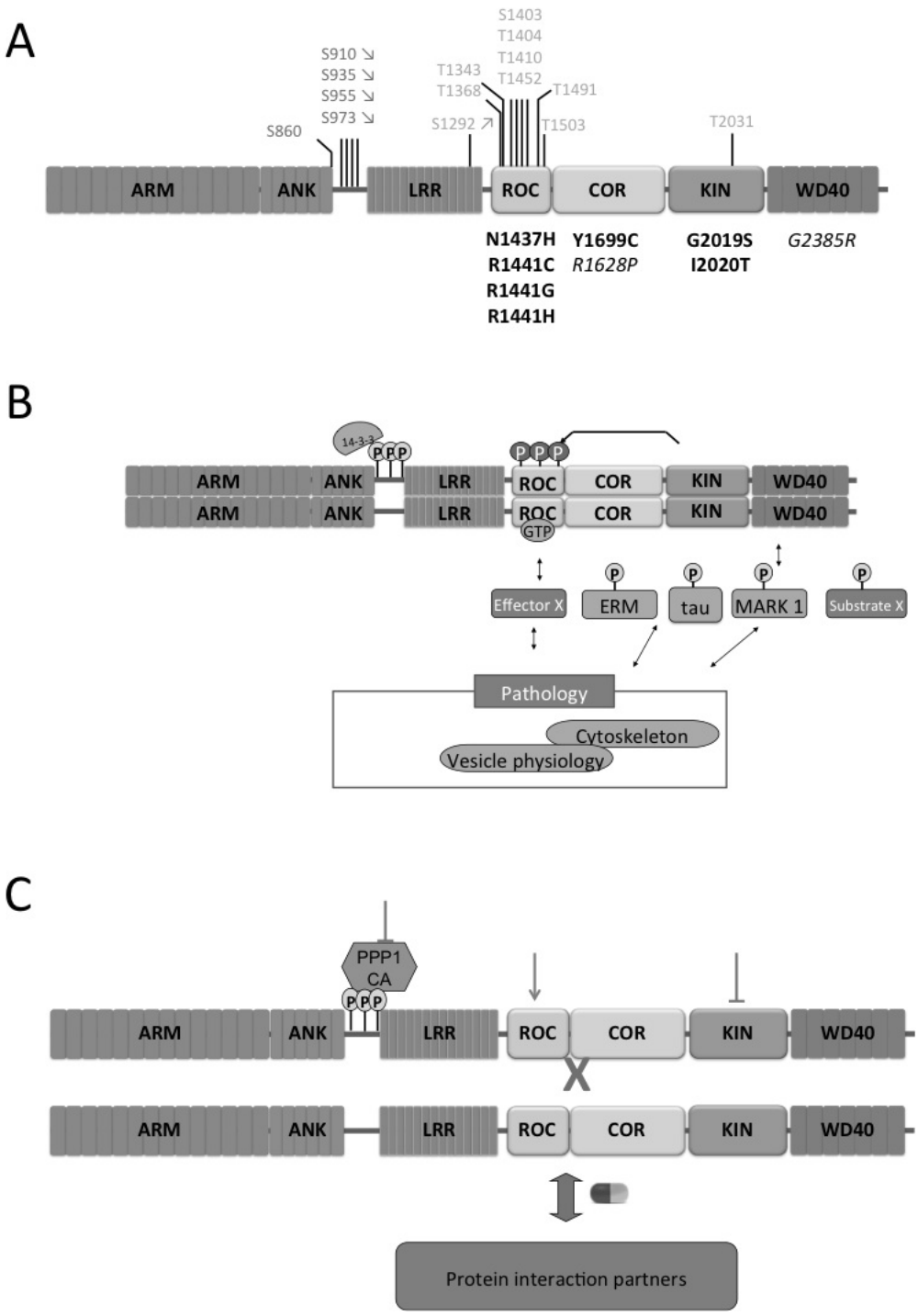

Fig. (1). A) Schematic of LRRK2 domain structure and phosphorylation sites. Sites above the schematic include cellular transphosphorylation sites (at ANK-LRR interdomain region) and autophosphorylation sites (light grey). Please note that phosphosites shown are confirmed in at least 2 independent studies, additional reported phosphosites can be found in references [27, 32, 36, 65, 115, 116]. Below the schematic, disease mutants are indicated in black, substitutions in italics are risk factor mutations. Arrows (for S910/S935/S955/S973/S1292) indicate the most common regulation of phosphorylation (up or down) observed across most disease mutants. ARM, armadillo domain; ANK, ankyrin repeat domain; LRR, leucine rich repeat domain; ROC, Ras of Complex proteins domain; COR, C-terminal of ROC domain; Kin, kinase domain; WD40, WD40 domain. **B)** Schematic of the LRRK2 signaling cascade. The structure of the LRRK2 dimer is depicted with its 7 domains (including ROC-GTPase and Kinase domains), with its phosphorylation (black P on light background) and autophosphorylation sites (white P on dark background). Kinase activity is represented by both autophosphorylation and phosphorylation of substrate. **C)** Schematic of strategies for LRRK2 targeting, including targeting kinase and GTPase functions, targeting phosphoreguylators of LRRK2 such as phosphatases, or targeting LRRK2 dimerization or interaction with cellular partners. Thus far, compounds have been reported targeting LRRK2 kinase and GTPase functions (see text for more details).

Drosophila, which possesses only one LRRK gene (LRK-1), into mammals, which instead have 2 LRRKs, LRRK1 and LRRK2. In fact, phosphorylation of 4E-BP1 could not be clearly observed in mammalian cells or brain expressing LRRK2 [61, 62], leaving 4E-BP1 a controversial substrate of human LRRK2. A link between LRRK2 and protein translation, however, has been further corroborated by the observation that Drosophila and human LRRK2 phosphorylates the ribosomal protein S15 at threonine 136 to regulate protein synthesis, with mutant LRRK2 impairing this process [63]. Interestingly, a recent chemical genetics screen for LRRK2 substrates has also revealed protein translation factors as candidate substrates for LRRK2, including eIF3C and eIF4B [36].

In conclusion, although the list of putative LRRK2 substrates is long, none of them has been independently validated or unambiguously proved using LRRK2 knock-out cells and/or phospho-specific antibodies. This does not mean that these substrates are necessary false positive, but more likely that their phosphorylation is modest and we might still miss a way (*i.e.* growth factors, hormones, neurotransmitters *etc*.) to activate LRRK2 kinase above a consistent detection threshold. A clear knowledge of LRRK2 substrates will be very important to define the detailed cellular pathways that could serve as alternative targets to LRRK2 inhibition, which, as discussed below, may not be the safest option for a therapeutic approach.

SMALL MOLECULES AGAINST LRRK2 KINASE ACTIVITY

Soon after the confirmation of LRRK2 kinase activity *in vitro*, it was found that several existing compounds, such as broad spectrum kinase inhibitors staurosporine, H-1152 or K252A, could inhibit LRRK2 kinase activity (for an overview of existing compounds which inhibit LRRK2 please see reference [64]). Two types of activity assays have primarily been used to assess LRRK2 *in vitro* kinase activity:autophosphorylation as well as phosphorylation of heterologous *in vitro* substrates, both generic or LRRK2 related. For the *in vitro* substrates, these include generic substrates such as myelin basic protein and myosin light chain as well as candidate LRRK2 substrates. However, one *in vitro* substrate has overwhelmingly been adopted, the so called lrrktide substrate derived from ERM proteins initially identified in a kinase substrate tracking and elucidation (KESTREL) screen for LRRK2 substrates [39]. At present, the lrrktide substrate *in vitro* assay or the lrrktide derived Nictide are the gold standard to test *in vitro* LRRK2 kinase activity.

Despite the large number of LRRK2 substrates reported (see above), these have disappointingly had no use in screening for cellular activity of LRRK2 kinase inhibitors, either because of difficulties in validating these assays or for lack of substrate specific reagents. Rather, cellular testing has been based on testing phosphorylation changes on LRRK2 itself. LRRK2 is highly phosphorylated including autophosphorylation sites clustering in or near the ROC domain and cellular phosphorylation sites in the ANK-LRR interdomain region (see Fig. **1A**, **1B**). Evidence for the importance of LRRK2 phosphorylation has accumulated in recent years with disease mutants showing hypophosphorylation of ANK-LRR interdomain phosphorylation sites. Also, although most autophosphorylation sites remain to be confirmed *in vivo*, several LRRK2 mutants show hyperphosphorylation of the S1292 autophosphorylation site. Dephosphorylation also leads to dynamic changes such as loss of 14-3-3 binding and shuttling to skein like structures [65, 66].

Interestingly, Dzamko, Nichols, Alessi and colleagues first found that compounds that inhibit LRRK2 kinase activity *in vitro* can induce dephosphorylation of LRRK2 at the S910-S935 sites in cells [66]. This inhibitor induced dephosphorylation of LRRK2 also affects the other phosphosites of the ANK-LRR interdomain cluster such as S955 and S973 [67], and results in a reduction of overall LRRK2 phosphorylation as measured by metabolic labeling with radioactive phosphates [65, 68]. Although this appears at first glance to be logical, it is actually counter intuitive given that S910-S935 are not autophosphorylation sites. Indeed, studies testing several LRRK2 variants with a broad range of kinase activities have found that the phosphorylation state (at the S935 cluster) is not correlated with *in vitro* kinase activity of LRRK2 mutants [28, 69].

In contrast, reports thus far have shown a good correlation between *in vitro* activity of LRRK2 kinase inhibitors and their ability to dephosphorylate LRRK2 in cells. This apparent paradox was addressed by Vancraenenbroeck *et al*. who used a kinome-wide panel of kinase inhibitors with the initial intent to identify the classes of upstream kinases involved in phosphorylating LRRK2 in cells. In order to discern inhibitors that are acting on upstream kinases from those acting on LRRK2 itself, the *in vitro* potency of the kinase panel was tested in LRRK2 using the *in vitro* peptide (lrrktide) based radiometric assay. Comparing the *in vitro* results to cellular dephosphorylation at S935 led to the conclusion that the compounds which most potently dephosphorylated LRRK2 are those which act on LRRK2 itself, a finding confirmed by *in silico* docking of the most potent compounds to the LRRK2 ATP-binding site [70]. Given that the S935 site is not an autophosphorylation site, this result was quite unexpected and suggests that binding of inhibitors to the LRRK2 kinase pocket induces recruitment of phosphatases to dephosphorylate LRRK2, similar to the observation of the induction of PP1 binding to LRRK2 after treatment with the LRRK2 inhibitor LRRK2-IN1 [68]. Although this suggests that the S935 cellular dephosphorylation is a good readout of cellular activity of LRRK2 compounds, it remains an indirect measure of LRRK2 cellular kinase activity and future efforts should be directed towards strategies to complement the S935 dephosphorylation assay, which still requires further validation for use as a readout in potential inhibitor trials [71].

In the past 5 years, these *in vitro* and cellular tests have been deployed in drug discovery and compound development studies, yielding more than 100 LRRK2 kinase inhibitors [72, 73], including compounds with strong potency (low nanomolar range), promising selectivity (*i.e.* affecting few other kinases in testing in kinome panels) and good brain permeability. Table **1** gives an overview of a selection of

small molecule LRRK2 kinase inhibitors whose structures are published. For a more detailed overview of LRRK2 kinase inhibitors, please refer to specifically dedicated reviews and patent overviews [72-76].

The recent availability of LRRK2 tool compounds induced an acceleration in our understanding of LRRK2 biology and pharmacology. The very first LRRK2 specific compound published allowed the confirmation of cellular and *in vivo* phosphorylation changes in LRRK2. LRRK2-IN1 was found to be active in peripheral tissues, but is not brain penetrant [77]. LRRK2-IN1 was also found for some cellular readouts such as neurite outgrowth in primary neurons to have identical cellular effects in normal and LRRK2 KO cells, suggesting some off-target activity of this compound [76, 78]. These observations stimulated further development of more specific and brain penetrant compounds.

As can be seen in Table **1**, these next generation LRRK2 kinase inhibitors include compounds with improvements in all areas: potency, selectivity and brain availability. For instance, while all compounds given in Table **1** show nanomolar range activity *in vitro* and in cells, the more recently developed compounds demonstrate activity at single digit nanomolar concentrations, while also showing brain penetrance and fewer off target kinase. These compounds demonstrate therefore the basic qualities required for safety and efficacy testing in preclinical models.

EFFICACY AND SAFETY OF LRRK2 INHIBITION

One of the main challenges in testing efficacy of LRRK2 kinase inhibitors is the question of which is the appropriate disease model. LRRK2 is transmitted in an autosomal dominant fashion; therefore a first strategy to develop models of LRRK2 PD is to overexpress LRRK2 mutants in cell culture and transgenic animal models. In cell culture, several authors have reported detrimental effects of LRRK2 overexpression on cell viability, however these effects are highly variable from study to study, which may explain why cellular toxicity readouts have not been widely adopted in screening of LRRK2 kinase inhibitors (reviewed in [8]). In Drosophila, neuronal degeneration has been observed both in knockout animals of the drosophila LRRK1/2 homolog LRK-1 [79] as well as in LRRK2 overexpressors [80]. Also, in small mammals, key phenotypes of disease such as dopaminergic cell loss and motor deficits are lacking or mild after LRRK2 overexpression (for detailed reviews of LRRK2 animal models, please refer to references [22, 81, 82]). Strategies of LRRK2 overexpression which do lead to nigral degeneration involve viral vector mediated overexpression of LRRK2 [30, 83] as well as combined LRRK2 and alpha-synuclein transgenic overexpression mice [84] although the combined LRRK2-alpha-synuclein toxicity phenotype is not observed in all overexpression conditions [85]. Further development of these and novelcellular and *in vivo* disease models of LRRK2 mediated cellular toxicity is required for future LRRK2 inhibitor testing.

An intermediate step in testing efficacy of LRRK2 inhibitors is to measure alternative LRRK2 cellular and *in vivo* phenotypes. In this regard, LRRK2 effects on cytoskeleton or cellular motility has attracted much interest, given the many reports that LRRK2 affects neurite complexity in cultured neurons (see above). In cell culture, a potential readout is therefore the measure of neurite complexity in cultured neurons that is expected to be increased in the presence of LRRK2 kinase inhibitors. Alternatively, a scratch assay of cellular motility of fibroblasts has been reported, in which cellular motility is reduced in the presence of LRRK2-IN1 [86]. In transgenic animal models, LRRK2 inhibitor readouts may be related to such reported phenotypes as changes in synaptic functions or dopamine release in the striatum (see above), although these readouts have yet to be tested.

Table 1. Overview of key LRRK2 kinase inhibitors.

Compound ID	*In Vitro* Activity (IC50 WT, nM)	Cellular Activity S935 (IC50 WT, nM)	Brain Penetrance	Off Target Kinases/ Total Tested	Refs.
LRRK2-IN1	13	280	No	12/442	[77]
CZC25146	5	44	Low	5/185	[106]
HG-10-102-01	20	650	Yes	2/451	[107]
GSK2578215A	11	992	Low	2/451	[108]
GNE7915	37	194	Yes	2/451	[109]
GNE-0877	1	3	Yes	4/190	[110]
GNE-9605	2	19	Yes	-	[110]
Indolinone 5 (= Nov-LRRK2-11)	9	-	Yes	-	[111]
Indolinone 15b	15	-	-	0/46	[112]
PF-06447475	3	25	Yes	3/39	[113]
JH-II-127	6,6	100-300	Yes	-	[114]

Another major issue is to address safety of LRRK2 kinase inhibition. One of the strategies to begin to assess the physiological effects of pharmacological inhibition is to study phenotypes of knockout animals. In this regard, one of the main observations in LRRK2 KO mice is a striking age-dependent impairment of protein degradation pathways in kidneys, which is reflected macroscopically by darkened kidneys in aged LRRK2 KO mice [87]. This phenotype was replicated in several other studies [24, 25, 88], which also report lung and liver abnormalities. At the cellular level, the observed abnormalities in kidney of LRRK2 KO mice and rats are an increase in size and number of secondary lysosomes in kidney proximal tubule cells. Lung type II cells of LRRK2 KOs display increases in size and number of lamellar bodies, while livers display increased accumulation of lipid droplets in hepatocytes and stellate cells.

One caveat in using LRRK2 knockouts to mimick LRRK2 kinase inhibition phenotypes is that kinase is only one of LRRK2's many functions; therefore phenotypes observed in KO animals may result from the loss of its non-kinase functions such as GTPase, dimerization or scaffolding functions. Interestingly, a first study of the effects of administering a LRRK2 kinase inhibitor to rodents (5 day treatment) showed that these reduced overall LRRK2 protein levels [25], suggesting that kinase inhibition may lead to LRRK2 protein loss and associated phenotypes. In this view, heterozygous LRRK2 mice may better represent kinase inhibition than complete knockout and, interestingly, these mice have no kidney phenotype [25, 89]. Recently, Fuji and colleagues reported a safety study in rodents and non-human primates for two selective, potent and brain penetrant compounds, administered daily for up to 29 days (see Table **2**). This study confirms the presence of dysfunctional lamellar bodies in type II lung cells of non-human primates, suggesting that abnormalities observed in LRRK2 KO animals are also induced by LRRK2 kinase inhibition.

While it can be noted that these abnormalities are not radical disturbance of normal kidney or lung functions, it remains to be assessed in how far these abnormalities in peripheral tissues constitute a safety risk relative to the potential therapeutic benefit of LRRK2 kinase inhibition in PD. These studies also suggest that alternative strategies of targeting LRRK2 should be assessed.

ALTERNATIVE STRATEGIES BEYOND KINASE INHIBITION

Given the reported side effects of LRRK2 kinase inhibition, it is important to evaluate alternative strategies to target LRRK2 function. Alternative targeting strategies for LRRK2 are schematically depicted in Fig. **1C**. The GTPase activity of LRRK2 has been so far less studied compared to its kinase activity. Mutations in the ROC-COR module have been shown to diminish GTP hydrolysis [90]. Reduced GTP hydrolysis would result in a protein locked into an active state and, presumably, in aberrant signal transduction. Moreover, several LRRK2 binding partners interact with LRRK2 ROC domain, including Sec16, a protein involved in the formation of endoplasmic reticulum exit sites (ERES) [91], PKARIIβ [92] and tubulins [93]. Notably, interaction between ROC and both PKA and Sec16 are impaired in the presence of the pathogenic mutation R1441C, further supporting a role of this domain in the pathogenesis of LRRK2-PD. GTPases such as Ras and heterotrimeric G proteins have been explored as therapeutic targets in cancer. While most kinase inhibitors are ATP-competitive, GTPase inhibitors that directly target the active site of Ras are conceptually difficult given the very high binding affinity of Ras for GTP and GDP, together with the high concentration of GTP in the cell [94]. In the case of Ras, alternative approaches have been undertaken including targeting protein-protein interaction or other regulatory mechanisms. For LRRK2, this is hindered by the limited knowledge of proteins that modulate guanosine nucleotide hydrolysis or exchange. A candidate LRRK2 GTPase activating protein (GAP), ArfGAP1, has been reported by two independent studies [95, 96], however, both studies mapped the interaction in regions other than ROC, raising the question as to whether ArfGAP1 is a *bona fide* LRRK2 GAP or, alternately, LRRK2 scaffolds ArfGAP1 in a more complex signaling network. Independent studies reported ARHGEF7 as a LRRK2 guanine nucleotide exchange factor (GEF) [38,

Table 2. Overview of studies of potential safety liability of LRRK2 kinase inhibitors in mammalian animal models.

Compound	Dose	Species	Potential Safety Liability	Refs.
GNE-7915	200 – 300 mg/kg p.o. twice daily for 15 days	Mouse	No microscopic effects observed in lungs or kidneys	[23]
	10, 50 or 100 mg/kg p.o. daily for 7 days	Rat	No microscopic effects observed in lungs or kidneys	[23]
	10, 25 or 65 mg/kg p.o. daily for 7 days or 30 mg/kg for 29 days	Cynomolgus monkey	Increased vacuolation of type II lung cells	[23]
GNE-0877	30 – 65 mg/kg p.o. twice daily for 15 days	Mouse	No microscopic effects observed in lungs or kidneys	[23]
	30, 75 or 200 mg/kg p.o. once daily for 7 days	Rat	No microscopic effects observed in lungs or kidneys	[23]
	6 or 20 mg/kg p.o. daily for 29 days	Cynomolgus monkey	Increased vacuolation of type II lung cells	[23]
Indolinone 5 (= Nov-LRRK2-11)	30 mg/kg p.o. twice daily for 5 days	Mouse	Loss of LRRK2 expression, cellular effects not tested	[25]

97], with the R1441C mutant in the ROC domain displaying reduced binding [38]. A fine mapping of the interaction interface between LRRK2-ROC and ARHGEF7 would provide important information for rationale design of allosteric compounds interfering with ARHGEF7 binding to ROC. Other studies, however, postulate that the regulation of LRRK2 GTPase activity occurs upon protein dimerization suggesting GEFs and GAPs are not required (reviewed in references [90, 98]). Further work is required to fully understand the exact mechanism of ROC/GTPase regulation.

In contrast to Ras or other small GTPases, the low affinity of LRRK2 for guanine nucleotides (in the μM range) [99] may provide the opportunity to successfully develop GTP-competitive inhibitors. Recent studies highlighted that LRRK2 GTPase inhibition may indeed represent a feasible strategy alternative to kinase inhibition. In particular, one GTP-competitive inhibitor (termed 68) was shown to reduce GTP binding, attenuate neuronal degeneration *in vitro* and decrease LPS-induced LRRK2 upregulation and microglia activation in a mouse model of neuroinflammation [100]. Although this study is interesting and demonstrates the concrete possibility of targeting LRRK2 ROC, the selectivity of this compound and the potential side effects *in vivo* still remain to be determined. The same authors recently reported the design and synthesis of a novel analog of 68, FX2149, with potent GTP and kinase inhibition activity and higher brain penetration compared to 68 [101]. It will be interesting to determine whether targeting LRRK2 GTPase activity results in similar side effects in lungs and kidneys similar to kinase inhibition or instead is less toxic.

Therapeutic strategies alternative to kinase and GTPase inhibition may be directed at blocking or interfering with LRRK2 dimerization or targeting LRRK2 substrates. LRRK2 dimerization is, at least in part, mediated by self-interaction of the ROC-COR module [8, 102], and can be monitored by native gels, size exclusion chromatography, yeast 2 hybrid, gradient centrifugation or transmission electron microscopy coupled with immunogold staining [8, 99, 102-105]. As several LRRK2 interactions have been mapped at the level of the ROC domain, future challenges may be directed at mapping the interaction at the aminoacid level to design allosteric molecules that prevent dimerization. When and if future studies will provide solid evidence of LRRK2 *in vivo* substrates, it will be also possible and desirable to consider inhibition of kinases or other enzymes downstream of LRRK2 as alternative and possibly more specific targets to turn off pathogenic LRRK2 signal transduction cascades.

CONCLUDING REMARKS

The field of LRRK2 kinase inhibition has come a long way since the first tool compounds were reported only 4 years ago. At least 100 compounds targeting LRRK2 kinase activity have been reported, and several of these have very desirable features such as low nanomolar range potency, excellent selectivity profiles and good brain penetrance. The availability of these experimental drugs is contributing to filling in the gaps in our knowledge on the safety and efficacy of LRRK2 kinase inhibition. Recent studies of LRRK2 kinase inhibition in preclinical models point to potential undesired effects in peripheral tissues such as lung and kidney. Also, while strategies are now emerging to measure target engagement of LRRK2 inhibitors, there remains an important need to expand efficacy studies in preclinical models of progressive PD. Future work in the LRRK2 inhibition field must therefore be directed towards developing molecules and treatment regimens which demonstrate efficacy in mammalian models of disease in conditions where safety liabilities are reduced to a minimum.

Outstanding Questions and Issues

- What is the best way to assess preclinical efficacy of kinase inhibitors?
- What are the cellular mechanism(s) of action of LRRK2 kinase inhibitors?
- What are the *bona fide* LRRK2 heterologous substrates?
- How is LRRK2 kinase activity regulated in the cell?
- Can the observed safety liabilities of existing compounds be improved in chronic treatment paradigms?
- Can novel efficacious LRRK2 kinase inhibitors be developed with improved safety profiles?
- Will LRRK2 inhibitors have efficacy outside of G2019S associated PD?
- Are non-kinase inhibitors more safe and/or efficacious than kinase inhibitors?

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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REFERENCES

[1] Lees, A.J.; Hardy, J.; Revesz, T. Parkinson's disease. *Lancet*, **2009**, *373*, 2055-2066. doi:10.1016/S0140-6736(09)60492-X.

[2] Schulte, C.; Gasser, T. Genetic basis of Parkinson's disease: inheritance, penetrance, and expression. *Appl. Clin. Genet.*, **2011**, *4*, 67-80. doi: 10.2147/TACG.S11639.

[3] Nalls, M.A.; Pankratz, N.; Lill, C.M.; Do, C.B.; Hernandez, D.G.; Saad, M.; DeStefano, A.L.; Kara, E.; Bras, J.; Sharma, M.; Schulte, C.; Keller, M.F.; Arepalli, S.; Letson, C.; Edsall, C.; Stefansson, H.; Liu, X.; Pliner, H.; Lee, J.H.; Cheng, R.; Ikram, M.A.; Ioannidis, J.P.A.; Hadjigeorgiou, G.M.; Bis, J.C.; Martinez, M.; Perlmutter, J.S.; Goate, A.; Marder, K.; Fiske, B.; Sutherland, M.; Xiromerisiou, G.; Myers, R.H.; Clark, L.N.; Stefansson, K.; Hardy, J.A.; Heutink, P.; Chen, H.; Wood, N.W.; Houlden, H.; Payami, H.; Brice, A.; Scott, W.K.; Gasser, T.; Bertram, L.; Eriksson, N.; Foroud, T.; Singleton, A.B. Large-scale meta-analysis of genome-

wide association data identifies six new risk loci for Parkinson's disease. *Nat. Genet.*, **2014**, *56*, 1-7. doi: 10.1038/ng.3043.

[4] Lill, C.M.; Roehr, J.T.; McQueen, M.B.; Kavvoura, F.K.; Bagade, S.; Schjeide, B.M.M.; Schjeide, L.M.; Meissner, E.; Zauft, U.; Allen, N.C.; Liu, T.; Schilling, M.; Anderson, K.J.; Beecham, G.; Berg, D.; Biernacka, J.M.; Brice, A.; DeStefano, A.L.; Do, C.B.; Eriksson, N.; Factor, S.A.; Farrer, M.J.; Foroud, T.; Gasser, T.; Hamza, T.; Hardy, J.A.; Heutink, P.; Hill-Burns, E.M.; Klein, C.; Latourelle, J.C.; Maraganore, D.M.; Martin, E.R.; Martinez, M.; Myers, R.H.; Nalls, M.A.; Pankratz, N.; Payami, H.; Satake, W.; Scott, W.K.; Sharma, M.; Singleton, A.B.; Stefansson, K.; Toda, T.; Tung, J.Y.; Vance, J.; Wood, N.W.; Zabetian, C.P.; Young, P.; Tanzi, R.E.; Khoury, M.J.; Zipp, F.; Lehrach, H.; Ioannidis, J.P.A.; Bertram, L. Comprehensive research synopsis and systematic meta-analyses in Parkinson's disease genetics: The PDGene database. *PLoS Genet.*, **2012**, *8*(3), e1002548. doi: 10.1371/journal.pgen.1002548

[5] Cookson, M.R. The role of leucine-rich repeat kinase 2 (LRRK2) in Parkinson's disease. *Nat. Rev. Neurosci.*, **2010**, *11*, 791-797. doi: 10.1038/nrn2935.

[6] Civiero, L.; Dihanich, S.; Lewis, P.A.; Greggio, E. Genetic, Structural, and Molecular Insights into the Function of Ras of Complex Proteins Domains. *Chem. Biol.*, **2014**, *21*, 809-818. doi: 10.1016/j.chembiol.2014.05.010.

[7] Gotthardt, K.; Weyand, M.; Kortholt, A.; Van Haastert, P.J.M.; Wittinghofer, A. Structure of the Roc-COR domain tandem of C. tepidum, a prokaryotic homologue of the human LRRK2 Parkinson kinase. *EMBO J.*, **2008**, *27*, 2239-2249. doi: 10.1038/emboj.2008.150.

[8] Daniëls, V.; Vancraenenbroeck, R.; Law, B.M.H.; Greggio, E.; Lobbestael, E.; Gao, F.; De Maeyer, M.; Cookson, M.R.; Harvey, K.; Baekelandt, V.; Taymans, J.M. Insight into the mode of action of the LRRK2 Y1699C pathogenic mutant. *J. Neurochem.*, **2011**, *116*, 304-315. doi: 10.1111/j.1471-4159.2010.07105.x.

[9] Paisán-Ruíz, C.; Jain, S.; Evans, E.W.; Gilks, W.P.; Simón, J.; van der Brug, M.; López de Munain, A.; Aparicio, S.; Gil, A.M.; Khan, N.; Johnson, J.; Martinez, J.R.; Nicholl, D.; Carrera, I.M.; Pena, A.S.; de Silva, R.; Lees, A.; Martí-Massó, J.F.; Pérez-Tur, J.; Wood, N.W.; Singleton, A.B. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. *Neuron*, **2004**, *44*, 595-600. http://dx.doi.org/10.1016/j.neuron.2004.10.023

[10] Zimprich, A.; Biskup, S.; Leitner, P.; Lichtner, P.; Farrer, M.; Lincoln, S.; Kachergus, J.; Hulihan, M.; Uitti, R.J.; Calne, D.B.; Stoessl, A.J.; Pfeiffer, R.F.; Patenge, N.; Carbajal, I.C.; Vieregge, P.; Asmus, F.; Müller-Myhsok, B.; Dickson, D.W.; Meitinger, T.; Strom, T.M.; Wszolek, Z.K.; Gasser, T. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. Neuron, **2004**, *44*(4), 601-607. http://dx.doi.org/10.1016/j.neuron.2004.11.005

[11] Di Fonzo, A.; Rohé, C.F.; Ferreira, J.; Chien, H.F.; Vacca, L.; Stocchi, F.; Guedes, L.; Fabrizio, E.; Manfredi, M.; Vanacore, N.; Goldwurm, S.; Breedveld, G.; Sampaio, C.; Meco, G.; Barbosa, E.; Oostra, B.A.; Bonifati, V. A frequent LRRK2 gene mutation associated with autosomal dominant Parkinson's disease. *Lancet*, **2005**, *365*, 412-415. http://dx.doi.org/10.1016/S0140-6736(05)17829-5

[12] Gilks, W.P.; Abou-Sleiman, P.M.; Gandhi, S.; Jain, S.; Singleton, A.; Lees, A.J.; Shaw, K.; Bhatia, K.P.; Bonifati, V.; Quinn, N.P.; Lynch, J.; Healy, D.G.; Holton, J.L.; Revesz, T.; Wood, N.W. A common LRRK2 mutation in idiopathic Parkinson's disease. *Lancet*, **2005**, *365*, 415-416. http://dx.doi.org/10.1016/s0140-6736(05)17830-1

[13] Simón-Sánchez, J.; Schulte, C.; Bras, J.M.; Sharma, M.; Gibbs, J.R.; Berg, D.; Paisan-Ruiz, C.; Lichtner, P.; Scholz, S.W.; Hernandez, D.G.; Krüger, R.; Federoff, M.; Klein, C.; Goate, A.; Perlmutter, J.; Bonin, M.; Nalls, M.A.; Illig, T.; Gieger, C.; Houlden, H.; Steffens, M.; Okun, M.S.; Racette, B.A.; Cookson, M.R.; Foote, K.D.; Fernandez, H.H.; Traynor, B.J.; Schreiber, S.; Arepalli, S.; Zonozi, R.; Gwinn, K.; van der Brug, M.; Lopez, G.; Chanock, S.J.; Schatzkin, A.; Park, Y.; Hollenbeck, A.; Gao, J.; Huang, X.; Wood, N.W.; Lorenz, D.; Deuschl, G.; Chen, H.; Riess, O.; Hardy, J.A.; Singleton, A.B.; Gasser, T.; Brug, M. Van Der; Simon-sanchez, J.; Gasse, T. Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nat. Genet.*, **2009**, *41*, 1308-1312. http://dx.doi.org/10.1038/ng.487

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

[15] Correia Guedes, L.; Ferreira, J.J.; Rosa, M.M.; Coelho, M.; Bonifati, V.; Sampaio, C. Worldwide frequency of G2019S LRRK2 mutation in Parkinson's disease: a systematic review. *Parkinsonism Relat. Disord.*, **2010**, *16*, 237-242. http://dx.doi.org/10.1016/j.parkreldis.2009.11.004

[16] Hulihan, M.M.; Ishihara-Paul, L.; Kachergus, J.; Warren, L.; Amouri, R.; Elango, R.; Prinjha, R.K.; Upmanyu, R.; Kefi, M.; Zouari, M., Sassi, S. Ben, Yahmed, S. Ben, El Euch-Fayeche, G., Matthews, P.M.; Middleton, L.T.; Gibson, R.A; Hentati, F.; Farrer, M.J. LRRK2 Gly2019Ser penetrance in Arab-Berber patients from Tunisia: a case-control genetic study. *Lancet Neurol.*, **2008**, *7*, 591-594. http://dx.doi.org/10.1016/S1474-4422(08)70116-9

[17] Ozelius, L.J.; Senthil, G.; Saunders-Pullman, R.; Ohmann, E.; Deligtisch, A.; Tagliati, M.; Hunt, A.L.; Klein, C.; Henick, B.; Hailpern, S.M.; Lipton, R.B.; Soto-Valencia, J.; Risch, N.; Bressman, S.B. LRRK2 G2019S as a cause of Parkinson's disease in Ashkenazi Jews. *N. Engl. J. Med.*, **2006**, *354*, 424-425. http://dx.doi.org/10.1056/NEJMc055509

[18] Lesage, S.; Dürr, A.; Tazir, M.; Lohmann, E.; Leutenegger, A.L.; Janin, S.; Pollak, P.; Brice, A. LRRK2 G2019S as a cause of Parkinson's disease in North African Arabs. *N. Engl. J. Med.*, **2006**, *354*, 422-423. http://dx.doi.org/10.1056/NEJMc055540

[19] Healy, D.G.; Falchi, M.; O'Sullivan, S.S.; Bonifati, V.; Durr, A.; Bressman, S.; Brice, A.; Aasly, J.; Zabetian, C.P.; Goldwurm, S.; Ferreira, J.J.; Tolosa, E.; Kay, D.M.; Klein, C.; Williams, D.R.; Marras, C.; Lang, A.E.; Wszolek, Z.K.; Berciano, J.; Schapira, A.H.V.; Lynch, T.; Bhatia, K.P.; Gasser, T.; Lees, A.J.; Wood, N.W. Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. *Lancet Neurol.*, **2008**, *7*, 583-590. http://dx.doi.org/10.1016/S1474-4422(08)70117-0

[20] West, A.B.; Moore, D.J.; Biskup, S.; Bugayenko, A.; Smith, W.W.; Ross, C.A.; Dawson, V.L.; Dawson, T.M. Parkinson's disease-associated mutations in leucine-rich repeat kinase 2 augment kinase activity. *Proc. Natl. Acad. Sci. U.S.A.*, **2005**, *102*, 16842-16847. http://dx.doi.org/10.1073/pnas.0507360102

[21] Greggio, E.; Jain, S.; Kingsbury, A.; Bandopadhyay, R.; Lewis, P.; Kaganovich, A.; van der Brug, M.P.; Beilina, A.; Blackinton, J.; Thomas, K.J.; Ahmad, R.; Miller, D.W.; Kesavapany, S.; Singleton, A.; Lees, A.; Harvey, R.J.; Harvey, K.; Cookson, M.R. Kinase activity is required for the toxic effects of mutant LRRK2/dardarin. *Neurobiol. Dis.*, **2006**, *23*, 329-341. http://dx.doi.org/10.1016/j.nbd.2006.04.001

[22] Daniëls, V.; Baekelandt, V.; Taymans, J.M. On the road to leucine-rich repeat kinase 2 signalling: evidence from cellular and *in vivo* studies. *Neurosignals.*, **2011**, *19*, 1-15. http://dx.doi.org/10.1159/000324488

[23] Fuji, R.N.; Flagella, M.; Baca, M.; S Baptista, M.A.; Brodbeck, J.; Chan, B.K.; Fiske, B.K.; Honigberg, L.; Jubb, A.M.; Katavolos, P.; Lee, D.W.; Lewin-Koh, S.C.; Lin, T.; Liu, X.; Liu, S.; Lyssikatos, J.P.; O'Mahony, J.; Reichelt, M.; Roose-Girma, M.; Sheng, Z.; Sherer, T.; Smith, A.; Solon, M.; Sweeney, Z.K.; Tarrant, J.; Urkowitz, A.; Warming, S.; Yaylaoglu, M.; Zhang, S.; Zhu, H.; Estrada, A.A.; Watts, R.J. Effect of selective LRRK2 kinase inhibition on nonhuman primate lung. *Sci. Transl. Med.*, **2015**, *7*, 273ra15.

[24] Baptista, M.A.S.; Dave, K.D.; Frasier, M.A.; Sherer, T.B.; Greeley, M.; Beck, M.J.; Varsho, J.S.; Parker, G.A.; Moore, C.; Churchill, M.J.; Meshul, C.K.; Fiske, B.K. Loss of Leucine-Rich Repeat Kinase 2 (LRRK2) in Rats Leads to Progressive Abnormal Phenotypes in Peripheral Organs. *PLoS One*, **2013**, *8*, e80705. http://dx.doi.org/10.1371/journal.pone.0080705

[25] Herzig, M.C.; Kolly, C.; Persohn, E.; Theil, D.; Schweizer, T.; Hafner, T.; Stemmelen, C.; Troxler, T.J.; Schmid, P.; Danner, S.; Schnell, C.R.; Mueller, M.; Kinzel, B.; Grevot, A.; Bolognani, F.; Stirn, M.; Kuhn, R.R.; Kaupmann, K.; van der Putten, P.H.; Rovelli, G.; Shimshek, D.R. LRRK2 protein levels are determined

by kinase function and are crucial for kidney and lung homeostasis in mice. *Hum. Mol. Genet.*, **2011**, *20*, 4209-4223. http://dx.doi.org/10.1093/hmg/ddr348

[26] Greggio, E.; Cookson, M.R. Leucine-rich repeat kinase 2 mutations and Parkinson's disease: three questions. *ASN Neuro1*., **2009**, 13-24. http://dx.doi.org/10.1042/AN20090007

[27] Kamikawaji, S.; Ito, G.; Iwatsubo, T.; Accepted, J. Identification of the autophosphorylation sites of LRRK2. Biochemistry, **2009**, *48*, 10963-10975. http://dx.doi.org/10.1021/bi9011379

[28] Ito, G.; Fujimoto, T.; Kamikawaji, S.; Kuwahara, T.; Iwatsubo, T. Lack of Correlation between the Kinase Activity of LRRK2 Harboring Kinase-Modifying Mutations and Its Phosphorylation at Ser910, 935, and Ser955. *PLoS One*, **2014**, *9*, e97988. http://dx.doi.org/10.1371/journal.pone.0097988

[29] Smith, W.W.; Pei, Z.; Jiang, H.; Dawson, V.L.; Dawson, T.M.; Ross, C.A. Kinase activity of mutant LRRK2 mediates neuronal toxicity. *Nat. Neurosci.*, **2006**, *9*, 1231-1233. http://dx.doi.org/10.1038/nn1776

[30] Lee, B.D.; Shin, J.H.; Vankampen, J.; Petrucelli, L.; West, A.B.; Ko, H.S.; Lee, Y.I.; Maguire-Zeiss, K.A.; Bowers, W.J.; Federoff, H.J.; Dawson, V.L.; Dawson, T.M. Inhibitors of leucine-rich repeat kinase-2 protect against models of Parkinson's disease. *Nat. Med.*, **2010**, *16*, 998-1000. http://dx.doi.org/10.1038/nm.2199

[31] Greggio, E.; Taymans, J.; Zhen, E.Y.; Ryder, J.; Vancraenenbroeck, R.; Beilina, A.; Sun, P.; Deng, J.; Jaffe, H.; Baekelandt, V.; Merchant, K.; Cookson, M.R.; Yuejun, E. The Parkinson's disease kinase LRRK2 autophosphorylates its GTPase domain at multiple sites. *Biochem. Biophys. Res. Commun.*, **2009**, *389*, 449-454. http://dx.doi.org/10.1016/j.bbrc.2009.08.163

[32] Gloeckner, C.J.; Boldt, K.; von Zweydorf, F.; Helm, S.; Wiesent, L.; Sarioglu, H.; Ueffing, M. Phosphopeptide analysis reveals two discrete clusters of phosphorylation in the N-terminus and the Roc domain of the Parkinson-disease associated protein kinase LRRK2. *J. Proteome Res.*, **2010**, *9*, 1738-1745. http://dx.doi.org/10.1021/pr9008578

[33] Sheng, Z.; Zhang, S.; Bustos, D.; Kleinheinz, T.; Le Pichon, C.E.; Dominguez, S.L.; Solanoy, H.O.; Drummond, J.; Zhang, X.; Ding, X.; Cai, F.; Song, Q.; Li, X.; Yue, Z.; van der Brug, M.P.; Burdick, D. J.; Gunzner-Toste, J.; Chen, H.; Liu, X.; Estrada, A.A.; Sweeney, Z.K.; Scearce-Levie, K.; Moffat, J.G.; Kirkpatrick, D.S.; Zhu, H. Ser1292 autophosphorylation is an indicator of LRRK2 kinase activity and contributes to the cellular effects of PD mutations. *Sci. Transl. Med.*, **2012**, *4*, 164ra161. http://dx.doi.org/10.1126/scitranslmed.3004485

[34] Kawakami, F.; Yabata, T.; Ohta, E.; Maekawa, T.; Shimada, N.; Suzuki, M.; Maruyama, H.; Ichikawa, T.; Obata, F. LRRK2 phosphorylates tubulin-associated tau but not the free molecule: LRRK2-mediated regulation of the tau-tubulin association and neurite outgrowth. *PLoS One*, **2012**, *7*, e30834. http://dx.doi.org/10.1371/journal.pone.0030834

[35] Bailey, R.M.; Covy, J.P.; Melrose, H.L.; Rousseau, L.; Watkinson, R.; Knight, J.; Miles, S.; Farrer, M.J.; Dickson, D.W.; Giasson, B.I.; Lewis, J. LRRK2 phosphorylates novel tau epitopes and promotes tauopathy. *Acta Neuropathol.*, **2013**, *126*, 809-827. http://dx.doi.org/10.1007/s00401-013-1188-4

[36] Krumova, P.; Reyniers, L.; Meyer, M.; Lobbestael, E.; Stauffer, D.; Gerrits, B.; Muller, L.; Hoving, S.; Kaupmann, K.; Voshol, J.; Fabbro, D.; Bauer, A.; Rovelli, G.; Taymans, J.M.; Bouwmeester, T.; Baekelandt, V. Chemical genetic approach identifies microtubule affinity-regulating kinase 1 as a leucine-rich repeat kinase 2 substrate. *FASEB J.*, **2015**, 1-13. http://dx.doi.org/10.1096/fj.14-262329

[37] Gillardon, F. Leucine-rich repeat kinase 2 phosphorylates brain tubulin-beta isoforms and modulates microtubule stability--a point of convergence in parkinsonian neurodegeneration? *J. Neurochem.* **2009**, *110*, 1514-1522. http://dx.doi.org/10.1111/j.1471-4159.2009.06235.x

[38] Haebig, K.; Gloeckner, C.J.; Miralles, M.G.; Gillardon, F.; Schulte, C.; Riess, O.; Ueffing, M.; Biskup, S.; Bonin, M. ARHGEF7 (Beta-PIX) acts as guanine nucleotide exchange factor for leucine-rich repeat kinase 2. *PLoS One*, **2010**, *5*, e13762. http://dx.doi.org/10.1371/journal.pone.0013762

[39] Jaleel, M.; Nichols, R.J.; Deak, M.; Campbell, D.G.; Gillardon, F.; Knebel, A.; Alessi, D.R. LRRK2 phosphorylates moesin at threonine-558: characterization of how Parkinson's disease mutants affect kinase activity. *Biochem. J.*, **2007**, *405*, 307-317. http://dx.doi.org/10.1042/BJ20070209

[40] Parisiadou, L.; Xie, C.; Cho, H.J.; Lin, X.; Gu, X.L.; Long, C.X.; Lobbestael, E.; Baekelandt, V.; Taymans, J.M.; Sun, L.; Cai, H. Phosphorylation of ezrin/radixin/moesin proteins by LRRK2 promotes the rearrangement of actin cytoskeleton in neuronal morphogenesis. *J. Neurosci.*, **2009**, *29*, 13971-13980. http://dx.doi.org/10.1523/JNEUROSCI.3799-09.2009

[41] Lee, S.; Liu, H.P.; Lin, W.Y.; Guo, H.; Lu, B. LRRK2 kinase regulates synaptic morphology through distinct substrates at the presynaptic and postsynaptic compartments of the Drosophila neuromuscular junction. *J. Neurosci.*, **2010**, *30*, 16959-16969. http://dx.doi.org/10.1523/JNEUROSCI.1807-10.2010

[42] Meixner, A.; Boldt, K.; Van Troys, M.; Askenazi, M.; Gloeckner, C. J.; Bauer, M.; Marto, J.A.; Ampe, C.; Kinkl, N.; Ueffing, M. A QUICK screen for Lrrk2 interaction partners--leucine-rich repeat kinase 2 is involved in actin cytoskeleton dynamics. *Mol. Cell. Proteomics*, **2011**, *10*, M110.001172.

[43] Gandhi, P.N.; Wang, X.; Zhu, X.; Chen, S.G.; Wilson-Delfosse, A. L. The Roc domain of leucine-rich repeat kinase 2 is sufficient for interaction with microtubules. *J. Neurosci. Res.*, **2008**, *86*, 1711-1720. http://dx.doi.org/10.1002/jnr.21622

[44] Caesar, M.; Felk, S.; Aasly, J.O.; Gillardon, F. Changes in actin dynamics and F-actin structure both in synaptoneurosomes of LRRK2(R1441G) mutant mice and in primary human fibroblasts of LRRK2(G2019S) mutation carriers. *Neuroscience*, **2015**, *284*, 311-324. http://dx.doi.org/10.1016/j.neuroscience.2014.09.070

[45] Häbig, K.; Gellhaar, S.; Heim, B.; Djuric, V.; Giesert, F.; Wurst, W.; Walter, C.; Hentrich, T.; Riess, O.; Bonin, M. LRRK2 guides the actin cytoskeleton at growth cones together with ARHGEF7 and Tropomyosin 4. *Biochim. Biophys. Acta*, **2013**, *1832*, 2352-2367.

[46] Law, B.M.H.; Spain, V.A.; Leinster, V.H.L.; Chia, R.; Beilina, A.; Cho, H.J.; Taymans, J.M.; Urban, M.K.; Sancho, R.M.; Ramírez, M.B.; Biskup, S.; Baekelandt, V.; Cai, H.; Cookson, M.R.; Berwick, D.C.; Harvey, K. A Direct Interaction between Leucine-rich Repeat Kinase 2 and Specific β-Tubulin Isoforms Regulates Tubulin Acetylation. *J. Biol. Chem.*, **2014**, *289*, 895-908. http://dx.doi.org/10.1074/jbc.M113.507913

[47] Godena, V.K.; Brookes-Hocking, N.; Moller, A.; Shaw, G.; Oswald, M.; Sancho, R.M.; Miller, C.C.J.; Whitworth, A.J.; De Vos, K.J. Increasing microtubule acetylation rescues axonal transport and locomotor deficits caused by LRRK2 Roc-COR domain mutations. *Nat. Commun.*, **2014**, *5*, 5245. http://dx.doi.org/10.1038/ncomms6245

[48] Macleod, D.; Dowman, J.; Hammond, R.; Leete, T.; Inoue, K.; Abeliovich, A. The familial Parkinsonism gene LRRK2 regulates neurite process morphology. *Neuron*, **2006**, *52*, 587-593. http://dx.doi.org/10.1016/j.neuron.2006.10.008

[49] Winner, B.; Melrose, H.L.; Zhao, C.; Hinkle, K.M.; Yue, M.; Kent, C.; Braithwaite, A.T.; Ogholikhan, S.; Aigner, R.; Winkler, J.; Farrer, M.J.; Gage, F.H. Adult neurogenesis and neurite outgrowth are impaired in LRRK2 G2019S mice. *Neurobiol. Dis.*, **2011**, *41*, 706-716. http://dx.doi.org/10.1016/j.nbd.2010.12.008

[50] Piccoli, G.; Condliffe, S.B.; Bauer, M.; Giesert, F.; Boldt, K.; De Astis, S.; Meixner, A.; Sarioglu, H.; Vogt-Weisenhorn, D.M.; Wurst, W.; Gloeckner, C.J.; Matteoli, M.; Sala, C.; Ueffing, M. LRRK2 controls synaptic vesicle storage and mobilization within the recycling pool. *J. Neurosci.*, **2011**, *31*, 2225-2237. http://dx.doi.org/10.1523/JNEUROSCI.3730-10.2011

[51] Cirnaru, M.D.; Marte, A.; Belluzzi, E.; Russo, I.; Gabrielli, M.; Longo, F.; Arcuri, L.; Murru, L.; Bubacco, L.; Matteoli, M.; Fedele, E.; Sala, C.; Passafaro, M.; Morari, M.; Greggio, E.; Onofri, F.; and Piccoli, G. LRRK2 kinase activity regulates synaptic vesicle trafficking and neurotransmitter release through modulation of LRRK2 macro-molecular complex. *Front. Mol. Neurosci.*, **2014**, *7*, 49. http://dx.doi.org/10.3389/fnmol.2014.00049

[52] Yun, H.J.; Park, J.; Ho, D.H.; Kim, H.; Kim, C.H.; Oh, H.; Ga, I.; Seo, H.; Chang, S.; Son, I.; Seol, W. LRRK2 phosphorylates Snapin and inhibits interaction of Snapin with SNAP-25. *Exp. Mol. Med.*, **2013**, *45*, e36 http://dx.doi.org/10.1038/emm.2013.68.

[53] Matta, S.; Van Kolen, K.; da Cunha, R.; van den Bogaart, G.; Mandemakers, W.; Miskiewicz, K.; De Bock, P.J.; Morais, V.A.; Vilain, S.; Haddad, D.; Delbroek, L.; Swerts, J.; Chávez-Gutiérrez, L.; Esposito, G.; Daneels, G.; Karran, E.; Holt, M.; Gevaert, K.;

Moechars, D.W.; De Strooper, B.; Verstreken, P. LRRK2 controls an EndoA phosphorylation cycle in synaptic endocytosis. *Neuron*, **2012**, *75*, 1008-1021. http://dx.doi.org/10.1016/j.neuron.2012.08.022

[54] Arranz, A.M.; Delbroek, L.; Van Kolen, K.; Guimarães, M.R.; Mandemakers, W., Daneels, G., Matta, S., Calafate, S., Shaban, H., Baatsen, P.; De Bock, P.J.; Gevaert, K.; Vanden Berghe, P.; Verstreken, P.; De Strooper, B.; Moechars, D. LRRK2 functions in synaptic vesicle endocytosis through a kinase-dependent mechanism. *J. Cell Sci.*, **2014**, *44*, 1-11.

[55] Yun, H.J.; Kim, H.; Ga, I.; Oh, H.; Ho, D.H.; Kim, J.; Seo, H.; Son, I.; Seol, W. An early endosome regulator, Rab5b, is an LRRK2 kinase substrate. *J. Biochem.*, **2015**. http://dx.doi.org/10.1093/jb/mvv005

[56] Yun, H. J.; Park, J.; Ho, D.H.; Kim, H.; Kim, C.H.; Oh, H.; Ga, I.; Seo, H.; Chang, S.; Son, I.; Seol, W. LRRK2 phosphorylates Snapin and inhibits interaction of Snapin with SNAP-25. *Exp. Mol. Med.*, **2013**, *45*, e36. http://dx.doi.org/10.1038/emm.2013.68

[57] Schapansky, J.; Nardozzi, J.D.; Felizia, F.; Lavoie, M.J. Membrane recruitment of endogenous LRRK2 precedes its potent regulation of autophagy. *Hum. Mol. Genet.*, **2014**, 1-14. http://dx.doi.org/10.1093/hmg/ddu138

[58] Gómez-Suaga, P.; Luzón-Toro, B.; Churamani, D.; Zhang, L.; Bloor-Young, D.; Patel, S.; Woodman, P.G.; Churchill, G.C.; Hilfiker, S. Leucine-rich repeat kinase 2 regulates autophagy through a calcium-dependent pathway involving NAADP. *Hum. Mol. Genet.*, **2012**, *21*, 511-525. http://dx.doi.org/10.1093/hmg/ddr481

[59] Manzoni, C.; Mamais, A.; Dihanich, S.; Abeti, R.; Soutar, M.P.M.; Plun-Favreau, H.; Giunti, P.; Tooze, S.A.; Bandopadhyay, R.; Lewis, P.A. Inhibition of LRRK2 kinase activity stimulates macroautophagy. *Biochim. Biophys. Acta*, **2013**, *1833*, 2900-2910.

[60] Imai, Y.; Gehrke, S.; Wang, H.Q.; Takahashi, R.; Hasegawa, K.; Oota, E.; Lu, B. Phosphorylation of 4E-BP by LRRK2 affects the maintenance of dopaminergic neurons in Drosophila. *EMBO J.*, **2008**, *27*, 2432-2443. http://dx.doi.org/10.1038/emboj.2008.163

[61] Trancikova, A.; Mamais, A.; Webber, P.J.; Stafa, K.; Tsika, E.; Glauser, L.; West, A.B.; Bandopadhyay, R.;Moore, D.J. Phosphorylation of 4E-BP1 in the mammalian brain is not altered by LRRK2 expression or pathogenic mutations. *PLoS One*, **2012**, *7*, e47784. http://dx.doi.org/10.1371/journal.pone.0047784

[62] Kumar, A.; Greggio, E.; Beilina, A.; Kaganovich, A.; Chan, D.; Taymans, J.M.; Wolozin, B.; Cookson, M.R. The Parkinson's disease associated LRRK2 exhibits weaker *in vitro* phosphorylation of 4E-BP compared to autophosphorylation. *PLoS One*, **2010**, *5*, e8730. http://dx.doi.org/10.1371/journal.pone.0008730

[63] Martin, I.; Kim, J.W.; Lee, B.D.; Kang, H.C.; Xu, J.C.; Jia, H.; Stankowski, J.; Kim, M.S.; Zhong, J.; Kumar, M.; Andrabi, S.A.; Xiong, Y.; Dickson, D.W.; Wszolek, Z.K.; Pandey, A.; Dawson, T.M.; Dawson, V.L. Ribosomal protein s15 phosphorylation mediates LRRK2 neurodegeneration in Parkinson's disease. *Cell*, **2014**, *157*, 472-485. http://dx.doi.org/10.1016/j.cell.2014.01.064

[64] Vancraenenbroeck, R.; Lobbestael, E.; Maeyer, M.; De, Baekelandt, V.; Taymans, J.M. Kinases as targets for Parkinson's disease: from genetics to therapy. *CNS Neurol. Disord. Drug Targets*, **2011**, *10*, 724-740. http://dx.doi.org/10.2174/187152711797247858

[65] Reyniers, L.; Del Giudice, M.G.; Civiero, L.; Belluzzi, E.; Lobbestael, E.; Beilina, A.; Arrigoni, G.; Derua, R.; Waelkens, E.; Li, Y.; Crosio, C.; Iaccarino, C.; Cookson, M.R.; Baekelandt, V.; Greggio, E.; Taymans, J.M. Differential protein-protein interactions of LRRK1 and LRRK2 indicate roles in distinct cellular signaling pathways. *J. Neurochem.*, **2014**, 1-12. http://dx.doi.org/10.1111/jnc.12798

[66] Dzamko, N.; Deak, M.; Hentati, F.; Reith, A.D.; Prescott, A.R.; Alessi, D.R.; Nichols, R.J. Inhibition of LRRK2 kinase activity leads to dephosphorylation of Ser(910)/Ser(935), disruption of 14-3-3 binding and altered cytoplasmic localization. *Biochem. J.*, **2010**, *430*, 405-413. http://dx.doi.org/10.1042/BJ20100784

[67] Doggett, E.A.; Zhao, J.; Mork, C.N.; Hu, D.; Nichols, R.J. Phosphorylation of LRRK2 serines 955 and 973 is disrupted by Parkinson's disease mutations and LRRK2 pharmacological inhibition. *J. Neurochem.*, **2012**, *120*, 37-45. http://dx.doi.org/10.1111/j.1471-4159.2011.07537.x

[68] Lobbestael, E.; Zhao, J.; Rudenko, I.N.; Beylina, A.; Gao, F.; Wetter, J.; Beullens, M.; Bollen, M.; Cookson, M.R.; Baekelandt, V.; Nichols, R.J.; Taymans, J.M. Identification of protein phosphatase 1 as a regulator of the LRRK2 phosphorylation cycle. *Biochem. J.*, **2013**, *456*, 119-128. http://dx.doi.org/10.1042/BJ20121772

[69] Reynolds, A.; Doggett, E.A.; Riddle, S.M.; Lebakken, C.S.; Nichols, R.J. LRRK2 kinase activity and biology are not uniformly predicted by its autophosphorylation and cellular phosphorylation site status. *Front. Mol. Neurosci.*, **2014**, *7*, 1-14. http://dx.doi.org/10.3389/fnmol.2014.00054

[70] Vancraenenbroeck, R.; De Raeymaecker, J.; Lobbestael, E.; Gao, F.; De Maeyer, M.; Voet, A.; Baekelandt, V.; Taymans, J.M. In silico, *in vitro* and cellular analysis with a kinome-wide inhibitor panel correlates cellular LRRK2 dephosphorylation to inhibitor activity on LRRK2. *Front. Mol. Neurosci.*, **2014**, *7*, 51. http://dx.doi.org/10.3389/fnmol.2014.00051

[71] Dzamko, N.; Chua, G.; Ranola, M.; Rowe, D.B.; Halliday, G.M. Measurement of LRRK2 and Ser910/935 phosphorylated LRRK2 in peripheral blood mononuclear cells from idiopathic Parkinson's disease patients. *J. Parkinsons. Dis.*, **2013**, *3*, 145-152.

[72] Kethiri, R.R.; Bakthavatchalam, R. Leucine-rich repeat kinase 2 inhibitors: a review of recent patents (2011 - 2013). *Expert Opin. Ther. Pat.*, **2014**, *24*, 745-757. http://dx.doi.org/10.1517/13543776.2014.907275

[73] Deng, X.; Choi, H.G.; Buhrlage, S.J.; Gray, N.S. Leucine-rich repeat kinase 2 inhibitors: a patent review (2006 - 2011). *Expert Opin. Ther. Pat.*, **2012**, *22*, 1415-1426. http://dx.doi.org/10.1517/13543776.2012.729041

[74] Kramer, T.; Lo Monte, F.; Göring, S.; Okala Amombo, G.M.; Schmidt, B. Small molecule kinase inhibitors for LRRK2 and their application to Parkinson's disease models. *ACS Chem. Neurosci.*, **2012**, *3*, 151-160. http://dx.doi.org/10.1021/cn200117j

[75] Taymans, J. Can the increasing number of newly developed leucine-rich repeat kinase 2 inhibitors validate or invalidate a potential disease-modifying therapeutic approach for Parkinson's disease? *Expert Opin. Ther. Pat.*, **2014**, *24*, 727-730. http://dx.doi.org/10.1517/13543776.2014.915945

[76] Galatsis, P.; Henderson, J.L.; Kormos, B.L.; Hirst, W.D. Leucine-Rich Repeat Kinase 2 (LRRK2) Inhibitors, in *Topics in Medicinal Chemistry*, **2014**.

[77] Deng, X.; Dzamko, N.; Prescott, A.; Davies, P.; Liu, Q.; Yang, Q.; Lee, J.D.; Patricelli, M.P.; Nomanbhoy, T.K.; Alessi, D.R.; Gray, N.S. Characterization of a selective inhibitor of the Parkinson's disease kinase LRRK2. *Nat. Chem. Biol.*, **2011**, Nature Publishing Group.

[78] Luerman, G.C.; Nguyen, C.; Samaroo, H.; Loos, P.; Xi, H.; Hurtado-Lorenzo, A.; Needle, E.; Stephen Noell, G.; Galatsis, P.; Dunlop, J.; Geoghegan, K.F.; Hirst, W.D. Phosphoproteomic evaluation of pharmacological inhibition of leucine-rich repeat kinase 2 reveals significant off-target effects of LRRK-2-IN-1. *J. Neurochem.*, **2014**, *128*, 561-576. http://dx.doi.org/10.1111/jnc.12483

[79] Lee, S.B.; Kim, W.; Lee, S.; Chung, J. Loss of LRRK2/PARK8 induces degeneration of dopaminergic neurons in Drosophila. *Biochem. Biophys. Res. Commun.*, **2007**, *358*, 534-539. http://dx.doi.org/10.1016/j.bbrc.2007.04.156

[80] Ng, C.H.; Mok, S.Z.S.; Koh, C.; Ouyang, X.; Fivaz, M.L.; Tan, E.K.; Dawson, V.L.; Dawson, T.M.; Yu, F.; Lim, K.L. Parkin protects against LRRK2 G2019S mutant-induced dopaminergic neurodegeneration in Drosophila. *J. Neurosci.*, **2009**, *29*, 11257-11262. http://dx.doi.org/10.1523/JNEUROSCI.2375-09.2009

[81] Baptista, M.A.S.; Dave, K.D.; Sheth, N.P.; De Silva, S.N.; Carlson, K.M.; Aziz, Y.N.; Fiske, B.K.; Sherer, T.B.; Frasier, M.A. A strategy for the generation, characterization and distribution of animal models by The Michael J. Fox Foundation for Parkinson's Research. *Dis. Model. Mech.*, **2013**, *000*, 1-9. http://dx.doi.org/10.1242/dmm.011940

[82] Yue, Z.; Lachenmayer, M.L. Genetic LRRK2 models of Parkinson's disease: Dissecting the pathogenic pathway and exploring clinical applications. *Mov. Disord.*, **2011**, *26*, 1386-1397. http://dx.doi.org/10.1002/mds.23737

[83] Dusonchet, J.; Kochubey, O.; Stafa, K.; Young, S.M.; Zufferey, R.; Moore, D.J.; Schneider, B.L.; Aebischer, P. A rat model of progressive nigral neurodegeneration induced by the Parkinson's disease-associated G2019S mutation in LRRK2. *J. Neurosci.*, **2011**, *31*, 907-912. http://dx.doi.org/10.1523/JNEUROSCI.5092-10.2011

[84] Lin, X.; Parisiadou, L.; Gu, X.L.; Wang, L.; Shim, H.; Sun, L.; Xie, C.; Long, C.X.; Yang, W.J.; Ding, J.; Chen, Z.Z.; Gallant, P.E.; Tao-Cheng, J.H.; Rudow, G.; Troncoso, J.C.; Liu, Z.; Li, Z.; Cai, H. Leucine-rich repeat kinase 2 regulates the progression of neuropathology induced by Parkinson's-disease-related mutant alpha-synuclein. *Neuron*, **2009**, *64*, 807-827. http://dx.doi.org/10.1016/j.neuron.2009.11.006

[85] Herzig, M.C.; Bidinosti, M.; Schweizer, T.; Hafner, T.; Stemmelen, C.; Weiss, A.; Danner, S.; Vidotto, N.; Stauffer, D.; Barske, C.; Mayer, F.; Schmid, P.; Rovelli, G.; van der Putten, P.H.; Shimshek, D.R. High LRRK2 levels fail to induce or exacerbate neuronal alpha-synucleinopathy in mouse brain. *PLoS One*, **2012**, *7*, e36581. http://dx.doi.org/10.1371/journal.pone.0036581

[86] Caesar, M.; Zach, S.; Carlson, C.B.; Brockmann, K.; Gasser, T.; Gillardon, F. Leucine-rich repeat kinase 2 functionally interacts with microtubules and kinase-dependently modulates cell migration. *Neurobiol. Dis.*, **2013**, *54*, 280-288. http://dx.doi.org/10.1016/j.nbd.2012.12.019

[87] Tong, Y.; Yamaguchi, H.; Giaime, E.; Boyle, S.; Kopan, R.; Kelleher, R.J.; Shen, J. 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.S.A.*, **2010**, *107*, 9879-9884. http://dx.doi.org/10.1073/pnas.1004676107

[88] Ness, D.; Ren, Z.; Gardai, S.; Sharpnack, D.; Johnson, V.J.; Brennan, R.J.; Brigham, E.F.; Olaharski, A.J. Leucine-rich repeat kinase 2 (LRRK2)-deficient rats exhibit renal tubule injury and perturbations in metabolic and immunological homeostasis. *PLoS One*, **2013**, *8*, e66164. http://dx.doi.org/10.1371/journal.pone.0066164

[89] Hinkle, K.M.; Yue, M.; Behrouz, B.; Dächsel, J.C.; Lincoln, S.J.; Bowles, E.E.; Beevers, J.E.; Dugger, B.; Winner, B.; Prots, I.; Kent, C.B.; Nishioka, K.; Lin, W.L.; Dickson, D.W.; Janus, C.J.; Farrer, M.J.; Melrose, H.L. LRRK2 knockout mice have an intact dopaminergic system but display alterations in exploratory and motor co-ordination behaviors. *Mol. Neurodegener*, **2012**. *http*://dx.doi.org/10.1186/1750-1326-7-25

[90] Taymans, J.M. The GTPase function of LRRK2. *Biochem. Soc. Trans.*, **2012**, *40*, 1063-1069. http://dx.doi.org/10.1042/BST20120133

[91] Cho, H.J.; Yu, J.; Xie, C.; Rudrabhatla, P.; Chen, X.; Wu, J.; Parisiadou, L.; Liu, G.; Sun, L.; Ma, B.; Ding, J.; Liu, Z.; Cai, H. Leucine-rich repeat kinase 2 regulates Sec16A at ER exit sites to allow ER-Golgi export. *EMBO J.*, **2014**, *33*, 2314-2331. http://dx.doi.org/10.15252/embj.201487807

[92] Parisiadou, L.; Yu, J.; Sgobio, C.; Xie, C.; Liu, G.; Sun, L.; Gu, X.L.; Lin, X.; Crowley, N.A.; Lovinger, D.M.; Cai, H. LRRK2 regulates synaptogenesis and dopamine receptor activation through modulation of PKA activity. *Nat. Neurosci.*, **2014**, 1-12. http://dx.doi.org/10.1038/nn.3636

[93] Gandhi, P.N.; Wang, X.; Zhu, X.; Chen, S.G.; Wilson-Delfosse, A.L. The Roc domain of leucine-rich repeat kinase 2 is sufficient for interaction with microtubules. *J. Neurosci. Res.*, **2008**, *86*, 1711-1720. http://dx.doi.org/10.1002/jnr.21622

[94] Downward, J. Targeting RAS signalling pathways in cancer therapy. *Nat. Rev. Cancer*, **2003**, *3*, 11-22. http://dx.doi.org/10.1038/nrc969

[95] Stafa, K.; Trancikova, A.; Webber, P.J.; Glauser, L.; West, A.B.; Moore, D.J. GTPase activity and neuronal toxicity of Parkinson's disease-associated LRRK2 is regulated by ArfGAP1. *PLoS Genet.*, **2012**, *8*, e1002526. http://dx.doi.org/10.1371/journal.pgen.1002526

[96] Xiong, Y.; Yuan, C.; Chen, R.; Dawson, T.M.; Dawson, V.L. ArfGAP1 is a GTPase activating protein for LRRK2: reciprocal regulation of ArfGAP1 by LRRK2. *J. Neurosci.*, **2012**, *32*, 3877-3886. http://dx.doi.org/10.1523/JNEUROSCI.4566-11.2012

[97] Chia, R.; Haddock, S.; Beilina, A.; Rudenko, I.N.; Mamais, A.; Kaganovich, A.;Li, Y.; Kumaran, R.; Nalls, M.A.; Cookson, M.R. Phosphorylation of LRRK2 by casein kinase 1α regulates trans-Golgi clustering *via* differential interaction with ARHGEF7. *Nat. Commun.*, **2014**, *5*, 5827. http://dx.doi.org/10.1038/ncomms6827

[98] Gasper, R.; Meyer, S.; Gotthardt, K.; Sirajuddin, M.; Wittinghofer, A. It takes two to tango: regulation of G proteins by dimerization. *Nat. Rev. Mol. Cell Biol.*, **2009**, *10*, 423-429. http://dx.doi.org/10.1038/nrm2689

[99] Civiero, L.; Vancraenenbroeck, R.; Belluzzi, E.; Beilina, A.; Lobbestael, E.; Reyniers, L.; Gao, F.; Micetic, I.; De Maeyer, M.; Bubacco, L.; Baekelandt, V.; Cookson, M.R.; Greggio, E.; Taymans, J.M. Biochemical characterization of highly purified leucine-rich repeat kinases 1 and 2 demonstrates formation of homodimers. *PLoS One*, **2012**, *7*, e43472. http://dx.doi.org/10.1371/journal.pone.0043472

[100] Li, T.; Yang, D.; Zhong, S.; Thomas, J.M.; Xue, F.; Liu, J.; Kong, L.; Voulalas, P.; Hassan, H.E.; Park, J.S.; MacKerell, A.D.; Smith, W.W. Novel LRRK2 GTP-binding inhibitors reduced degeneration in Parkinson's disease cell and mouse models. *Hum. Mol. Genet.*, **2014**, *23*, 6212-6222. http://dx.doi.org/10.1093/hmg/ddu341

[101] Li, T.; He, X.; Thomas, J.M.; Yang, D.; Zhong, S.; Xue, F.; Smith, W.W. A Novel GTP-Binding Inhibitor, FX2149, Attenuates LRRK2 Toxicity in Parkinson's Disease Models. *PLoS One* (Andrabi, S. A., Ed.), **2015**, *10*, e0122461. http://dx.doi.org/10.1371/journal.pone.0122461

[102] Greggio, E.; Zambrano, I.; Kaganovich, A.; Beilina, A.; Taymans, J.M.; Daniëls, V.; Lewis, P.; Jain, S.; Ding, J.; Syed, A.; Thomas, K.J.; Baekelandt, V.; Cookson, M.R. The Parkinson disease-associated leucine-rich repeat kinase 2 (LRRK2) is a dimer that undergoes intramolecular autophosphorylation. *J. Biol. Chem.*, **2008**, *283*, 16906-14. http://dx.doi.org/10.1074/jbc.M708718200

[103] Taymans, J.M.; Vancraenenbroeck, R.; Ollikainen, P.; Beilina, A.; Lobbestael, E.; De Maeyer, M.; Baekelandt, V.; Cookson, M.R. LRRK2 kinase activity is dependent on LRRK2 GTP binding capacity but independent of LRRK2 GTP binding. *PLoS One*, **2011**, *6*, e23207. http://dx.doi.org/10.1371/journal.pone.0023207

[104] Ito, G.; Iwatsubo, T. Re-examination of the dimerization state of leucine-rich repeat kinase 2: predominance of the monomeric form. *Biochem. J.*, **2012**, *441*, 987-994. http://dx.doi.org/10.1042/bj20111215

[105] Berger, Z.; Smith, K.A.; Lavoie, M.J. Membrane localization of LRRK2 is associated with increased formation of the highly active LRRK2 dimer and changes in its phosphorylation. *Biochemistry*, **2010**, *49*, 5511-5523. http://dx.doi.org/10.1021/bi100157u

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

[107] Choi, H.G.; Zhang, J.; Deng, X.; Hatcher, J.M.; Patricelli, M.P.; Zhao, Z.; Alessi, D.R.; Gray, N.S. Brain Penetrant LRRK2 Inhibitor. *ACS Med. Chem. Lett.*, **2012**, *3*, 658-662. http://dx.doi.org/10.1021/ml300123a

[108] Saez-Atienzar, S.; Bonet-Ponce, L.; Blesa, J.R.; Romero, F.J.; Murphy, M.P.; Jordan, J.; Galindo, M.F. The LRRK2 inhibitor GSK2578215A induces protective autophagy in SH-SY5Y cells: involvement of Drp-1-mediated mitochondrial fission and mitochondrial-derived ROS signaling. *Cell Death Dis.*, **2014**, *5*, e1368. http://dx.doi.org/10.1038/cddis.2014.320

[109] Estrada, A.A; Liu, X.; Baker-Glenn, C.; Beresford, A.; Burdick, D.J.; Chambers, M.;Chan, B.K.; Chen, H.; Ding, X.; DiPasquale, A.G.; Dominguez, S.L.; Dotson, J.; Drummond, J.; Flagella, M.; Flynn, S.; Fuji, R.; Gill, A.; Gunzner-Toste, J.; Harris, S.F.; Heffron, T.P.; Kleinheinz, T.; Lee, D.W.; Le Pichon, C.E.; Lyssikatos, J.P.; Medhurst, A.D.; Moffat, J.G.; Mukund, S.; Nash, K.; Scearce-Levie, K.; Sheng, Z.; Shore, D.G.; Tran, T.; Trivedi, N.; Wang, S.; Zhang, S.; Zhang, X.; Zhao, G.; Zhu, H.; Sweeney, Z.K. Discovery of highly potent, selective, and brain-penetrable leucine-rich repeat kinase 2 (LRRK2) small molecule inhibitors. *J. Med. Chem.*, **2012**, *55*, 9416-9433. http://dx.doi.org/10.1021/jm301020q

[110] Estrada, A.A.; Chan, B.K.; Baker-Glenn, C.; Beresford, A.; Burdick, D. J.; Chambers, M.; Chen, H.; Dominguez, S.L.; Dotson, J.; Drummond, J.; Flagella, M.; Fuji, R.; Gill, A.; Halladay, J.; Harris, S.F.; Heffron, T.P.; Kleinheinz, T.; Lee, D.W.; Le Pichon, C.E.; Liu, X.; Lyssikatos, J.P.; Medhurst, A.D.; Moffat, J.G.; Nash, K.; Scearce-Levie, K.; Sheng, Z.; Shore, D.G.; Wong, S.; Zhang, S.; Zhang, X.; Zhu, H.; Sweeney, Z.K. Discovery of highly potent, selective, and brain-penetrant aminopyrazole leucine-rich repeat kinase 2 (LRRK2) small molecule inhibitors. *J. Med. Chem.*, **2014**, *57*, 921-36. http://dx.doi.org/10.1021/jm401654j

[111] Troxler, T.; Greenidge, P.; Zimmermann, K.; Desrayaud, S.; Drückes, P.; Schweizer, T.; Stauffer, D.; Rovelli, G.; Shimshek, D.R. Discovery of novel indolinone-based, potent, selective and

brain penetrant inhibitors of LRRK2. *Bioorg. Med. Chem. Lett.*, **2013**, *23*, 4085-4090. http://dx.doi.org/10.1016/j.bmcl.2013.05.054

[112] Göring, S.; Taymans, J.M.; Baekelandt, V.; Schmidt, B. Indolinone based LRRK2 kinase inhibitors with a key hydrogen bond. *Bioorg. Med. Chem. Lett.*, **2014**, *24*, 4630-4637. http://dx.doi.org/10.1016/j.bmcl.2014.08.049

[113] Henderson, J.L.; Kormos, B.L.; Hayward, M.M.; Co, K.J.; Jasti, J.; Kurumbail, R.G.; Wager, T.T.; Verhoest, P.R.; Noell, G.S.; Chen, Y.; Needle, E.; Berger, Z.; Steyn, S.J.; Houle, C.; Hirst, W.D.; Galatsis, P. 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*, **2015**.

[114] JM, H.; Zhang, J.; Choi, H.; Ito, G.; Alessi, D.; Gray, N. Discovery of a Pyrrolopyrimidine (JH-II-127), a Highly Potent, Selective, and Brain Penetrant LRRK2 Inhibitor. *ACS Med. Chem. Lett.*, **2015**, *6*, 584-589. http://dx.doi.org/10.1021/acsmedchemlett.5b00064

[115] Nichols, R.J.; Dzamko, N.; Morrice, N.A.; Campbell, D.G.; Deak, M.; Ordureau, A.; Macartney, T.; Tong, Y.; Shen, J.; Prescott, A.R.; Alessi, D.R. 14-3-3 binding to LRRK2 is disrupted by multiple Parkinson's disease-associated mutations and regulates cytoplasmic localization. *Biochem. J.*, **2010**, *430*, 393-404. http://dx.doi.org/10.1042/BJ20100483

[116] Lobbestael, E.; Baekelandt, V.; Taymans, J.M. Phosphorylation of LRRK2: from kinase to substrate. *Biochem. Soc. Trans.*, **2012**, *40*, 1102-1110. http://dx.doi.org/10.1042/BST20120128

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