ARTICLE

The ongoing pursuit of neuroprotection in Parkinson 's disease.

D. Athauda, T. Foltynie
Sobell Department of Motor Neuroscience, UCL Institute of Neurology & The National Hospital for Neurology and Neurosurgery, Queen Square, London, WC1N 3BG
Email address: t.foltynie@ucl.ac.uk
Telephone: +44 203 448 8726, Fax: +44 203 448 8642

Abstract
Despite the great promise that many potential neuroprotective agents have shown in the laboratory, to date these have not translated to positive results in patients with Parkinson's disease (PD), with several recent high profile clinical trials (Coenzyme Q10, Creatine, Cogane among others) all failing to show any clinical benefits. This "failure to translate" is likely related primarily to our incomplete understanding of the pathogenic mechanisms underlying PD together with excessive reliance on data from the toxin-based animal models of PD to judge which agents to take to double blind clinical trial evaluation. In addition restricted resources inevitably mean difficult compromises must be made in terms of optimal trial design, made harder by the absence of validated biomarkers of disease progression. To date, drug development in PD dementia has been mostly unsuccessful; however, emerging biochemical, genetic and pathological evidence suggests a link between tau and A-Beta deposition and cognitive decline in PD – potentially opening up new targets for therapeutic intervention. In this context, this review discusses some of the most important existing current and future potential therapeutic agents for motor and cognitive impairments on the horizon to help inform whether there is greater reason to expect positive results in the short or medium term future.

Key Points
- Currently obstacles impeding the development of effective neuroprotective therapies for PD include a lack of understanding regarding the pathogenesis of PD, lack of accurate animal models of PD, limitations in clinical trial design, insensitive trial endpoints, a lack of validated biomarkers and inhibitory costs of developing new drugs.
- Advances in the development of transgenic animal models, newer adaptive and delayed start trial designs, identification of possible serum, CSF or neuroimaging biomarkers and re-positioning of existing drugs for use in PD are making inroads to the goal of identifying and testing an effective disease modifying therapy.
- Alpha-synuclein plays a key role in the pathogenesis of PD and targeting the formation and clearance of this protein by directly blocking alpha synuclein aggregation, enhancing lysosomal clearance systems or immunisation against alpha-

synuclein show promising results in pre-clinical models but are still very early in development.

- As 50% of PDD patients have comorbid AD pathology which can confer a worse prognosis, targeting tau and AB may be useful avenues to attempt to reduce or halt cognitive dysfunction, and disease modifying therapies under investigation in AD may also be of benefit in PD.

Introduction

Despite the obvious need for neuroprotective or disease modifying agents and the great promise many potential candidates have shown in preclinical studies, there are currently no treatments that have been licensed as neuroprotective agents in Parkinson's disease (PD). Several high profile phase I/II clinical trials of notable potential neuroprotective agents have recently failed to show benefits despite strong laboratory and in vivo animal data seeming to support their potential. These are summarised in Table 1. This discrepancy between encouraging preclinical data and failure of translation in clinical trials requires continuous reappraisal, however rather than performing an individualised analysis of why each of these specific agents may have failed in clinical trials, this review focuses on a general critique of the current obstacles that continue to impede the development of neuroprotective agents. In this context, we present a summary of the current status of major potential therapeutic candidates currently being assessed in clinical trials for neuroprotective or disease modifying effects in PD and assessment of their therapeutic potential. Although clinical trials assessing potential disease modifying therapies have mainly focused on preserving motor function (historically easier to assess and because validated tools to assess non-motor symptoms have only relatively recently appeared); non-motor symptoms of PD, (in particular cognitive dysfunction), are increasingly recognised as having a significant contribution to patients' quality of life, are only partially responsive to dopaminergic therapy and mostly remain refractory to current intervention[1,2]. Of specific relevance is the growing amount of evidence suggesting that AD-type pathology – amyloid-B plaques and hyperphosphorylated tau-containing neurofibrillary tangles, contributes to cognitive dysfunction in PD. To this end, we also discuss whether targeting tau and utilising disease modifying treatments in AD to improve cognitive dysfunction may be of benefit to patients with PD also.

Current obstacles impeding development of neuroprotective therapies in PD and potential solutions

<u>Lack of understanding regarding the pathogenesis of PD</u>

It is currently believed that neurodegeneration in PD is due to a combination of cell-autonomous and non cell-autonomous processes. The cell autonomous mechanisms include mitochondrial dysfunction, dysfunction of the autophagy/lysosomal processes and dysregulation of calcium homeostasis, while non-cell autonomous processes include neuroinflammation, loss of trophic support and the trans-synaptic transmission of misfolded alpha synuclein.[3,4] The interaction between these pathways remains unclear, and the long term impact on interfering with these fundamental processes for normal healthy cell function is not understood, but this process of neuronal degeneration has been speculated to occur in four stages: from the initial molecular prodrome, leading to cell damage, resulting in decompensation and dysfunction, and finally to degeneration. Elucidation of these

pathogenetic details is facilitated by; 1) The creation of human cell lines from patients with the various Mendelian forms of PD, helping to explain the interactions between lysosomes, mitochondria and abnormal protein aggregation in single cell systems, 2) the development of transgenic animals with human PD genes helping to demonstrate how these processes impact on cell-to cell transmission of alpha synuclein. This knowledge can identify whether patients at each or any stage of neurodegeneration, might gain benefit from potential "target specific" neuroprotective or neurorestorative therapies (for review[3,5])

Lack of accurate animal models:

The most widely used animal models of PD are based on administration of locally administered neurotoxin 6-hydroxydopamine (6-OHDA), systemically administered toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice and non-human primates, or direct infusion of bacterial endotoxin lipopolysaccharide (LPS) into the nigrostriatal pathway of rats to produce models of selective degeneration of substantia nigra pars compacta (SNpc) dopamine (DA) neurons to produce a parkinsonian syndrome.[6,7] Other neurotoxins less often also used include rotenone, paraquat, isoquinoline derivatives, and methamphetamine.[8] Though adequate at replicating the motor aspects of PD in the later stages, the very selectivity of these models means they often do not exhibit the complex extra-dopaminergic involvement and non-motor dysfunction seen in the early stages of PD. Also, the pathological hallmark of PD – the presence of Lewy bodies (LB), has not consistently been observed in these neurotoxin based animal models.[8] Though some MPTP models in rhesus monkeys have been shown to mimic the extra-striatal dopamine, noradrenaline, and serotonin changes often observed in humans,[9] these models require the acute administration of toxins which likely does not reflect the chronic and progressive nature of the neurodegenerative process in PD. Recent refinements to this technique however using low concentrations or an intermittent dosing schedules are now being successfully used to replicate the more progressive pathology seen in humans and are also being used to study compensatory mechanisms[10,11]

Transgenic animal models, which recapitulate monogenic mutations seen in familial PD patients, have an advantage over neurotoxin based models is that these models produce the pathological abnormalities seen in PD and provide insights into selected molecular aspects of PD pathogenesis and early stages of the disease - the MitoPark mouse has been developed in which mitochondrial function is selectively disrupted in dopaminergic neurons – producing a mouse exhibiting a progressive PD-like phenotype. Though not reproducing the genetic mutations seen in humans with PD, these models could be useful in large scale screening of new therapeutic agents.[7] A major limitation of these models however is that they do not consistently reproduce the expected nigrostriatal degeneration and phenotypes seen in PD.[12]

Due to the growing amount of evidence implicating the mis-folding of alpha synuclein as a key event involved in the pathogenesis of PD, new mouse models have been developed in which a single injection of synthetic mis-folded alpha-synuclein can initiate a cascade of events leading to the presence of LB-like inclusions, selective loss of neurons in the substantia nigra pars compacta, and motor impairments.[13,14]

While it is clear that these animal models have led to great steps forward in the treatment of the motor symptoms and complications of PD, it is early to judge whether they will be any more successful in aiding the development of disease modifying or neuroprotective strategies. There is currently no "best" model that perfectly captures the chronic neurodegeneration and clinical phenotypes seen in PD – the current animal models have advantages and disadvantages; to most effectively develop a neuroprotective agent in pre-clinical trials, investigators need to understand the limitations of various models and may need to choose a specific model based on specific research needs or develop an agent that has reproducible efficacy across multiple animal models.

Limitations in trial design:

A further potential contribution to the lack of translation between encouraging results in preclinical models and clinical trials is the trial design. Current designs may either not be sensitive enough to identify disease modifying effects, or may struggle to convincingly separate such effects from long-lasting symptomatic relief.

Washout trial designs attempt to eliminate and separate any potential symptomatic effects from disease modifying effects of the studied drug treatment; however, there often remains underlying uncertainty as to whether any of the beneficial effects seen could be due to unanticipated long lasting symptomatic effects if the washout period is too short –one putative explanation for levodopa's apparent disease modifying effect in the ELLDOPA trial.[15] However using a long washout period of several months or longer may be confounded by the progressive nature of PD, and risks high levels of patient drop out.

More promising are delayed start designs, as used in the ADAGIO trial to assess rasagiline's disease modifying potential, although these require an initial period long enough to allow a neuroprotective effect to occur (which also raises ethical concerns of drug naïve patients remaining on placebo for a long period), and again differential patient dropout between randomised groups can affect the statistical analysis of results, while the expected differences between the early and delayed start groups are often small due to the slowly progressive nature of PD.[16]

To truly assess neuroprotective or disease modifying effects, a long term simple / longitudinal study designs is most effective - using composite global measures to provide a multi-dimensional assessment of disease progression over a long time period – typically a number of years.[17] Though this is the most reliable at differentiating disease modifying effects on various outcomes, it requires considerable investment and extensive collaboration between drug companies, research institutes and clinical centres to manage the significant costs.

Insensitive endpoints/outcome measures of clinical trials:

Objective endpoints in PD based on biomarkers in CSF, blood or imaging are yet to have documented validity and reliability, so clinical trials have to rely on clinical endpoints or markers by which to assess disease progression, which have numerous limitations. The time

taken for drug naïve PD patients to require dopaminergic or symptomatic therapy as a specific endpoint as evidence of disease progression is often used in clinical trials, and although this is a well-defined, measurable endpoint, not confounded by any possible symptomatic effects from other drugs,[18] a notable limitation is that in reality, the decision to start symptomatic therapy relies on a number of complex factors between the patient and the physician and often does not correlate with disease stage or rate of progression, and it is often impossible to differentiate whether delayed need for symptomatic treatment reflects symptomatic or neuroprotective effects of a study drug[18 19]

Another commonly used method is the use of repeated assessment with the Unified PD rating scale (UPDRS). While also subject to intra- and inter- rater variability, the most recent version of this scale (MDS-UPDRS), when combined with scales to evaluate gait and dyskinesia and multiple other non-motor measures of cognition, mood and sleep, can provide multiple opportunities to detect a signal of effect, perhaps more likely to indicate clinically relevant advantage if all are indicating positive beneficial effects.

Lack of validated biomarkers

A validated, sensitive biomarker to diagnose PD and monitor response to therapeutic intervention does not currently exist, though research is ongoing.   The Parkinson's Progression Markers Initiative (PPMI), have recently published encouraging results that have shown that levels of CSF Aβ1-42, T-tau, P-tau181, and α-synuclein have prognostic and diagnostic potential in early-stage PD, with lower levels seen in PD compared with health controls.[20] Groups trying to improve upon imaging biomarkers using 7T MRI, transcranial ultrasound, novel MRI sequences or novel PET ligands have shown promise in differentiating PD patients from controls, but are still at an early stage in development.[21]

Suboptimal patient population choice for clinical trials

Commonly, studies have chosen to include early untreated PD patients as there is thought to be a higher percentage of remaining neurons that might be salvaged compared to patients with more advanced disease. [22] A recent study quantifying nigrostriatal degeneration in PD patients from time of diagnosis showed that dopamine markers in the fibres of the dorsal putamen are however already variably reduced at diagnosis and are virtually absent as little as 4 years after diagnosis.[23] Any clinical deterioration after this time is thought to represent a loss of a compensatory mechanisms or degeneration of non-dopaminergic neurons. This data has obvious implications for patient selection for future trials and may also suggest that previous trials with seemingly negative results may have been conducted in patients with little surviving dopaminergic neurons. Ideally, patients enrolled for potential neuroprotective trials should be at an early enough stage of their disease so as to have a greater number of surviving neurons from which to derive benefit. A caveat to this is that these patients typically take longer to identify, and there is a higher rate of misdiagnosis, or risk of including patients with atypical forms of parkinsonism such as PSP, MSA, CBD etc., while 10% of patients diagnosed clinically with early PD have normal dopaminergic functional imaging (so called SWEDDS (scans without evidence of dopaminergic deficit)[24]. Patients may also have widely different severity of disease even at presentation and have variable rates of

progression - increasing age at onset is associated with higher levels of disability and Hoehn and Yahr stage, while tremor dominant PD is associated with slower disease progression and less cognitive impairment than patients with akinetic rigid phenotypes.[25]

Conversely, including patients with moderate/advanced PD who are more likely to have measurable endpoints on validated scales, may have such advanced neurodegeneration that the effect of introducing neuroprotective agents may be too little, too late. However, it has been recently shown that there is a continued persistence of populations of melanin containing neurons in the SNpc in comparison to the number of tyrosine hydroxylase-immunoreactive neurons for decades after diagnosis, which may suggests that trophic or regenerative therapies might still have value even in the later stages of the illness.[23]

In summary, although there is a little doubt that intervening early as possible will provide the greatest likelihood of a response to any neuroprotective treatment, the compromise position however is that there will likely always be a need to identify a neuroprotective treatment that is also helpful in the population with clinically manifest disease, and a need to identify whether it is tolerated in conjunction with symptomatic agents such as L-dopa, and if it has any effects on the development on the major disabling milestones of PD such as freezing, falls, and dementia.

Choosing the most effective dose(s)

Doses of experimental therapies for use in human trials are often chosen to replicate plasma concentrations obtained from previous animal models; however, this may not necessarily correlate with concentrations seen in the brain and at receptor level. Adding to the uncertainty is that results from various animal models are often conflicting, or may only be effective within a narrow range. As no validated biomarker exists currently, it is difficult to titrate varying doses or ascertain the most effective dose range. The use of adaptive trial designs however, can allow a large number of varying doses to be tested in the initial stages, without negatively affecting the longterm results.[26]

Inhibitory development costs

The development of a new therapy takes approximately 15 years and costs around $1.2billion.[27] In spite of the potential commercial value of an effective disease-modifying therapy for PD, this area of research is considered high-risk within the pharmaceutical industry due to the high costs of trials and the lack of any positive results to date. Novel methods being used to overcome this include using high throughput screening methods which utilise phenotypic cell-based drug screens. Yeast cells engineered to express alpha synuclein in a way that re-captures the cellular pathology in PD have recently been used to screen more than 190,000 compounds for potential neuroprotective effects – which led to the identification of a new potential pharmaceutical target, the E3 ubiquitin ligase, and a novel molecule, NAB2, an N-aryl benzimidazole, which showed protective effects in three different PD models.[28,29] High throughput screening can also be combined with a focus on drugs already licensed for use in humans for re-purposing to assess their effects on mitochondrial function - ursodeoxycholic acid, already licensed for use in liver disease, has been recently identified using this method and phase 2a trials are already planned.[30]

Other non-mammalian transgenic models of PD using drosophila and zebrafish are also being used to as low-cost, high throughput method to screen novel compounds that may counteract pathological mechanisms of PD.[7]

Current neuroprotective strategies/ candidates

Based on the various cellular mechanisms involved in the pathogenesis of PD, growing evidence implicates the modulation of calcium homeostasis, oxidative stress, mitochondrial function, autophagy, and formation and clearance of alpha-synuclein as potential targets for putative neuroprotective therapies (Figure 1). These targets and the main candidates for neuroprotective therapies currently in clinical trial testing are summarised in Table 2.

Calcium channel blockade

It has been shown that the apparent selective vulnerability and degeneration of dopaminergic neurons of the SNpc in PD may be related to high energy demands associated with calcium influx through CaV1.3 L-type calcium channels (LTCC) during their autonomous pacemaking, leading to increased mitochondrial-mediated oxidative stress and subsequent cell death. [31] Furthermore, increased CaV1.3 subtype expression occurs in the cerebral cortex of early stage PD patients before the appearance of pathological changes, suggesting that disturbed calcium homeostasis may be an early event in the pathogenesis and not just a consequence of neurodegeneration, therefore offering a potentially attractive target for neuroprotective therapies.[32]

Epidemiological data shows that patients treated with centrally acting calcium channel blockers may have a reduced risk of developing PD,[33–35] although this remains controversial.[36] Isradipine, licensed for the treatment of hypertension, has a high affinity for CaV1.3 LTCC, and while there is strong evidence of benefit in pre-clinical studies of PD;[37,38] a recent randomised trial (RCT), though not powered for efficacy, suggested only a very modest advantage (~1 point in the total UPDRS score) in patients treated with 10mg isradipine for 12 months of questionable clinical relevance.[39] Furthermore, pre-clinical data suggests that most robust neuroprotective effects of isradipine are seen at doses much higher than would be tolerated in humans – possibly limiting isradipine's therapeutic potential. [37] Nevertheless, a fully powered phase III trial is planned to assess efficacy of isradipine 10 mg in PD. Emerging candidates from pre-clinical studies including novel, highly selective CaV1.3 antagonists may offer more promising results in the future without producing side effects that accompany general antagonism of L-type calcium channels.[40]

Oxidative stress pathways

Evidence of oxidative damage is seen in the SN of PD patients in post mortem studies,[41] and is thought to play an important role in the pathogenesis of PD, occurring as a result of failure of endogenous protective mechanisms, DA metabolism, defective mitophagy and accumulation of mtDNA mutations. Though previous efforts to reduce oxidative stress using antioxidants selegiline, rasagiline and vitamin E have not convincingly or consistently demonstrated disease modifying effects,[42,43]current clinical trials are underway investigating the potential of other various anti-oxidants.

Based on epidemiological data showing that patients with high levels of serum urate, a natural anti-oxidant, have a 33% reduction in the risk of developing PD, [44–46] and high levels of urate in serum and CSF of patients with early PD are associated with slower rates of clinical and radiological progression,[47–49] efforts are underway to investigate whether raising serum urate - using an orally administered precursor inosine,[50] may serve as a potentially neuroprotective therapy. In a recent RCT, inosine was well tolerated,[51] and was associated with a favourable rate of progression based on changes in UPDRS scores over 24 months; but after adjustment for baseline differences, this amounted to only ~1 point per year on total UPDRS scale. Although the trial data was not powered to determine efficacy, there was no difference between the time to requiring dopaminergic therapy between groups, and few patients reached the 2 year analysis time point. Furthermore, elevated serum urate has also been shown to increase the risk of hypertension, coronary heart disease, gout and stroke over the longer term, and these side effects are potentially problematic for older patients with PD, potentially limiting its ultility.[52] A larger phase III trial is currently being planned.

Another potential target being investigated to lessen oxidative stress is by targeting iron accumulation. Levels of iron in excess of that expected in normal ageing have has been found in the SNpc of patients with sporadic PD and autosomal recessive juvenile parkinsonism[53] and in vivo studies using MRI and transcranial ultrasonography show that increased levels of iron in the basal ganglia and SN correlate to PD motor severity[54] potentially by inducing oxidative stress and aggregation of misfolded alpha synuclein.[55] There is strong evidence from pre-clinical studies that deferiprone, a chelating agent licensed for the treatment of peripheral iron overload disorders, is neuroprotective.[56 57 58] A recent delayed start design trial of deferiprone demonstrated a reduction in the UPDRS motor subscale in the early start group (-2.3 +/- 0.6) compared to the delayed start group (+1.0 +/- 0.7) which was sustained at 12 months – though improvements waned after 18 months continuous treatment.[59] Whether excessive iron represents a cause or consequence of dopaminergic neuronal cell death is uncertain and though efficacy results are highly encouraging, it remains to be seen whether observed clinical benefits in PD patients occurred as a result of iron chelation alone or via permissive effects of chelation on dopaminergic treatments. Two further phase II/III trials are now underway (Clinical trials.gov identifier NCT01539837 and NCT00943748).

Glutathione (GSH) is a tripeptide that acts as the brain's primary anti-oxidant system (and also as a neurotransmitter)[60] Prolonged depletion of GSH inhibits mitochondrial complex I activity[61] and depletion of GSH is seen in the SN of PD patients,[62,63] but also patients with incidental Lewy Body disease – suggesting that loss of GSH occurs early in the pathogenesis of PD.[64] Previous efforts to increase GSH levels in patients with PD intravenously have been unsuccessful as GSH crosses the blood brain barrier via a saturable mechanism and is not taken up by neurons, presumably explaining why an RCT administering intravenous GSH to 21 PD patients failed to show any evidence of clinical efficacy[60,65].

N-acetylcysteine (NAC), used as a mucolytic, is an oral precursor to GSH, and decreases alpha-synuclein aggregation in transgenic models,[66]. In a pilot human trial, NAC was well tolerated, and could raise levels of GSH in CSF.[67] Though data from transgenic models is encouraging, there is as yet no efficacy data in humans, so it remains to be seen whether

orally administered NAC will offer positive effects. Notably a trial using oral administered NAC in patients with early Alzheimer's disease (AD) failed to alter any primary outcome measures.[68] Given that GSH depletion seems to occur early in the pathogenesis of PD, GSH replacement may be best trialled among patients at the earliest or pre-symptomatic stage of disease.

Zonisamide, is a drug used for the adjunctive treatment of epilepsy thought to act via inhibition of voltage-gated sodium channels and calcium channels, mediating neuronal depolarization-induced glutamate release.[69] It also enhances dopamine release and is licensed in Japan as an adjunctive treatment in PD to aid motor symptoms, and is currently being investigated in parallel for its neuroprotective potential.[70] While zonisamide has demonstrated symptomatic benefits in doses from 25mg-100mg, pre-clinical data regarding neuroprotective equivalent doses is conflicting – some investigators used serum concentrations well above that which could be achieved with maximal human dosing,[71]. While separating proven symptomatic benefits from any neuroprotective effects may also prove difficult, a Phase 2 trial is currently underway (Clinical Trials.gov identifier NCT01766128).

Enhancing mitochondrial function
Considerable evidence from genetic, animal and post mortem studies implicate mitochondrial dysfunction in the pathogenesis of PD (see for review[72]) - mitochondrial toxins can cause parkinsonism, autosomal recessive forms of PD related to mutations in Parkin, DJ-1 and PINK1 impair mitochondrial autophagy and the selective vulnerability of nigro-striatal neurons may be related to the high metabolic demands placed on the cells. Though enhancing mitochondrial biogenesis to counteract the effects of neurodegeneration is a reasonable strategy, recent trials using mitochondrial enhancers such as creatine, co-enzyme Q10 and MitoQ have all failed to convincingly demonstrate disease modifying effects. Possible reasons speculated to account for this failure include poor brain penetrance of the compound, patients selected with too far advanced degeneration to benefit and also growing evidence to suggest that mitophagy and mitochondrial calcium homeostasis may play larger roles in maintaining mitochondrial biogenesis than previously thought, and may offer better potential targets for intervention.[73]

Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) is a protein that interacts with the peroxisome proliferator-activated receptor gamma (PPARγ) and controls mitochondrial and microglial function, and acts with SIRT1 to upregulate mitochondrial biogenesis[74] and is therefore a potentially attractive target for intervention. Encouraging data from pre-clinical studies of resveratrol, a SIRT1 activator, has shown it protects dopaminergic neurons from MPTP and 6-OHDA induced deficits in vivo, possibly via modulation of autophagy and pro-inflammatory pathways, and further in vivo testing is planned.[75,76] Though no clinical data exists, there is a sufficient indication of potential benefit to merit further in vivo studies better elucidate the importance of these mechanisms which could lead to a pilot study in patients with PD.

A recent novel high throughput screening method to identify potential compound's that can rescue mitochondrial dysfunction directly in PD patient tissue identified urosdeoxycholic acid, which was subsequently shown to rescue mitochondrial function in parkin & LRRK2 mutant fibroblasts in vitro, and is neuroprotective in transgenic mouse model of AD, and has led to this compound being fast tracked to a phase 1 trial currently being planned.[30]

Neuroinflammation

Inflammation is increasingly recognised as playing an important role in the pathogenesis of PD based on i) epidemiological studies suggesting NSAID use confers a lower risk of developing PD;[77] ii) the presence of increased microglial activation seen in PD patients using PET imaging;[78] iii) increased pro-inflammatory mediators seen in the SN post-mortem tissue of PD patients[79] and an association between the HLA locus and PD risk from genome-association studies.[80] Agents that target these inflammatory pathways are currently being investigated in clinical trials.

Pioglitazone, licensed for use in patients with type 2 diabetes, is thought to act via activation of the PPARγ [81] and has demonstrated anti-inflammatory-mediated neuroprotective effects in models of AD,[82] epilepsy,[83] stroke[84] and ALS.[85] Due to its demonstrated efficacy across multiple toxin animal models and influence across multiple cellular pathways,[86–88] a Phase 2 trial is currently underway (Clinical Trials.gov Identifier NCT01280123); however, its future potential may be limited by adverse effects and an association with the development of bladder cancers.[89–92] It has recently been discovered that many of the metabolic effects of pioglitazones may occur independently of PPARγ and involve binding to a complex on the inner mitochondrial membrane - identified as mTOT (mitochondrial target of thiazolidinones) directly influencing mitochondrial function[93,94] and novel compounds are in early development in testing against models of PD, which may offer similar benefits of neuroprotection with limited side effects.[95]

Myeloperoxidase (MPO), a heme enzyme expressed in a variety of phagocytic cells, is thought to play an important role in alpha synuclein-mediated toxicity. Following alpha synuclein induced activation of microglia, MPO mediates production of pro-inflammatory molecules, promoting misfolding of wild type alpha synuclein, leading to further aggregate formation in unaffected neurons.[96] MPO expression in microglia is found to be up regulated in the ventral midbrain of human PD patients and mice exposed to MPTP[97] and it has been speculated that inhibition of MPO may slow down neurodegeneration in PD given that ventral midbrain dopaminergic neurons of mutant mice deficient in MPO are more resistant to MPTP-induced cytotoxicity. Though a RCT of AZD3241, a novel MPO inhibitor, has recently been completed which demonstrated that it was well tolerated, and decreased plasma MPO activity,[98] there is no efficacy data, and the evidence from in vitro or in vivo studies that this particular compound may have neuroprotective effects in PD patients is still limited.

Statins, as well having effects on lowering cholesterol, have marked anti-inflammatory effects. In vitro studies show that simvastatin can reduce alpha-synuclein aggregation, inhibit the formation of TNF-alpha and peroxynitrite in activated microglia, and via modulation of the NMDA receptor, protect dopaminergic neurons from inflammatory damage, while in LPS and MPTP animal models of PD, statins prevents dopaminergic degeneration.[99–104] Data from epidemiological data regarding statin use and risk of developing PD is variable, with some studies identifying a reduced incidence of PD with statin use[105–107] while others found no association[108–110] or an inverse association with LDL cholesterol[111] – though this variance is possibly explained by early studies not accounting for confounders such as co-morbid diabetes, and use of NSAID's and calcium channel blockers which can modulate the risk of PD. However, a recent population-based study, adjusted for

most confounders, showed that patients on continued lipophilic statin therapy had a reduced incidence of PD compared to people who stopped their medication, though more longitudinal studies are needed.[112] While no clinical data of statin treatment in PD exist, there is promising in vivo and in vitro data highlighting potential mechanism and this should fuel further clinical studies.

Targeting alpha synuclein:

The pathological hallmark of PD is the presence of LB's and Lewy neurites (LN), of which alpha-synuclein constitutes a major component. Substantial evidence suggests that the conversion of alpha-synuclein from soluble monomers to aggregated, insoluble forms is a key event in the pathogenesis of PD.[113] Usually cleared from the cell by the ubiquitin proteasome or autophagy-lysosomal systems, defects of these systems have been identified in patients with PD. Furthermore, post mortem studies of fetal mesencephalic brain tissue grafted into brains of PD patients showed evidence of LB-like inclusions in the host tissue, suggesting that alpha-synuclein can transfer from unaffected cells and may act as a prion type protein – findings which subsequently have been confirmed in vitro an in vivo[13,114–117]. Mutations and variations in the alpha-synuclein gene can cause familial PD and contribute to sporadic PD, and overexpression in transgenic models leads to a parkinsonian phenotype; and therefore targeting the formation and clearance of alpha-synuclein as therapeutic measures to protect against neurodegeneration are promising avenues gathering great interest.

Reducing the formation of alpha-synuclein is one potential strategy and pre-clinical studies using small peptides, vector mediated RNAi interference and novel molecules such as ELN484228 that can directly block alpha synuclein aggregation[118] are promising avenues but are still early in development.

Another strategy is to reduce the amount of insoluble toxic alpha-synuclein, and both active immunisation, or passive immunisation with monoclonal antibodies against alpha synuclein, given to transgenic mice overexpressing human alpha synuclein have promoted reduction and clearance of the protein and rescued behavioural deficits.[119,120] This data has led to 2 commercial programs i) PRX02, which is an antibody against the 9E4 C-terminus of alpha synuclein, has major support pledged by Roche, with a phase I trial testing the safety and tolerability of PRX02 commencing in healthy individuals (Clinical trials.gov NCT02095171); and ii) Affitope (PD01), a peptide-carrier conjugate vaccine developed to induce antibodies selectively against alpha synuclein, currently in phase I clinical trial testing in patients with PD (Clinical trials.gov NCT01568099). On a cautionary note, similar efforts using immunization are more advanced in AD. Previous trials targeting beta amyloid in patients with AD have led to early termination due to significant meningoencephalitis, and led to concerns regarding antibodies altering the physiological function of other synucleins or triggering autoimmunity, potentially exacerbating Parkinson's pathology. However, unlike b-amyloid, a-synuclein does not deposit in leptomeningeal and cortical blood vessels, and by using vaccines with short peptides that do not trigger T cell autoimmunity or cross react with other synucleins, these risks are theoretically lower.[121]

Phosphorylation of alpha-synuclein at serine residue position 129 represents the most abundant form of alpha-synuclein found in LB's and targeting these pathways underlie the

mechanisms behind kinase inhibitors.[122]However, as several different kinases are capable of phosphorylating alpha-synuclein, a significant production of phosphor-Ser129 alpha-synuclein would still occur with drugs targeting a single kinase alone, and therefore targeting enzymes such as protein phosphatase 2A (PP2A) that enhance de-phosphorylation of Ser129 may represent a potentially valuable therapeutic strategy.[123] Metformin has recently been shown to lower levels of alpha-synuclein in vitro and in vivo by inhibiting mammalian target of rapamycin (mTOR) and enhancing PP2A activity – leading to reduced levels of alpha-synuclein.[124] Interestingly, although epidemiological data regarding the association between PD and diabetes is conflicting, a recent prospective cohort study showed that Type 2 diabetes increased the incidence of PD in a Taiwanese population almost 2-fold, which was exacerbated by the use of sulphonureas, but the risk was avoided by the use of metformin therapy.[125] This emerging data is prompting further studies to investigate metformin's disease modifying properties.

Enhancing clearance of alpha-synuclein by modulating ubiquitin and lysosomal systems is another strategy with promising early results. The identification of heat shock proteins that act as "chaperones" by promoting the transfer of excessive alpha-synuclein to the ubiquitin and lysosomal systems for clearance has led to testing compounds that could enhance this pathway, such as latrepidine, an anti-histamine, which has recently been shown to stimulate autophagy and reduce accumulation of α-synuclein in vitro and in vivo, and further testing is underway[126] NAB2, a novel small molecule, has recently been shown to promote endosomal transport events via action on the ubiquitin ligase Rsp5/Nedd4 – "resetting" vesicle tracking homeostasis and reversing multiple pathological phenotypes caused by alpha-synuclein in vitro.[28,29]

GCase, an enzyme involved in modulating lysosomal function and folding of alpha-synuclein, is encoded by the gene GBA1; mutations of which account for up to 25% of young onset PD patients,[127] are associated with reduced GCase activity and increased neocortical accumulation of aggregates of αlpha-synuclein.[128] Reduced levels of GCase activity are seen in the SN of patients with sporadic PD, while increased expression of alpha-synuclein in cell models is associated with reduced GCase activity. Therefore, targeting GCase to increase its activity may increase alpha-synuclein metabolism and reduce its accumulation. Intracerebral injection of GCase via a AAV viral vector delivery method increased activity of GCase and reduced accumulation of alpha-synuclein and tau and improved and reversed cognitive deficits,[129] while ambroxol, a secretolytic agent licensed for used in the treatment of respiratory diseases, has been shown to act as a chaperone molecule and thus increase the clearance of alpha synuclein.[130–132] Further testing in a Thy1-Alpha-Synuclein mouse model is planned.

Though the identification of these new potential targets and preliminary results are encouraging, studies with these novel compounds are still very early in development.

Other / uncertain / multi-pathway targeting:
A number of neuroprotective agents currently in clinical trials have multiple or uncertain mechanisms that may act on several pathways related to pathological processes in PD.

Data from in vitro and in vivo studies suggest that GM1 ganglioside, a component of neuronal cell membranes, affects multiple pathways implicated in the pathogenesis of PD - modulating intracellular calcium homeostasis, mitochondrial function, lysosomal integrity and preventing aggregation of alpha synuclein,[133,134,135] while two subsequent RCT's showed encouraging effects on motor function in PD.[135,136] While purified GM1 is well tolerated, and has shown encouraging results through careful trial design and placebo control, the existing data cannot conclude that the major long term effects are more than symptomatic only. Given that this agent is currently produced from bovine brain tissue, GM1 has also raised public safety concerns and alternative methods of production using synthetic analogues or ovine sources are currently being investigated.

Exenatide is a synthetic glucagon-like peptide-1 (GLP-1) agonist licensed for the treatment of type 2 diabetes, and improves glycaemic control via activation of the GLP-1 receptor.[137] Though their role in the CNS is unclear, GLP-1 receptor stimulation using exenatide in vitro has neuroprotective, anti-inflammatory and anti-apoptotic effects, and exerts neurotrophic effects, stimulates mitochondrial biogenesis and enhances synaptic plasticity.[138,139] In views of these effects, exenatide has been investigated in multiple animal toxin models of PD and demonstrated neuroprotective and neurorestorative effects in comparable human doses possibly via anti-inflammatory effects[140,141] or related to stimulation of neurogenesis.[142] It also demonstrated positive effects on non-dopaminergic impairments, reversed non-motor behavioural impairments, and improved learning and memory performance.[143,144] In a small, open label RCT, exenatide exposure led to a mean advantage of 7.0 points on the MDS-UPDRS Part III which persisted after a 12 month "wash-out" period, together with improvements in the Mattis Dementia Rating scale and well as other non-motor areas.[145,146] While clearly encouraging, the existing trial data is open label, and thus should not be interpreted as proof of efficacy in PD. Furthermore there is a great deal of mechanistic uncertainty regarding this agent, and exenatide has also been linked to a small increased risk of pancreatitis in patients with diabetes. A larger, double blind trial using a once weekly, long acting form of exenatide is underway (Clinical trials.gov Identifier NCT01971242).

In view of consistent epidemiological studies suggesting a 50% reduced risk of developing PD in smokers,[147,148] nicotine is being investigated for its potentially disease modifying properties. While in vitro data suggest possible mechanism of protection - nicotine inhibits alpha-synuclein fibrillation and reduce oxidative stress[149,150] while stimulation of the a7, a4B2 and a4 subunit of the nicotinic Ach receptor modulates inflammatory pathways and calcium homeostasis,[151,152] nicotine's effects in animal models of PD are conflicting, with discrepancies regarding dose, level of protection and dosing regimen [153,154,155,156]. Though conflicting results from pre-clinical trials may be explained by differences in methodology – at best the results are inconclusive. Results from human trials suggest a beneficial role in reducing dyskinesia, but evidence for neuroprotection is as yet unconvincing. Nevertheless, an RCT using transdermal nicotine patches in 40 PD has been completed and results awaited (Clinical trials.gov NCT00873392).

Neuroprotection against cognitive decline:
Cognitive impairments have a significant impact on quality of life - dementia is common in the advanced stage of PD, affecting up to 80% of patients,[157] and is often used as a milestone heralding impending residential care and mortality, but cognitive deficits are seen

in 24% of patients even at diagnosis[158], and mild cognitive impairment can occur early in the course of PD.[2] These deficits can worsen motor disabilities seen in PD, and at all stages are a significant factor contributing to a poorer quality of life.[2] Historically, the augmentation of neurotransmitter deficits have represented more relevant pharmaceutical targets for treatments, and currently, the acetylcholinesterase inhibitor rivastigmine is the only licensed therapy for cognitive symptoms in PD dementia (PDD) or Dementia with Lewy bodies (DLB), with evidence suggesting positive effects on cognition, behavioural disturbance and ability to perform activities of daily living,[159] (evidence for the use of menantine in PDD is currently unclear and further studies are needed). Unfortunately, acetylcholinesterase inhibitors can often worsen motor deficits, and only aim to correct the end result of neurodegeneration, and therefore do little to affect the progression of the disease. Despite the unmet need for additional treatments of cognitive dysfunction in PDD and DLB, there are only two advanced stage clinical trials underway - donepezil, an acetylcholinesterase inhibitor, and atomoxetine, a selective noradrenalin reuptake inhibitor, are in phase 2/3 clinical trial testing in patients with PDD based on previous favourable effects on cognition and behaviour. To date, there have been no clinical trials to so far investigate potential disease modifying therapies in PDD or DLB.

The neuropathology underlying PDD is heterogeneous and results from previous studies have been variable, but it is strongly speculated that alpha-synuclein spread from the brainstem to limbic and neocortical structures is the major contributor to emerging dementia in PD.[160] Therefore, it is a reasonable to assume that strategies to inhibit the formation and propagation of alpha-synuclein may halt cognitive and motor decline. However, 50% of patients with PDD also have amyloid-B plaques and hyperphosphorylated tau-containing neurofibrillary tangles – usually seen in the brains of patients with AD and furthermore, this co-morbid pathology may act synergistically with LB's and LN's t and confer a worse prognosis.[161–164] Further evidence from genetic studies lend support to suggest a link between tau and PD - the H1/H1 subhaplotype of the gene that encodes tau, MAPT, has been associated with increased tau expression in humans,[165] is associated with poor memory performance in PD,[166] and, through recent genome-wide association studies, has been identified as an independent risk factor for the development of PD[165167] and PDD.[168]

This growing evidence supporting the role of tau in Parkinson's disease had led some to speculate that targeting tau phosphorylation and formation may be of benefit in preventing cognitive decline, and that disease modifying therapy or strategies to target tau in AD may also be of benefit to a subset of patients with PDD.

Glycogen synthase-kinase 3b (GSK-3), is an enzyme involved in the phosphorylation of tau, and efforts at inhibition with compounds in human trials has so far proved disappointing. Valproate showed no clinical efficacy in Phase 3 trials,[169] while micro-doses of lithium, previously shown to prevent oxidative-stress-induced alpha-synuclein accumulation and neurodegeneration in a transgenic model of PD,[170] demonstrated stabilisation of cognitive function in AD patients,[171] but adverse effects affected subsequent follow up. Efforts are further advanced with derivatives of methylene blue – a compound that has already demonstrated neuroprotection and reversed behavioural deficits in a rotenone model of PD possibly by rescuing mitochondrial function and reducing free-radical formation.[172] TRx0237, a derivative of methylene blue, has been shown to reduce tau aggregation and inflammation with associated positive effects on behaviour in vivo, although studies are conflicting,[173174]while in subsequent Phase II trials of AD patients, it reduced the rate of

cognitive decline (though no benefit was seen at high doses).[175] Phase 3 trials are currently underway.

Tau hyperphosporylation has been speculated to reduce stabilisation of microtubules (MT), exacerbating neuronal dysfunction. In view of this, small molecule MT stabilizing drugs previously used in the treatment of cancers are being re-purposed to investigate their utility in the treatment of tauopathies. In aged transgenic mouse models with established tau pathology (hereby modelling a human setting) epothilone D significantly reduced tau pathology, prevented the loss of hippocampal neurons and synapses, and improved cognitive performance.[176]Though these results are encouraging, and may lead to the identification of further brain penetrating small molecule MT stabilising compounds, they are still very early in development and have not yet been tested in clinical trials.

Despite the encouraging number of compounds with potentially positive effects on cognition, there are numerous hurdles that need to be addressed before effective disease modifying therapies targeting cognition can be achieved. Identifying an appropriate target for intervention is required through greater understanding of the pathological process underlying cognitive dysfunction. Despite the large amount of evidence suggesting a role for alpha-synuclein in the development of cognitive (and motor) symptoms, there is a marked heterogeneity in PDD patients – some non-demented patients with PD have been shown to have large amounts of cortical alpha-synuclein,[177] while some demented individuals have demonstrated minimal cortical alpha-synuclein at post-mortem,[178]. Furthermore the interaction between AB and alpha-synuclein and correlation of cognitive function is yet to be elucidated. In addition, the timing of the intervention is important, as it remains to be seen whether halting alpha-synuclein aggregation or tau hyperphosphorylation in already symptomatic individuals could halt cognitive decline. Biomarker studies have recently indicated PDD patients are more likely to have increased CSF levels of t-tau and decreased AB1-42 than in PD,[179] while non-tremor dominant PD patients are more likely to develop dementia and manifest concurrent AB pathology[180,181] and it is reasonable to assume that further advances in biomarker studies, incorporating CSF, neuroimaging and biochemical markers together with further identification of clinical subtypes of PDD patients more likely to develop dementia, may help clinicians and trialists select the subgroup of individuals most appropriate for early intervention.

Conclusion
As things stand, double blind data supporting major clinically relevant effects have not yet been found for any agent, but there are several drugs that have been taken to clinical trials based on the existing neurotoxin animal models that may yet be shown to have relevant neuroprotective effects.

Many agents that appear to have beneficial effects on neuronal cell biology and may be useful in PD often do not survive beyond Phase 1 clinical trials because of toxicity. However, this hurdle can to some extent be avoided by prioritising agents already licensed for human use i.e. drug repositioning/repurposing. The linked clinical trials initiative[182] launched by the Cure Parkinson's trust is a way of identifying which licensed drugs may be effectively repositioned for PD and provides a rapid and cost efficient signal of effect to add to animal model data to allow subsequent prioritisation of larger scale investment. This said,

agents with major effects on neurodegenerative processes may also have long-term consequences for e.g. inflammation and/or immunosurveillance for malignant change in other body tissues, and these aspects must be considered in the follow up of patients exposed to these drug classes.

High throughput screening approaches are also useful to help select which of many thousands of compounds offers the most promise using in vitro assays e.g. measuring effects on alpha synuclein aggregation or mitochondrial function. Indeed it appears increasingly likely that direct targeting of alpha synuclein aggregation may become possible, while other studies demonstrate that up regulation of proteosomal or lysosomal systems, and/or promotion of chaperone molecules that can reduce alpha synuclein aggregation may be feasible, thus opening further potential new therapeutic targets.

Efforts are underway to accurately identify an "at risk" population of PD before significant clinical symptoms occur – possibly an essential first step before neurodegeneration progresses beyond the reach of any possible neuroprotective agent. This parallels the need to identify a highly sensitive and specific biomarker of PD to allow high-risk individuals to be distinguished from people with little or no risk of developing neurodegeneration. This is of utmost importance not only to be confident about efficacy but also to minimise the risks of exposing healthy people to potential adverse events that may accompany exposure to potential disease modifying drugs. The progression of symptoms in PD is heavily influenced by factors including clinical phenotype, age, and genotype and so in future, it may be more helpful to classify PD and other neurodegenerative diseases as clinicopathological entities rather than clinical syndromes - which could help define subsets of the PD population with more individualised biomarkers of disease activity, more accurately gauge prognosis and aid selection of specific disease modifying therapies e.g. patients at higher risk of PDD.

Although PDD can have a significant impact on quality of life, there is only a single, modestly effective symptomatic therapy for it and no treatments proven to slow its progression. Growing genetic, biochemical and pathological evidence implicates that tau and alpha-synuclein are involved in or share converging pathways in the pathogenesis of PD, and can influence development of cognitive impairment in patients with PD. These developments have helped identify new targets for potential disease modifying therapies and it is clear that agents that only aim to prevent nigro-striatal degeneration may only have limited potential or may need to be combined with agents that target multiple pathways.

Many of the agents discussed have potential for further investigation – however it remains to be seen whether any will prove to effective neuroprotective therapies for PD. This said, the rate of continued advances in many areas suggests, it is reasonable to remain optimistic that PD may be transformed from a continually progressive, disabling disease to a chronic, more manageable course.

Review criteria

PubMed was searched for articles published between September 1989 and July 2014. MeSH search terms were "Parkinson's disease" alone and in combination with "disease modification", "neuroprotection", "clinical trials", "alpha-synuclein", "dementia", "cognitive impairment", "pathogenesis", "animal models", "mitochondrial", "biomarkers", "treatments". Only papers in English were reviewed. Articles were selected for their relevance, with a preference for new papers. Some other relevant papers known by the authors were also included.

Display items:

Table 1. Recent high profile trials of potential neuroprotective agents in PD. While it remains possible that 1 or more of these approaches has beneficial neuroprotective effects for PD, none have data to convincingly support clinically relevant neuroprotection when administered in isolation according to the clinical trial protocols used. DS- Delayed Start. PA-Parallel group. UPDRS – Unified Parkinson's disease rating scale. DA – Dopaminergic.

Drug and class of evidence	Mechanism of action	Evidence of neuroprotection from pre-clinical studies	Evidence of neuroprotection from clinical trials and level of evidence
Rasagiline	Antioxidant MAO inhibitor Anti-apoptotic	Protected DA neurons in MPTP and 6-OHDA models of PD Increased expression of BDNF, GDNF, and NGF[183]	Phase 3 (DS) Inconclusive. 1mg rasagiline demonstrated significance but was not repeated at 2mg (Class 1b). Small change in UPDRS made results difficult to interpret[43]
Creatine	Improves mitochondrial bioenergetics Antioxidant	Protected DA neurons in MPTP and 6-OHDA models of PD[184]	Phase 3 (PA) Trial involving 1,741 patients terminated early due to lack of efficacy (Class 1b)[17]
Co-enzyme Q10 (ubiquinone)	Electron carrier for mitochondrial complex I & II Antioxidant	Protected DA neurons in MPTP mouse model and delayed progression of PD in Earlier phase II study - non-significant advantage in UPDRS scores[121]	Phase 3 (PA) Trial terminated due to lack of efficacy over placebo (Class 1b)[185]
Cogane (PYM50028)	Promotes release of GDNF and BDNF	Reverses MPP+-induced neuronal atrophy in mesencephalic neurons in vitro[186] Protected DA neurons in MPTP models of	Phase 3 (PA) Trial halted. No benefits over placebo identified (Class 1b)[187]

		PD[186]	
Mitoquinone (MitoQ)	Antioxidant Mitochondrial bioenergetic	Protected DA neurons from MPTP and 6-OHDA induced toxicity[188]	Phase 3 (PA) No benefit over placebo. However, study had small sample size and may not have reached adequate levels of brain penetrance (Class 2b)[189]
TCH346	Anti-apoptotic	Protected DA neurons in vitro and MPTP model[18]	Phase 2 There were no significant differences between groups (Class 1b)[18]
CERE-120 (AAV neurturin)	Intraputaminal and intranigral injection of GDNF analogue	GDNF protects DA neurons in vitro and mouse and non-human primate models of PD[190] Intraputaminal AAV2-neurturin injection in RCT superior to sham surgery[190]	Phase 2b (PA) Intraputaminal and SNpc AAV2-neurturin injection in RCT: No statistically significant efficacy on the primary endpoint (Class 2c). Secondary endpoint – Off periods in self-reported motor diaries did significantly improve (Class 2c)[191]

Fig.1 Proposed mechanisms involved in the pathogenesis of PD, with the identification of potential targets for intervention highlighted and the relevant drugs thought to act on these pathways. (1) Targeting native coils of alpha-synuclein expression, (2) upregulation of chaperone molecules to promote clearance of alpha-synuclein (e.g. latrepirdine, ambroxol), (3) targeting enzymes such as protein phosphatase 2A (PP2A) that enhance de-phosphorylation of alpha-synuclein at Ser129 (e.g. metformin), (4) facilitation of the UPS system to clear unwanted alpha-synuclein (e.g.NAB2), (5) enhancing liposomal function and metabolism of alpha-synuclein by increasing GCase activity (e.g. resveratrol), (6) directly targeting toxic alpha-nuclein by active/passive immunisation (e.g. PRX02, Affitope) or directly blocking its aggregation (e.g. ELN484228), (7) prevent the propagation of alpha-synuclein from affected to unaffected cells, (8) targeting Ca1.3V LTCC to reduce calcium influx (e.g. isradipine), (9) modulation of the NMDA receptor to limit activation of excitatory pathways (e.g. simvastatin), (10) targeting inflammatory pathways (e.g. pioglitazone, AZD3241), (11) modulation of SIRT1 to enhance mitochondrial biogenesis and reduce activation of proinflammatory pathways (e.g. resveratrol), (12) reducing oxidative stress pathways (e.g. inosine, N-acetylcysteine, zonisamide), (13) reducing iron accumulation (e.g. deferiprone), (14) targeting apoptotic pathways (e.g. GCSF).

UPS, ubiquitin-proteasome system; LTCC, L-type calcium channels; NMDA, N-methyl-D-aspartate; ROS, reactive oxygen species; SIRT1, sirtuin1; PGC-1-alpha, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; P-129, serine residue position 129.

Table 2. Summary of evidence of the current candidates in clinical trial testing

Drug	Proposed mechanism(s) of action	Summary of pre-clinical evidence	Summary of clinical evidence
Isradipine	Calcium channel blocker	In vitro and vivo evidence of neuroprotection in MPTP and 6-OHDA models by reverting DA neurons to juvenile sodium channel pacemaking mechanism[37,38] Brain penetrance[192]	10mg was highest dose tolerated in RCT of 99 PD patients – modest advantage of UPDRS III[39]
Inosine	Antioxidant Raises serum urate levels[50]	In vitro and in vivo evidence in 6-OHDA and MPTP of neuroprotection[193]	Able to raise serum and CSF urate in RCT of 75 PD patients, and associated with favourable rate of progression of UPDRS after 24 months[51]
Deferiprone	Anti-oxidant Iron chelator	In vitro evidence for protection against MPP+ toxin related degeneration[57] In vivo evidence in 6-OHDA models preserving DA neurons by reducing hydroxl formation[58] Established brain penetrance[194]	RCT (DS design) of 40 PD patients reduced iron levels in SN with reduced UPDRS III scores in ES group sustained at 12 months, though waned after 18 months[59]
NAC	Antioxidant Precursor of GSH	In vivo evidence in MPTP and transgenic mice overexpressing α-synuclein, of increased GSH levels associated with prevention of DA cell death and motor abnormalities, with reduction of alpha-synuclein levels in brain[66,195,196]	Phase 1 trial of 12 PD patients raised levels of CSF GSH and well tolerated[67]
Zonisamide	Anti-oxidant	In vitro and vivo evidence in 6-OHDA, MPTP and rotenone models in dose dependent manner of	RCT of PD patients symptomatic improvement in motor function[200]

		neuroprotection of DN neurons via modulation of calcium homeostasis, enhancing GABA release and upregulation of expression of mRNA encoding astrocytic anti-oxidative and neurotrophic factors but conflicting doses[71,197–199]	
Pioglitazone	Anti-inflammatory Enhances mitochondrial biogenesis	In vivo evidence in LPS, rotenone and non-human MPTP primate models of improved motor and behavioural effects , with reduced microglial activation, DA cell loss[86,87,201]	No clinical data - Phase II trial currently underway
AZD3241	Anti-inflammatory Inhibits MPO Influences alpha-synuclein aggregation	In vitro evidence and in vivo evidence in 6-OHDA model of reduced inflammatory cytokines[97,202]	RCT of 51 PD patients – well tolerated, decreased MPO activity[98]
GM1		In vitro evidence of reducing alpha-synuclein aggregation, modulating lysosomal systems[133] In vivo evidence in MPTP non-human models of neurotropic effects with motor and cognitive improvements[134]	RCT of 26 PD patients showed symptomatic improvements of UPDRS III after 5 years[135] RCT (DS design) of 77 PD patients improved UPDRS III scores of ES group, but both groups worsened following washout[136]
Exenatide	GLP-1 agonist Anti-inflammatory, anti-apoptotic effects, neurotrophic effects, mitochondrial enhancer	In vitro evidence of effects on reducing inflammation, apoptosis, and enhancing neurotropic effects, mitochondrial biogenesis, and synaptic plasticity[138,139] In vivo evidence in 6-OHDA, MPTP, LPS models of neuroprotective and neurorestorative effects on DA neurons,	Open label RCT of 20+20 PD patients showed improvements in MDS-UPDRS III and memory, sustained after washout[145,203]

		with improved motor and memory performance[140–144]	
Transdermal nicotine	Reduced alpha-synuclein aggregation Modulates calcium homeostasis Modulates pro-inflammatory pathways	In vitro evidence to inhibit alpha-synuclein aggregation, reducing oxidative stress by modulating calcium homeostasis[149–152] In vivo evidence in 6-OHDA, MPTP, rotenone models of halting DA cell loss, but conflicting results, and doses[153–156]	Human trials conflicting – beneficial in 4 trials, no effect in 4 trials, worsening motor function in 1 trial[150]
GCSF	Anti-apoptotic Neurotropic factor	In vitro evidence of neuroprotective of DA cells via activation of anti-apoptotic STAT3 and AKT pathways,[204] neurotrophism,[205] stimulating neurogenesis through reciprocal interaction with VEGF activation[206] and modulation of pro-inflammatory pathways.[207] In vivo evidence in MPTP models models, though at doses higher than used in humans[208,209]	No clinical data yet
EPO	Anti-inflammatory	In vitro evidence of DA protection with proliferation of astrocytes[210] In vivo evidence in 6-OHDA models reduced inflammation in DA neurons[210]	Phase 1 trial of 10 PD patients showed improvements in UPDRS III, persisting for 30 weeks[211] RCT of 26 PD patients showed no effect on motor symptoms but some benefits on cardiovascular autonomic function and cognition[212]

References

1.   Duncan, G. W. *et al.* Health-related quality of life in early Parkinson's disease: the impact of nonmotor symptoms. *Mov. Disord.* **29,** 195–202 (2014).

2.   Lawson, R. A. *et al.* Severity of mild cognitive impairment in early Parkinson's disease contributes to poorer quality of life. *Parkinsonism Relat. Disord.* (2014). doi:10.1016/j.parkreldis.2014.07.004

3.   Schapira, A. H. V & Tolosa, E. Molecular and clinical prodrome of Parkinson disease: implications for treatment. *Nat. Rev. Neurol.* **6,** 309–17 (2010).

4.   Foltynie, T. & Kahan, J. Parkinson's disease: an update on pathogenesis and treatment. *J. Neurol.* **260,** 1433–40 (2013).

5.   Schapira, A. H. V, Olanow, C. W., Greenamyre, J. T. & Bezard, E. Slowing of neurodegeneration in Parkinson's disease and Huntington's disease: future therapeutic perspectives. *Lancet* **6736,** (2014).

6.   Olanow, C. W. & Kordower, J. H. Modeling Parkinson's disease. *Ann. Neurol.* **66,** 432–6 (2009).

7.   Blandini, F. & Armentero, M.-T. Animal models of Parkinson's disease. *FEBS J.* **279,** 1156–66 (2012).

8.   Bezard, E., Yue, Z., Kirik, D. & Spillantini, M. G. Animal models of Parkinson's disease: limits and relevance to neuroprotection studies. *Mov. Disord.* **28,** 61–70 (2013).

9.   Pifl, C., Schingnitz, G. & Hornykiewicz, O. Effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine on the regional distribution of brain monoamines in the rhesus monkey. *Neuroscience* **44,** 591–605 (1991).

10.   Mounayar, S. *et al.* A new model to study compensatory mechanisms in MPTP-treated monkeys exhibiting recovery. *Brain* **130,** 2898–914 (2007).

11.   Iravani, M. M. *et al.* A modified MPTP treatment regime produces reproducible partial nigrostriatal lesions in common marmosets. *Eur. J. Neurosci.* **21,** 841–54 (2005).

12.   Duty, S. & Jenner, P. Animal models of Parkinson's disease: a source of novel treatments and clues to the cause of the disease. *Br. J. Pharmacol.* **164,** 1357–91 (2011).

13.   Volpicelli-Daley, L. A. *et al.* Exogenous α-synuclein fibrils induce Lewy body pathology leading to synaptic dysfunction and neuron death. *Neuron* **72,** 57–71 (2011).

14.   Luk, K. C. & Lee, V. M.-Y. Modeling Lewy pathology propagation in Parkinson's disease. *Parkinsonism Relat. Disord.* **20 Suppl 1,** S85–7 (2014).

15. Fahn, S. *et al.* Levodopa and the progression of Parkinson's disease. *N. Engl. J. Med.* **351,** 2498–508 (2004).

16. D'Agostino, R. B. The delayed-start study design. *N. Engl. J. Med.* **361,** 1304–6 (2009).

17. Elm, J. J. Design innovations and baseline findings in a long-term Parkinson's trial: the National Institute of Neurological Disorders and Stroke Exploratory Trials in Parkinson's Disease Long-Term Study-1. *Mov. Disord.* **27,** 1513–21 (2012).

18. Olanow, C. W. *et al.* TCH346 as a neuroprotective drug in Parkinson's disease: a double-blind, randomised, controlled trial. *Lancet Neurol.* **5,** 1013–20 (2006).

19. Ward, C. D. Does selegiline delay progression of Parkinson's disease? A critical re-evaluation of the DATATOP study. *J. Neurol. Neurosurg. Psychiatry* **57,** 217–20 (1994).

20. Kang, J.-H. *et al.* Association of cerebrospinal fluid β-amyloid 1-42, T-tau, P-tau181, and α-synuclein levels with clinical features of drug-naive patients with early Parkinson disease. *JAMA Neurol.* **70,** 1277–87 (2013).

21. Agarwal, P. A. & Stoessl, A. J. Biomarkers for trials of neuroprotection in Parkinson's disease. *Mov. Disord.* **28,** 71–85 (2013).

22. FEARNLEY, J. M. & LEES, A. J. AGEING AND PARKINSON'S DISEASE: SUBSTANTIA NIGRA REGIONAL SELECTIVITY. *Brain* **114,** 2283–2301 (1991).

23. Kordower, J. H. *et al.* Disease duration and the integrity of the nigrostriatal system in Parkinson's disease. *Brain* **136,** 2419–31 (2013).

24. Schwingenschuh, P. *et al.* Distinguishing SWEDDs patients with asymmetric resting tremor from Parkinson's disease: a clinical and electrophysiological study. *Mov. Disord.* **25,** 560–9 (2010).

25. Foltynie, T., Brayne, C. & Barker, R. A. The heterogeneity of idiopathic Parkinson's disease. *J. Neurol.* **249,** 138–45 (2002).

26. Dragalin, V. An introduction to adaptive designs and adaptation in CNS trials. *Eur. Neuropsychopharmacol.* **21,** 153–8 (2011).

27. Sherer, T. B., Chowdhury, S., Peabody, K. & Brooks, D. W. Overcoming obstacles in Parkinson's disease. *Mov. Disord.* **27,** 1606–11 (2012).

28. Tardiff, D. F. *et al.* Yeast reveal a "druggable" Rsp5/Nedd4 network that ameliorates α-synuclein toxicity in neurons. *Science* **342,** 979–83 (2013).

29. Chung, C. Y. *et al.* Identification and rescue of α-synuclein toxicity in Parkinson patient-derived neurons. *Science* **342,** 983–7 (2013).

30. Mortiboys, H., Aasly, J. & Bandmann, O. Ursocholanic acid rescues mitochondrial function in common forms of familial Parkinson's disease. *Brain* **136,** 3038–50 (2013).

31. Chan, C. S., Gertler, T. S. & Surmeier, D. J. A molecular basis for the increased vulnerability of substantia nigra dopamine neurons in aging and Parkinson's disease. *Mov. Disord.* **25 Suppl 1,** S63–70 (2010).

32. Hurley, M. J., Brandon, B., Gentleman, S. M. & Dexter, D. T. Parkinson's disease is associated with altered expression of CaV1 channels and calcium-binding proteins. *Brain* **136,** 2077–97 (2013).

33. Becker, C., Jick, S. S. & Meier, C. R. Use of antihypertensives and the risk of Parkinson disease. *Neurology* **70,** 1438–44 (2008).

34. Ritz, B. *et al.* L-type calcium channel blockers and Parkinson disease in Denmark. *Ann. Neurol.* **67,** 600–6 (2010).

35. Pasternak, B. *et al.* Use of calcium channel blockers and Parkinson's disease. *Am. J. Epidemiol.* **175,** 627–35 (2012).

36. Marras, C. *et al.* Dihydropyridine calcium channel blockers and the progression of parkinsonism. *Ann. Neurol.* **71,** 362–9 (2012).

37. Ilijic, E., Guzman, J. N. & Surmeier, D. J. The L-type channel antagonist isradipine is neuroprotective in a mouse model of Parkinson's disease. *Neurobiol. Dis.* **43,** 364–71 (2011).

38. Chan, C. S. *et al.* "Rejuvenation" protects neurons in mouse models of Parkinson's disease. *Nature* **447,** 1081–6 (2007).

39. Phase II safety, tolerability, and dose selection study of isradipine as a potential disease-modifying intervention in early Parkinson's disease (STEADY-PD). *Mov. Disord.* **28,** 1823–31 (2013).

40. Kang, S. *et al.* CaV1.3-selective L-type calcium channel antagonists as potential new therapeutics for Parkinson's disease. *Nat. Commun.* **3,** 1146 (2012).

41. Alam, Z. I. *et al.* Oxidative DNA damage in the parkinsonian brain: an apparent selective increase in 8-hydroxyguanine levels in substantia nigra. *J. Neurochem.* **69,** 1196–203 (1997).

42. Pålhagen, S. *et al.* Selegiline slows the progression of the symptoms of Parkinson disease. *Neurology* **66,** 1200–6 (2006).

43. Olanow, C. W. *et al.* A double-blind, delayed-start trial of rasagiline in Parkinson's disease. *N. Engl. J. Med.* **361,** 1268–78 (2009).

44.   Weisskopf, M. G., O'Reilly, E., Chen, H., Schwarzschild, M. A. & Ascherio, A. Plasma urate and risk of Parkinson's disease. *Am. J. Epidemiol.* **166,** 561–7 (2007).

45.   Alonso, A., Rodríguez, L. A. G., Logroscino, G. & Hernán, M. A. Gout and risk of Parkinson disease: a prospective study. *Neurology* **69,** 1696–700 (2007).

46.   Shen, C., Guo, Y., Luo, W., Lin, C. & Ding, M. Serum urate and the risk of Parkinson's disease: results from a meta-analysis. *Can. J. Neurol. Sci.* **40,** 73–9 (2013).

47.   Schwarzschild, M. A. *et al.* Serum urate as a predictor of clinical and radiographic progression in Parkinson disease. *Arch. Neurol.* **65,** 716–23 (2008).

48.   Ascherio, A. *et al.* Urate as a predictor of the rate of clinical decline in Parkinson disease. *Arch. Neurol.* **66,** 1460–8 (2009).

49.   Andreadou, E. *et al.* Serum uric acid levels in patients with Parkinson's disease: their relationship to treatment and disease duration. *Clin. Neurol. Neurosurg.* **111,** 724–8 (2009).

50.   Yamamoto, T. *et al.* Effect of inosine on the plasma concentration of uridine and purine bases. *Metabolism.* **51,** 438–42 (2002).

51.   Schwarzschild, M. A. *et al.* Inosine to increase serum and cerebrospinal fluid urate in Parkinson disease: a randomized clinical trial. *JAMA Neurol.* **71,** 141–50 (2014).

52.   Grayson, P. C., Kim, S. Y., LaValley, M. & Choi, H. K. Hyperuricemia and incident hypertension: a systematic review and meta-analysis. *Arthritis Care Res. (Hoboken).* **63,** 102–10 (2011).

53.   Takanashi, M. *et al.* Iron accumulation in the substantia nigra of autosomal recessive juvenile parkinsonism (ARJP). *Parkinsonism Relat. Disord.* **7,** 311–314 (2001).

54.   Sian-Hülsmann, J., Mandel, S., Youdim, M. B. H. & Riederer, P. The relevance of iron in the pathogenesis of Parkinson's disease. *J. Neurochem.* **118,** 939–57 (2011).

55.   Crichton, R. & Ward, R. *Metal-Based Neurodegeneration: From Molecular Mechanisms to Therapeutic Strategies.* 440 (John Wiley & Sons, 2013). at <http://books.google.com/books?id=QROxAAAAQBAJ&pgis=1>

56.   Jamuar, S. S. & Lai, A. H. M. Safety and efficacy of iron chelation therapy with deferiprone in patients with transfusion-dependent thalassemia. *Ther. Adv. Hematol.* **3,** 299–307 (2012).

57.   Molina-Holgado, F., Gaeta, A., Francis, P. T., Williams, R. J. & Hider, R. C. Neuroprotective actions of deferiprone in cultured cortical neurones and SHSY-5Y cells. *J. Neurochem.* **105,** 2466–76 (2008).

58.	Dexter, D. T. *et al.* Clinically available iron chelators induce neuroprotection in the 6-OHDA model of Parkinson's disease after peripheral administration. *J. Neural Transm.* **118,** 223–31 (2011).

59.	Devos, D. *et al.* Targeting chelatable iron as a therapeutic modality in Parkinson's disease. *Antioxid. Redox Signal.* **21,** 195–210 (2014).

60.	Hauser, R. A., Lyons, K. E., McClain, T., Carter, S. & Perlmutter, D. Randomized, double-blind, pilot evaluation of intravenous glutathione in Parkinson's disease. *Mov. Disord.* **24,** 979–83 (2009).

61.	Chinta, S. J. & Andersen, J. K. Reversible inhibition of mitochondrial complex I activity following chronic dopaminergic glutathione depletion in vitro: implications for Parkinson's disease. *Free Radic. Biol. Med.* **41,** 1442–8 (2006).

62.	Sian, J. *et al.* Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. *Ann. Neurol.* **36,** 348–55 (1994).

63.	Riederer, P. *et al.* Transition metals, ferritin, glutathione, and ascorbic acid in parkinsonian brains. *J. Neurochem.* **52,** 515–20 (1989).

64.	Dexter, D. T. *et al.* Indices of oxidative stress and mitochondrial function in individuals with incidental Lewy body disease. *Ann. Neurol.* **35,** 38–44 (1994).

65.	Okun, M. S., Lang, A. & Jankovic, J. Reply: Based on the available randomized trial patients should say no to glutathione for Parkinson's disease. *Mov. Disord.* **25,** 961–2; author reply 962–3 (2010).

66.	Clark, J. *et al.* Oral N-acetyl-cysteine attenuates loss of dopaminergic terminals in alpha-synuclein overexpressing mice. *PLoS One* **5,** e12333 (2010).

67.	Katz, M., Swanson, R.A., Glass, G. A. Cerebrospinal fluid concentrations of N-acetylcysteine after oral administration: Phase I trial in Parkinson's disease [abstract]. *Mov. Disord.* **29,** S651–S681 (2014).

68.	Adair, J. C., Knoefel, J. E. & Morgan, N. Controlled trial of N-acetylcysteine for patients with probable Alzheimer's disease. *Neurology* **57,** 1515–7 (2001).

69.	Okada, M. *et al.* Exocytosis mechanism as a new targeting site for mechanisms of action of antiepileptic drugs. *Life Sci.* **72,** 465–73 (2002).

70.	Mochio, S. *et al.* Actigraphic study of tremor before and after treatment with zonisamide in patients with Parkinson's disease. *Parkinsonism Relat. Disord.* **18,** 906–8 (2012).

71.	Asanuma, M. *et al.* Neuroprotective effects of zonisamide target astrocyte. *Ann. Neurol.* **67,** 239–49 (2010).

72.   Schapira, A. H. V. Mitochondria in the aetiology and pathogenesis of Parkinson's disease. *Lancet Neurol.* **7,** 97–109 (2008).

73.   Tain, L. S. *et al.* Rapamycin activation of 4E-BP prevents parkinsonian dopaminergic neuron loss. *Nat. Neurosci.* **12,** 1129–35 (2009).

74.   Carta, A. R. & Pisanu, A. Modulating microglia activity with PPAR-γ agonists: a promising therapy for Parkinson's disease? *Neurotox. Res.* **23,** 112–23 (2013).

75.   Khan, M. M. *et al.* Resveratrol attenuates 6-hydroxydopamine-induced oxidative damage and dopamine depletion in rat model of Parkinson's disease. *Brain Res.* **1328,** 139–51 (2010).

76.   Lofrumento, D. D. *et al.* Neuroprotective effects of resveratrol in an MPTP mouse model of Parkinson's-like disease: possible role of SOCS-1 in reducing pro-inflammatory responses. *Innate Immun.* **20,** 249–60 (2014).

77.   Rees, K. *et al.* Non-steroidal anti-inflammatory drugs as disease-modifying agents for Parkinson's disease: evidence from observational studies. *Cochrane database Syst. Rev.* CD008454 (2011). doi:10.1002/14651858.CD008454.pub2

78.   Bartels, A. L. *et al.* [11C]-PK11195 PET: quantification of neuroinflammation and a monitor of anti-inflammatory treatment in Parkinson's disease? *Parkinsonism Relat. Disord.* **16,** 57–9 (2010).

79.   Imamura, K. *et al.* Distribution of major histocompatibility complex class II-positive microglia and cytokine profile of Parkinson's disease brains. *Acta Neuropathol.* **106,** 518–26 (2003).

80.   A two-stage meta-analysis identifies several new loci for Parkinson's disease. *PLoS Genet.* **7,** e1002142 (2011).

81.   Diani, A. R., Sawada, G., Wyse, B., Murray, F. T. & Khan, M. Pioglitazone preserves pancreatic islet structure and insulin secretory function in three murine models of type 2 diabetes. *Am. J. Physiol. Endocrinol. Metab.* **286,** E116–22 (2004).

82.   Heneka, M. T. *et al.* Acute treatment with the PPARgamma agonist pioglitazone and ibuprofen reduces glial inflammation and Abeta1-42 levels in APPV717I transgenic mice. *Brain* **128,** 1442–53 (2005).

83.   Okada, K., Yamashita, U. & Tsuji, S. Ameliorative effect of pioglitazone on seizure responses in genetically epilepsy-susceptible EL mice. *Brain Res.* **1102,** 175–8 (2006).

84.   Nakamura, T. *et al.* Pioglitazone exerts protective effects against stroke in stroke-prone spontaneously hypertensive rats, independently of blood pressure. *Stroke.* **38,** 3016–22 (2007).

85. Schütz, B. *et al.* The oral antidiabetic pioglitazone protects from neurodegeneration and amyotrophic lateral sclerosis-like symptoms in superoxide dismutase-G93A transgenic mice. *J. Neurosci.* **25,** 7805–12 (2005).

86. Dehmer, T., Heneka, M. T., Sastre, M., Dichgans, J. & Schulz, J. B. Protection by pioglitazone in the MPTP model of Parkinson's disease correlates with I kappa B alpha induction and block of NF kappa B and iNOS activation. *J. Neurochem.* **88,** 494–501 (2004).

87. Breidert, T. *et al.* Protective action of the peroxisome proliferator-activated receptor-gamma agonist pioglitazone in a mouse model of Parkinson's disease. *J. Neurochem.* **82,** 615–24 (2002).

88. Swanson, C. R. *et al.* The PPAR-γ agonist pioglitazone modulates inflammation and induces neuroprotection in parkinsonian monkeys. *J. Neuroinflammation* **8,** 91 (2011).

89. Suzuki, S. *et al.* Effects of pioglitazone, a peroxisome proliferator-activated receptor gamma agonist, on the urine and urothelium of the rat. *Toxicol. Sci.* **113,** 349–57 (2010).

90. Azoulay, L. *et al.* The use of pioglitazone and the risk of bladder cancer in people with type 2 diabetes: nested case-control study. *BMJ* **344,** e3645 (2012).

91. Ferwana, M. *et al.* Pioglitazone and risk of bladder cancer: a meta-analysis of controlled studies. *Diabet. Med.* **30,** 1026–32 (2013).

92. Consoli, A. & Formoso, G. Do thiazolidinediones still have a role in treatment of type 2 diabetes mellitus? *Diabetes. Obes. Metab.* **15,** 967–77 (2013).

93. Colca, J. R. *et al.* Identification of a mitochondrial target of thiazolidinedione insulin sensitizers (mTOT)--relationship to newly identified mitochondrial pyruvate carrier proteins. *PLoS One* **8,** e61551 (2013).

94. Colca, J. R. *et al.* Identification of a novel mitochondrial protein ("mitoNEET") cross-linked specifically by a thiazolidinedione photoprobe. *Am. J. Physiol. Endocrinol. Metab.* **286,** E252–60 (2004).

95. Colca, J. R. *et al.* Clinical proof-of-concept study with MSDC-0160, a prototype mTOT-modulating insulin sensitizer. *Clin. Pharmacol. Ther.* **93,** 352–9 (2013).

96. Appel, S. H. Inflammation in Parkinson's disease: cause or consequence? *Mov. Disord.* **27,** 1075–7 (2012).

97. Choi, D.-K. *et al.* Ablation of the inflammatory enzyme myeloperoxidase mitigates features of Parkinson's disease in mice. *J. Neurosci.* **25,** 6594–600 (2005).

98. Posener, J.A., Hauser, R.A., Stieber, M., Leventer, S.M., Eketjäll, S., Minkwitz, M.C., Ingersoll, E.W., Kugler, A. R. Safety, tolerability, and pharmacodynamics of AZD3241,

a myeloperoxidase inhibitor, in Parkinson's disease [abstract]. *Mov. Disord.* **29,** S682–S687 (2014).

99. Selley, M. L. Simvastatin prevents 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced striatal dopamine depletion and protein tyrosine nitration in mice. *Brain Res.* **1037,** 1–6 (2005).

100. Wang, Q., Wang, P. H., McLachlan, C. & Wong, P. T.-H. Simvastatin reverses the downregulation of dopamine D1 and D2 receptor expression in the prefrontal cortex of 6-hydroxydopamine-induced Parkinsonian rats. *Brain Res.* **1045,** 229–33 (2005).

101. Hernández-Romero, M. del C. *et al.* Simvastatin prevents the inflammatory process and the dopaminergic degeneration induced by the intranigral injection of lipopolysaccharide. *J. Neurochem.* **105,** 445–59 (2008).

102. Santiago, M., Hernández-Romero, M. C., Machado, A. & Cano, J. Zocor Forte (simvastatin) has a neuroprotective effect against LPS striatal dopaminergic terminals injury, whereas against MPP+ does not. *Eur. J. Pharmacol.* **609,** 58–64 (2009).

103. Yan, J., Sun, J., Huang, L., Fu, Q. & Du, G. Simvastatin prevents neuroinflammation by inhibiting N-methyl-D-aspartic acid receptor 1 in 6-hydroxydopamine-treated PC12 cells. *J. Neurosci. Res.* **92,** 634–40 (2014).

104. Bar-On, P. *et al.* Statins reduce neuronal alpha-synuclein aggregation in in vitro models of Parkinson's disease. *J. Neurochem.* **105,** 1656–67 (2008).

105. Gao, X., Simon, K. C., Schwarzschild, M. A. & Ascherio, A. Prospective study of statin use and risk of Parkinson disease. *Arch. Neurol.* **69,** 380–4 (2012).

106. Wahner, A. D., Bronstein, J. M., Bordelon, Y. M. & Ritz, B. Statin use and the risk of Parkinson disease. *Neurology* **70,** 1418–22 (2008).

107. Wolozin, B. *et al.* Simvastatin is associated with a reduced incidence of dementia and Parkinson's disease. *BMC Med.* **5,** 20 (2007).

108. Becker, C., Jick, S. S. & Meier, C. R. Use of statins and the risk of Parkinson's disease: a retrospective case-control study in the UK. *Drug Saf.* **31,** 399–407 (2008).

109. Hippisley-Cox, J. & Coupland, C. Unintended effects of statins in men and women in England and Wales: population based cohort study using the QResearch database. *BMJ* **340,** c2197 (2010).

110. Ritz, B. *et al.* Statin use and Parkinson's disease in Denmark. *Mov. Disord.* **25,** 1210–6 (2010).

111. Huang, X. *et al.* Lower low-density lipoprotein cholesterol levels are associated with Parkinson's disease. *Mov. Disord.* **22,** 377–81 (2007).

112. Lee, Y.-C. *et al.* Discontinuation of statin therapy associates with Parkinson disease: a population-based study. *Neurology* **81,** 410–6 (2013).

113. Martin, F. L., Williamson, S. J. M., Paleologou, K. E., Allsop, D. & El-Agnaf, O. M. A. Alpha-synuclein and the pathogenesis of Parkinson's disease. *Protein Pept. Lett.* **11,** 229–37 (2004).

114. Li, J.-Y. *et al.* Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. *Nat. Med.* **14,** 501–3 (2008).

115. Desplats, P. *et al.* Inclusion formation and neuronal cell death through neuron-to-neuron transmission of alpha-synuclein. *Proc. Natl. Acad. Sci. U. S. A.* **106,** 13010–5 (2009).

116. Hansen, C. *et al.* α-Synuclein propagates from mouse brain to grafted dopaminergic neurons and seeds aggregation in cultured human cells. *J. Clin. Invest.* **121,** 715–25 (2011).

117. Mougenot, A.-L. *et al.* Prion-like acceleration of a synucleinopathy in a transgenic mouse model. *Neurobiol. Aging* **33,** 2225–8 (2012).

118. Tóth, G. *et al.* Targeting the Intrinsically Disordered Structural Ensemble of α-Synuclein by Small Molecules as a Potential Therapeutic Strategy for Parkinson's Disease. *PLoS One* **9,** e87133 (2014).

119. Masliah, E. *et al.* Effects of alpha-synuclein immunization in a mouse model of Parkinson's disease. *Neuron* **46,** 857–68 (2005).

120. Masliah, E. *et al.* Passive immunization reduces behavioral and neuropathological deficits in an alpha-synuclein transgenic model of Lewy body disease. *PLoS One* **6,** e19338 (2011).

121. Schneeberger, a, Mandler, M., Mattner, F. & Schmidt, W. Vaccination for Parkinson's disease. *Parkinsonism Relat. Disord.* **18 Suppl 1,** S11–3 (2012).

122. Anderson, J. P. *et al.* Phosphorylation of Ser-129 is the dominant pathological modification of alpha-synuclein in familial and sporadic Lewy body disease. *J. Biol. Chem.* **281,** 29739–52 (2006).

123. Lee, K.-W. *et al.* Enhanced phosphatase activity attenuates α-synucleinopathy in a mouse model. *J. Neurosci.* **31,** 6963–71 (2011).

124. Pérez-Revuelta, B. I. *et al.* Metformin lowers Ser-129 phosphorylated α-synuclein levels via mTOR-dependent protein phosphatase 2A activation. *Cell Death Dis.* **5,** e1209 (2014).

125. Wahlqvist, M. L. *et al.* Metformin-inclusive sulfonylurea therapy reduces the risk of Parkinson's disease occurring with Type 2 diabetes in a Taiwanese population cohort. *Parkinsonism Relat. Disord.* **18,** 753–8 (2012).

126. Steele, J. W. *et al.* Latrepirdine stimulates autophagy and reduces accumulation of α-synuclein in cells and in mouse brain. *Mol. Psychiatry* **18,** 882–8 (2013).

127. Duran, R. *et al.* The glucocerobrosidase E326K variant predisposes to Parkinson's disease, but does not cause Gaucher's disease. *Mov. Disord.* **28,** 232–6 (2013).

128. Neumann, J. *et al.* Glucocerebrosidase mutations in clinical and pathologically proven Parkinson's disease. *Brain* **132,** 1783–94 (2009).

129. Sardi, S. P. *et al.* Augmenting CNS glucocerebrosidase activity as a therapeutic strategy for parkinsonism and other Gaucher-related synucleinopathies. *Proc. Natl. Acad. Sci. U. S. A.* **110,** 3537–42 (2013).

130. Zimran, A., Altarescu, G. & Elstein, D. Pilot study using ambroxol as a pharmacological chaperone in type 1 Gaucher disease. *Blood Cells. Mol. Dis.* **50,** 134–7 (2013).

131. Bendikov-Bar, I., Maor, G., Filocamo, M. & Horowitz, M. Ambroxol as a pharmacological chaperone for mutant glucocerebrosidase. *Blood Cells. Mol. Dis.* **50,** 141–5 (2013).

132. Olanow, C. W. & Schapira, A. H. V. Therapeutic prospects for Parkinson disease. *Ann. Neurol.* **74,** 337–47 (2013).

133. Wu, G., Lu, Z.-H., Kulkarni, N., Amin, R. & Ledeen, R. W. Mice lacking major brain gangliosides develop parkinsonism. *Neurochem. Res.* **36,** 1706–14 (2011).

134. Schneider, J. S. *et al.* Recovery from experimental parkinsonism in primates with GM1 ganglioside treatment. *Science* **256,** 843–6 (1992).

135. Schneider, J. S., Sendek, S., Daskalakis, C. & Cambi, F. GM1 ganglioside in Parkinson's disease: Results of a five year open study. *J. Neurol. Sci.* **292,** 45–51 (2010).

136. Schneider, J. S. *et al.* A randomized, controlled, delayed start trial of GM1 ganglioside in treated Parkinson's disease patients. *J. Neurol. Sci.* **324,** 140–8 (2013).

137. Parkes, D. G., Mace, K. F. & Trautmann, M. E. Discovery and development of exenatide: the first antidiabetic agent to leverage the multiple benefits of the incretin hormone, GLP-1. *Expert Opin. Drug Discov.* **8,** 219–44 (2013).

138. Perry, T., Haughey, N. J., Mattson, M. P., Egan, J. M. & Greig, N. H. Protection and reversal of excitotoxic neuronal damage by glucagon-like peptide-1 and exendin-4. *J. Pharmacol. Exp. Ther.* **302,** 881–8 (2002).

139. Perry, T. *et al.* A novel neurotrophic property of glucagon-like peptide 1: a promoter of nerve growth factor-mediated differentiation in PC12 cells. *J. Pharmacol. Exp. Ther.* **300,** 958–66 (2002).

140. Harkavyi, A. *et al.* Glucagon-like peptide 1 receptor stimulation reverses key deficits in distinct rodent models of Parkinson's disease. *J. Neuroinflammation* **5,** 19 (2008).

141. Kim, S., Moon, M. & Park, S. Exendin-4 protects dopaminergic neurons by inhibition of microglial activation and matrix metalloproteinase-3 expression in an animal model of Parkinson's disease. *J. Endocrinol.* **202,** 431–9 (2009).

142. Bertilsson, G. *et al.* Peptide hormone exendin-4 stimulates subventricular zone neurogenesis in the adult rodent brain and induces recovery in an animal model of Parkinson's disease. *J. Neurosci. Res.* **86,** 326–38 (2008).

143. Rampersaud, N. *et al.* Exendin-4 reverses biochemical and behavioral deficits in a pre-motor rodent model of Parkinson's disease with combined noradrenergic and serotonergic lesions. *Neuropeptides* **46,** 183–93 (2012).

144. Chen, S., Liu, A., An, F., Yao, W. & Gao, X. Amelioration of neurodegenerative changes in cellular and rat models of diabetes-related Alzheimer's disease by exendin-4. *Age (Dordr).* **34,** 1211–24 (2012).

145. Aviles-olmos, I. *et al.* Exenatide and the treatment of patients with Parkinson ' s disease. **123,** (2013).

146. Aviles-Olmos, I. *et al.* Motor and Cognitive Advantages Persist 12 Months After Exenatide Exposure in Parkinson's Disease. *J. Parkinsons. Dis.* (2014). doi:10.3233/JPD-140364

147. Chang, R. C.-C., Ho, Y.-S., Wong, S., Gentleman, S. M. & Ng, H.-K. Neuropathology of cigarette smoking. *Acta Neuropathol.* **127,** 53–69 (2014).

148. Tsuang, D. *et al.* Association between lifetime cigarette smoking and lewy body accumulation. *Brain Pathol.* **20,** 412–8 (2010).

149. Hong, D.-P., Fink, A. L. & Uversky, V. N. Smoking and Parkinson's disease: does nicotine affect alpha-synuclein fibrillation? *Biochim. Biophys. Acta* **1794,** 282–90 (2009).

150. Quik, M., Perez, X. a & Bordia, T. Nicotine as a potential neuroprotective agent for Parkinson's disease. *Mov. Disord.* **27,** 947–57 (2012).

151. Shimohama, S. Nicotinic receptor-mediated neuroprotection in neurodegenerative disease models. *Biol. Pharm. Bull.* **32,** 332–6 (2009).

152. Ward, R. J., Lallemand, F., de Witte, P. & Dexter, D. T. Neurochemical pathways involved in the protective effects of nicotine and ethanol in preventing the

development of Parkinson's disease: potential targets for the development of new therapeutic agents. *Prog. Neurobiol.* **85,** 135–47 (2008).

153. Costa, G., Abin-Carriquiry, J. A. & Dajas, F. Nicotine prevents striatal dopamine loss produced by 6-hydroxydopamine lesion in the substantia nigra. *Brain Res.* **888,** 336–342 (2001).

154. Ryan, R. E., Ross, S. A., Drago, J. & Loiacono, R. E. Dose-related neuroprotective effects of chronic nicotine in 6-hydroxydopamine treated rats, and loss of neuroprotection in alpha4 nicotinic receptor subunit knockout mice. *Br. J. Pharmacol.* **132,** 1650–6 (2001).

155. Parain, K., Marchand, V., Dumery, B. & Hirsch, E. Nicotine, but not cotinine, partially protects dopaminergic neurons against MPTP-induced degeneration in mice. *Brain Res.* **890,** 347–350 (2001).

156. Quik, M. *et al.* Chronic oral nicotine normalizes dopaminergic function and synaptic plasticity in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned primates. *J. Neurosci.* **26,** 4681–9 (2006).

157. Hely, M. A., Reid, W. G. J., Adena, M. A., Halliday, G. M. & Morris, J. G. L. The Sydney multicenter study of Parkinson's disease: the inevitability of dementia at 20 years. *Mov. Disord.* **23,** 837–44 (2008).

158. Muslimovic, D., Post, B., Speelman, J. D. & Schmand, B. Cognitive profile of patients with newly diagnosed Parkinson disease. *Neurology* **65,** 1239–45 (2005).

159. Rolinski, M., Fox, C., Maidment, I. & McShane, R. Cholinesterase inhibitors for dementia with Lewy bodies, Parkinson's disease dementia and cognitive impairment in Parkinson's disease. *Cochrane database Syst. Rev.* **3,** CD006504 (2012).

160. Irwin, D. J. *et al.* Neuropathologic substrates of Parkinson disease dementia. *Ann. Neurol.* **72,** 587–98 (2012).

161. Masliah, E. *et al.* beta-amyloid peptides enhance alpha-synuclein accumulation and neuronal deficits in a transgenic mouse model linking Alzheimer's disease and Parkinson's disease. *Proc. Natl. Acad. Sci. U. S. A.* **98,** 12245–50 (2001).

162. Jellinger, K. A., Seppi, K., Wenning, G. K. & Poewe, W. Impact of coexistent Alzheimer pathology on the natural history of Parkinson's disease. *J. Neural Transm.* **109,** 329–39 (2002).

163. Kotzbauer, P. T. *et al.* Pathologic accumulation of α-synuclein and Aβ in Parkinson disease patients with dementia. *Arch. Neurol.* **69,** 1326–31 (2012).

164. Compta, Y. *et al.* Lewy- and Alzheimer-type pathologies in Parkinson's disease dementia: which is more important? *Brain* **134,** 1493–505 (2011).

165. Simón-Sánchez, J. *et al.* Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nat. Genet.* **41,** 1308–12 (2009).

166. Morley, J. F. *et al.* Genetic influences on cognitive decline in Parkinson's disease. *Mov. Disord.* **27,** 512–8 (2012).

167. Edwards, T. L. *et al.* Genome-wide association study confirms SNPs in SNCA and the MAPT region as common risk factors for Parkinson disease. *Ann. Hum. Genet.* **74,** 97–109 (2010).

168. Goris, A. *et al.* Tau and alpha-synuclein in susceptibility to, and dementia in, Parkinson's disease. *Ann. Neurol.* **62,** 145–53 (2007).

169. Tariot, P. N. *et al.* Chronic divalproex sodium to attenuate agitation and clinical progression of Alzheimer disease. *Arch. Gen. Psychiatry* **68,** 853–61 (2011).

170. Zhang, X. *et al.* Long-term treatment with lithium alleviates memory deficits and reduces amyloid-β production in an aged Alzheimer's disease transgenic mouse model. *J. Alzheimers. Dis.* **24,** 739–49 (2011).

171. Nunes, M. A., Viel, T. A. & Buck, H. S. Microdose lithium treatment stabilized cognitive impairment in patients with Alzheimer's disease. *Curr. Alzheimer Res.* **10,** 104–7 (2013).

172. Wen, Y. *et al.* Alternative mitochondrial electron transfer as a novel strategy for neuroprotection. *J. Biol. Chem.* **286,** 16504–15 (2011).

173. Spires-Jones, T. L. *et al.* Methylene blue does not reverse existing neurofibrillary tangle pathology in the rTg4510 mouse model of tauopathy. *Neurosci. Lett.* **562,** 63–8 (2014).

174. Stack, C. *et al.* Methylene blue upregulates Nrf2/ARE genes and prevents tau-related neurotoxicity. *Hum. Mol. Genet.* **23,** 3716–32 (2014).

175. Wischik CM, Bentham P, W. D. & Al, E. Tau aggregation inhibitor (TAI) therapy with rember arrests disease progression in mild and moderate Alzheimer's disease over 50 weeks. *Alzheimers Dement* 4(4):T167 (2008). at <http://www.alzheimersanddementia.com/issue/S1552-5260(08)X0005-6>

176. Zhang, B. *et al.* The microtubule-stabilizing agent, epothilone D, reduces axonal dysfunction, neurotoxicity, cognitive deficits, and Alzheimer-like pathology in an interventional study with aged tau transgenic mice. *J. Neurosci.* **32,** 3601–11 (2012).

177. Braak, H., Rüb, U., Jansen Steur, E. N. H., Del Tredici, K. & de Vos, R. A. I. Cognitive status correlates with neuropathologic stage in Parkinson disease. *Neurology* **64,** 1404–10 (2005).

178. Hurtig, H. I. *et al.* Alpha-synuclein cortical Lewy bodies correlate with dementia in Parkinson's disease. *Neurology* **54,** 1916–21 (2000).

179. Montine, T. J. *et al.* CSF Aβ(42) and tau in Parkinson's disease with cognitive impairment. *Mov. Disord.* **25,** 2682–5 (2010).

180. Aarsland, D., Andersen, K., Larsen, J. P., Lolk, A. & Kragh-Sørensen, P. Prevalence and characteristics of dementia in Parkinson disease: an 8-year prospective study. *Arch. Neurol.* **60,** 387–92 (2003).

181. Selikhova, M. *et al.* A clinico-pathological study of subtypes in Parkinson's disease. *Brain* **132,** 2947–57 (2009).

182. Brundin, P. *et al.* Linked clinical trials--the development of new clinical learning studies in Parkinson's disease using screening of multiple prospective new treatments. *J. Parkinsons. Dis.* **3,** 231–9 (2013).

183. Weinreb, O., Amit, T., Riederer, P., Youdim, M. B. H. & Mandel, S. A. Neuroprotective profile of the multitarget drug rasagiline in Parkinson's disease. *Int. Rev. Neurobiol.* **100,** 127–49 (2011).

184. Beal, M. F. Neuroprotective effects of creatine. *Amino Acids* **40,** 1305–13 (2011).

185. Beal, M. F. *et al.* A randomized clinical trial of high-dosage coenzyme Q10 in early Parkinson disease: no evidence of benefit. *JAMA Neurol.* **71,** 543–52 (2014).

186. Visanji, N. P. *et al.* PYM50028, a novel, orally active, nonpeptide neurotrophic factor inducer, prevents and reverses neuronal damage induced by MPP+ in mesencephalic neurons and by MPTP in a mouse model of Parkinson's disease. *FASEB J.* **22,** 2488–97 (2008).

187. Investigation of Cogane (PYM50028) in Early-stage Parkinson's Disease (CONFIDENT-PD) - Full Text View - ClinicalTrials.gov. at <http://clinicaltrials.gov/show/NCT01060878>

188. Jin, H., Kanthasamy, A., Ghosh, A. & Anantharam, V. Biochimica et Biophysica Acta Mitochondria-targeted antioxidants for treatment of Parkinson ' s disease☐: Preclinical and clinical outcomes ☆. *BBA - Mol. Basis Dis.* (2013). doi:10.1016/j.bbadis.2013.09.007

189. Snow, B. J. *et al.* A double-blind, placebo-controlled study to assess the mitochondria-targeted antioxidant MitoQ as a disease-modifying therapy in Parkinson's disease. *Mov. Disord.* **25,** 1670–4 (2010).

190. Marks, W. J. *et al.* Gene delivery of AAV2-neurturin for Parkinson's disease: a double-blind, randomised, controlled trial. *Lancet Neurol.* **9,** 1164–72 (2010).

191. AAV2-GDNF for Advanced Parkinson s Disease - Full Text View - ClinicalTrials.gov. at <http://www.clinicaltrials.gov/ct2/show/NCT01621581>

192. Fitton, A. & Benfield, P. Isradipine. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in cardiovascular disease. *Drugs* **40,** 31–74 (1990).

193. Chen, X. *et al.* Disrupted and transgenic urate oxidase alter urate and dopaminergic neurodegeneration. *Proc. Natl. Acad. Sci. U. S. A.* **110,** 300–5 (2013).

194. Boddaert, N. *et al.* Selective iron chelation in Friedreich ataxia: biologic and clinical implications. *Blood* **110,** 401–8 (2007).

195. Berman, A. E. *et al.* N-acetylcysteine prevents loss of dopaminergic neurons in the EAAC1-/- mouse. *Ann. Neurol.* **69,** 509–20 (2011).

196. Pan, J. *et al.* Blockade of the translocation and activation of c-Jun N-terminal kinase 3 (JNK3) attenuates dopaminergic neuronal damage in mouse model of Parkinson's disease. *Neurochem. Int.* **54,** 418–25 (2009).

197. Choudhury, M. E. *et al.* Zonisamide-induced long-lasting recovery of dopaminergic neurons from MPTP-toxicity. *Brain Res.* **1384,** 170–8 (2011).

198. Choudhury, M. E. *et al.* Zonisamide up-regulated the mRNAs encoding astrocytic anti-oxidative and neurotrophic factors. *Eur. J. Pharmacol.* **689,** 72–80 (2012).

199. Yürekli, V. A., Gürler, S., Nazıroğlu, M., Uğuz, A. C. & Koyuncuoğlu, H. R. Zonisamide attenuates MPP+-induced oxidative toxicity through modulation of Ca2+ signaling and caspase-3 activity in neuronal PC12 cells. *Cell. Mol. Neurobiol.* **33,** 205–12 (2013).

200. Murata, M., Hasegawa, K. & Kanazawa, I. Zonisamide improves motor function in Parkinson disease: a randomized, double-blind study. *Neurology* **68,** 45–50 (2007).

201. Ulusoy, G. K. *et al.* Effects of pioglitazone and retinoic acid in a rotenone model of Parkinson's disease. *Brain Res. Bull.* **85,** 380–4 (2011).

202. Haddadi, R., Mohajjel Nayebi, A. & Brooshghalan, S. E. Pre-treatment with silymarin reduces brain myeloperoxidase activity and inflammatory cytokines in 6-OHDA hemi-parkinsonian rats. *Neurosci. Lett.* **555,** 106–11 (2013).

203. Foltynie, T. & Aviles-Olmos, I. Exenatide as a potential treatment for patients with Parkinson's disease: First steps into the clinic. *Alzheimers. Dement.* **10,** S38–S46 (2014).

204. Schneider, A. *et al.* The hematopoietic factor G-CSF is a neuronal ligand that counteracts programmed cell death and drives neurogenesis. *J. Clin. Invest.* **115,** 2083–98 (2005).

205. Watson, F. L. *et al.* Neurotrophins use the Erk5 pathway to mediate a retrograde survival response. *Nat. Neurosci.* **4,** 981–8 (2001).

206. Jung, K.-H. *et al.* Granulocyte colony-stimulating factor stimulates neurogenesis via vascular endothelial growth factor with STAT activation. *Brain Res.* **1073-1074,** 190–201 (2006).

207. Hartung, T. Anti-inflammatory effects of granulocyte colony-stimulating factor. *Curr. Opin. Hematol.* **5,** 221–5 (1998).

208. Meuer, K. *et al.* Granulocyte-colony stimulating factor is neuroprotective in a model of Parkinson's disease. *J. Neurochem.* **97,** 675–86 (2006).

209. Lee, S.-T. *et al.* Granulocyte-colony stimulating factor attenuates striatal degeneration with activating survival pathways in 3-nitropropionic acid model of Huntington's disease. *Brain Res.* **1194,** 130–7 (2008).

210. Xue, Y.-Q., Zhao, L.-R., Guo, W.-P. & Duan, W.-M. Intrastriatal administration of erythropoietin protects dopaminergic neurons and improves neurobehavioral outcome in a rat model of Parkinson's disease. *Neuroscience* **146,** 1245–58 (2007).

211. Pedroso, I. *et al.* Use of Cuban recombinant human erythropoietin in Parkinson's disease treatment. *MEDICC Rev.* **14,** 11–7 (2012).

212. Jang, W. *et al.* Safety and efficacy of recombinant human erythropoietin treatment of non-motor symptoms in Parkinson's disease. *J. Neurol. Sci.* **337,** 47–54 (2014).