REVIEW

CME

Disease-Modifying Strategies for Parkinson's Disease

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ABSTRACT: Parkinson's disease (PD) is an increasingly prevalent and progressively disabling neurodegenerative disease. The impact of PD on patients and their families as well as its burden on health care systems could be substantially reduced by disease-modifying therapies that slow the rate of neurodegeneration or stop the disease process. Multiple agents have been studied in clinical trials designed to assess disease modification in PD, but all have failed. Over the last 3 years, clinical trials investigating the potential of adeno-associated virus serotype 2 (AAV)-neurturin, coenzyme Q10, creatine, pramipexole, and pioglitazone reported negative findings or futility. Despite these disappointments, progress has been made by expanding our understanding of molecular pathways involved in PD to reveal new targets, and by developing novel animal models of PD for preclinical studies. Currently, at least eight ongoing clinical trials are testing the promise of isradipine, caffeine, nicotine, glutathione, AAV2-glial cell-line derived neurotrophic factor (GDNF), as well as active and passive immunization against α-synuclein (α-Syn). In this review, we summarize the clinical trials of disease-modifying therapies for PD that were published since 2013 as well as clinical trials currently in progress. We also discuss promising approaches and ongoing challenges in this area of PD research.

Key Words: autophagy; gene therapy; LRRK2; mitochondria; neuroprotection

One of the greatest unmet therapeutic needs in Parkinson's disease (PD) is disease-modifying therapy that reduces the rate of neurodegeneration or stops the disease process. Despite decades of research dedicated to discovering these therapies, multiple agents have failed in clinical trials.[1] As the complexities of the genotype, phenotype, and pathogenesis of PD continue to emerge,[2] the task of achieving disease modification in PD seems increasingly daunting. However, expansion in our understanding of PD has allowed for identification of new potential therapeutic targets, and recently developed animal models provide innovative tools for preclinical studies. Here we review clinical trials of disease-modifying therapies in PD that were published since 2013 as well as ongoing clinical trials. We also discuss promising approaches and continuing challenges for future research into disease-modifying strategies for PD.

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Recent Failures

AAV2-Neurturin

Neurotrophic factors have long been considered promising targets for neuroprotection in neurodegenerative

TABLE 1. Failed clinical trials of disease-modifying therapies for PD from 2013 to 2015

Study	Drug	Mechanism of Action	Trial Design	Subjects	Follow-up Period	Primary Outcome Measure(s)	Results
Olanow et al., 2015[6]	AAV2-Neuturin (injection into bilateral SNpc and putamen)	Neurotrophic factor	Multi-center, randomized, double-blind, sham surgery-controlled, phase 2 trial	Advanced PD subjects (n = 51)	15-24 months	Change in UPDRS part 3 in practically defined "off"-state	No statistically significant difference between treated and control groups
PSG et al., 2014 (QE3)[8]	Coenzyme Q10 (1200 mg/d or 2400 mg/d) + vitamin E (1200 IU/d)	Bioenergetic; Antioxidant	Multi-center, randomized, double-blind, placebo-controlled, phase 3 trial	Early PD subjects not requiring dopaminergic therapy (n = 600)	16 months (or until requiring dopaminergic therapy if sooner)	Change in total UPDRS score	Prematurely terminated due to futility
NET-PD et al., 2015 (LS1)[9]	Creatine (10 g/d)	Bioenergetic	Multi-center, randomized, double-blind, placebo-controlled, phase 3 trial	Early PD subjects receiving dopaminergic therapy (n = 1741)	4 years (median)	Difference in decline of clinical status defined by 5 outcome measures	Prematurely terminated due to futility
Schapira et al., 2013 (PROUD)[13]	Pramipexole (1.5 mg/day)	D2/D3 dopamine receptor agonist	Multi-center, randomized, double-blind, placebo-controlled, delayed-start trial	Early PD subjects not requiring dopaminergic therapy (n = 535)	15 months	Change in total UPDRS score	No statistically significant difference between early-start and delayed-start groups
NET-PD, 2015 (FS-ZONE)[15]	Pioglitazone (15 mg/d or 45 mg/d)	PPAR-γ agonist	Multi-center, randomized, double-blind, placebo-controlled, futility trial	Early PD subjects on rasagiline or selegiline (n = 210)	44 weeks	Change in total UPDRS score	Futility

Abbreviations: AAV2, adeno-associated virus serotype 2; LS1, Long-term Study 1; PD, Parkinson's disease; PPAR, peroxisome proliferator-activated receptor; PROUD, Pramipexole On Underlying Disease; QE3, Coenzyme Q10 in Early Parkinson Disease; SNpc, substantia nigra pars compacta; UPDRS, Unified Parkinson's Disease Rating Scale.

disorders but have yet to be translated into viable clinical therapies. Neurturin, a member of the glial cell line–derived neurotrophic factor (GDNF) family, was previously studied in PD with intraputaminal delivery by using stereotactic injection of a viral vector, adeno-associated virus serotype 2 (AAV). An open-label phase 1 trial of AAV2-neurturin (CERE-120) delivery to bilateral putamen in 12 subjects with moderately advanced PD demonstrated safety, tolerability, and some clinical improvement.[3] This was followed by a multicenter, double-blind, phase 2 randomized control trial (RCT) that demonstrated no significant difference between those who received AAV2-neurturin versus sham surgery in the primary endpoint of Unified Parkinson's Disease Rating Scale (UPDRS) part 3 scores in "off"-state at 12 months postprocedure, but some benefit in those followed for 18 months.[4] Pathological assessment of tissue from two patients who received AAV2-neurturin demonstrated very little in the substantia nigra pars compacta (SNpc), where it should have had its greatest impact after retrograde transport to remaining dopaminergic neuronal cell bodies.[5] Thus, an open-label phase 1 trial enrolled six PD subjects to receive bilateral injection of AAV2-neurturin into both the putamen and SNpc.[6] Over the 2-year follow-up period, this treatment strategy was safe and tolerable, with transient and clinically insignificant adverse events (e.g., incision site pain, headache, abnormal dreams, dyskinesia). Unfortunately, a subsequent phase 2 RCT found no significant difference in the primary endpoint (UPDRS part 3 score in "off"-state) and in most secondary endpoints (Table 1).[7]

Coenzyme Q10 and Creatine

Many lines of evidence, from the discovery of toxins that cause SNpc neurodegeneration (eg, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [MPTP], 6-hydroxydopamine [6-OHDA]) to the identification of genes associated with familial PD (eg, *parkin*, *PINK1*), converge on mitochondrial dysfunction as critical to PD pathogenesis. Accordingly, drugs capable of improving mitochondrial function or enhancing the bioenergetics of neurons with compromised mitochondria may be neuroprotective. Coenzyme Q10 is an electron carrier for complexes I/II of the mitochondrial electron transport chain and a free radical scavenger.[8] Coenzyme Q10 in Early PD (QE3) was a phase 3 RCT examining efficacy of coenzyme Q10 plus vitamin E in early PD subjects not on dopaminergic therapy (Table 1).[9] The primary clinical outcome was change in total UPDRS score from baseline to the 16-month visit, or to the last visit before development of sufficient symptoms requiring dopaminergic therapy if this occurred sooner. Creatine is a naturally occurring compound that, when converted to phosphocreatine, acts as a short-term energy source. Long-term Study 1 (LS-1) was a phase 3 RCT that enrolled early PD subjects to investigate creatine (Table 1).[10] The primary outcome measure was a difference in clinical decline from baseline to the planned 5-y follow-up time-point. Both QE3 and LS-1 were prematurely terminated when the results of planned interim analyses indicated futility. Failure of these drugs does not dismiss the major role that mitochondrial dysfunction plays in PD and thus should not exclude further investigation into mitochondrial

TABLE 2. Ongoing clinical trials of disease-modifying therapies for PD in 2015

Study	Drug	Mechanism of Action	Trial Design	Estimated Enrollment	Follow-up Period	Primary Outcome Measure(s)	Status
NCT02216188	PD01A + adjuvant (subcutaneous injection; 15μg or 75μg booster x 1)	Active immunization against α-synuclein	Single-center (Austria), randomized, single-blind, follow-up, phase 1 trial	PD subjects who previously received PD01A and untreated controls (n = 32)	6 months	Safety and tolerability	Enrolling by invitation
NCT01885494	PD01A + adjuvant (subcutaneous injection; 15 μg or 75 μg × 4)	Active immunization against α-synuclein	Single-center (Austria), observational, follow-up, phase 1 extension trial	PD subjects who previously received PD01A and untreated controls (n = 32)	52 weeks	Safety and tolerability	Active but not recruiting
NCT02267434	PD03A + adjuvant (subcutaneous injection; 15 μg or 75 μg × 4)	Active immunization against α-synuclein	Dual-center (Austria), randomized, single-blind, placebo-controlled, phase 1 trial	Early PD subjects (n = 36)	52 weeks	Safety and tolerability	Recruiting
NCT02157714	PRX002 (intravenous infusion)	Passive immunization against α-synuclein	Multi-center (United States), randomized, double-blind, placebo-controlled, phase 1 trial	PD subjects (n = 60)	6 months	Safety and tolerability; several pharmacokinetic parameters	Recruiting
NCT01738178	Caffeine (400 mg/d)	Nonspecific adenosine receptor antagonist	Multi-center (Canada, Brazil), randomized, double-blind, placebo-controlled, phase 3 trial with delayed-start component	PD subjects (n = 250)	5 years	MDS-UPDRS score	Recruiting
NCT01621581	AAV2-GDNF (convection enhanced delivery to bilateral putamen)	Neurotrophic factor	Single-center (United States), open-label, phase 1 trial	Advanced PD subjects (n = 24)	5 years	Safety and tolerability; several clinical measures	Recruiting
NCT02168842 (STEADY-PD III)	Isradipine (immediate release; 10 mg/d)	Dihydropyridine calcium channel blocker	Multi-center trial (United States, Canada), randomized, double-blind, placebo-controlled, phase 3 trial	Early PD subjects not requiring dopaminergic therapy (n = 336)	36 months	Change in total UPDRS score	Recruiting
NCT01560754 (NIC-PD)	Nicotine (transdermal patch; 7-28 mg/d)	Nicotinic acetylcholine receptor agonist	Multi-center (Germany, United States), randomized, double-blind, placebo-controlled, phase 2 trial with washout period	Early PD subjects not requiring dopaminergic therapy (n = 160)	12 months followed by 2-month washout period	Change in total UPDRS score	Recruiting
NCT02424708	GSH (intranasal; 300 mg/d or 600 mg/d)	Antioxidant	Dual-center (United States), randomized, double-blind, placebo-controlled, phase 2 trial	PD subjects (n = 45)	12 weeks	Change in total UPDRS score	Recruiting
NCT01470027	N-acetylcysteine (1800 mg/d or 3600 mg/d)	GSH precursor	Single-center (United States), randomized, double-blind, placebo-controlled, phase 1/2 trial	PD subjects on no medications for PD (n = 60)	4 weeks	Change in cerebral GSH levels measured by proton magnetic resonance spectroscopy	Recruiting
NCT01882010	Sagramostim (subcutaneous injection; 6 μg/kg/d)	GM-CSF	Dual-center (United States), randomized, double-blind, placebo-controlled phase 1 trial	PD subjects and non-PD controls (n = 32)	52 weeks	Safety and tolerability	Recruiting
NCT01453803	Adipose-derived stromal stem cells (intraarterial and intravenous infusion)	Multiple	Single-center (Mexico), open-label, phase 1/2 trial	PD subjects with motor complications (n = 10)	6 months	Safety and tolerability; UPDRS scores	Recruiting

Abbreviations: AAV2, adeno-associated virus serotype 2; GDNF, glial cell-line derived neurotrophic factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; GSH, glutathione; MDS-UPDRS, Movement Disorder Society–sponsored revision of the Unified Parkinson's Disease Rating Scale; PD, Parkinson's disease; UPDRS, Unified Parkinson's Disease Rating Scale.

pathways as potential therapeutic targets. If indeed parkin and PTEN-induced putative kinase 1 (PINK1) dysfunction are relevant to the pathogenesis of sporadic PD, upregulation of their function or augmentation of related pathways may be strategies to develop novel mitochondrial-targeted therapies.

Pramipexole

Studies using *in vitro* and *in vivo* models suggested that the dopamine D2/D3 receptor agonist pramipexole may have disease-modifying properties in PD.[11-13] PROUD (Pramipexole On Underlying Disease) was a RCT with a delayed start that enrolled early PD subjects

(Table 1).[14] Subjects were assigned to receive pramipexole for 15 months (early-start group), placebo for up to 9 months followed by pramipexole for 6 months (delayed-start group), or placebo for 15 months. Adjusted mean change in UPDRS total score showed no significant difference between early-start and delayed-start groups at 15 months. In a neuroimaging sub-study using single-photon emission computed tomography (SPECT), the adjusted mean 15-month change in striatal binding of ioflupane (^{123}I-FP-CIT), a presynaptic dopamine transporter (DAT) ligand, was not significantly different between the early-start (n = 62) and delayed-start (n = 61) groups. Results from the clinical and neuroimaging data of this well-designed and well-executed trial strongly suggest that pramipexole lacks disease-modifying effects. Regardless, pramipexole remains a well-established symptomatic treatment for PD.[15]

Pioglitazone

Pioglitazone is a peroxisome proliferator-activated receptor-γ (PPAR-γ) agonist initially developed for type II diabetes. Microglial activation is well established in PD but whether this contributes to the ongoing degenerative process (ie, detrimental) or somehow supports the function of diseased neurons is unclear. Activating PPAR-γ has the potential for reducing the production of pro-inflammatory cytokines by harmful activated microglia while sparing or inducing beneficial activated microglia. Furthermore, PPAR-γ activation might influence PPAR-γ coactivator 1-α, (PGC-1α) an important regulator of mitochondrial biogenesis and energy metabolism. A phase 2 RCT comparing change in total UPDRS scores from baseline to 44 weeks in early PD subjects treated with pioglitazone failed to show disease-modifying effects.[16]

Promising New Treatments

α-Synuclein Immunotherapy

Abundant evidence implicates α-synuclein (α-Syn) as a key contributor to neuronal death in PD.[17] Recent research supports the hypothesis that α-Syn also plays a critical role in propagating the neurodegenerative process via a prion-like mechanism.[18] Propagation is proposed to involve transport of toxic forms of α-Syn from a neuron's intracellular compartment to the extracellular space. This is followed by transmission to the intracellular compartment of a neighboring neuron, where permissive templating of α-Syn expressed by that neuron occurs. Thus, targeting extracellular α-Syn as a disease-modifying strategy for PD has become an appealing consideration that may be amenable to immunization-based therapies (also see Brundin et al, this issue). The first phase 1 trial to investigate this strategy enrolled 24 subjects with early PD for active immunization by subcutaneous injection of a vaccine composed of short immunogenic peptides that mimic the C-terminus of α-Syn (PD01A) with adjuvant. Results from this study are not published, but the company developing this vaccine announced that it was safe and well tolerated at two different doses over the 12-month study period. In addition, 50% of vaccinated subjects had anti–α-Syn antibodies in their serum, and antibodies were also detected in cerebrospinal fluid. Further testing of active immunization includes an ongoing phase 1 trial enrolling PD subjects who were previously vaccinated with PD01A to test safety and tolerability of one booster immunization (clinicaltrials.gov/ct2/show/NCT02216188) (Table 2). In addition, an observational extension study involving PD subjects from the initial PD01A trial assesses longer-term safety and tolerability as well as clinical (eg, MDS-UPDRS part 3), radiological (DAT-SPECT), and immunological measures (eg, antibody titers) (clinicaltrials.gov/ct2/show/NCT01885494). PD03A, a different peptide for active immunization against α-Syn, is being investigated in a phase 1 RCT for early PD (clinicaltrials.gov/ct2/show/NCT02267434) (Table 2).

A phase 1 trial testing passive immunization with a monoclonal anti–α-Syn antibody (PRX002) was recently completed. This trial enrolled 40 healthy volunteers to receive either PRX002 or placebo. Results are not published, but announcements from the companies developing this immunotherapy report no treatment-emergent adverse events in more than 10% of subjects and no drug-related serious adverse reactions. Administration of the antibody was associated with a rapid and dose-dependent reduction of free serum α-Syn levels. A phase 1 RCT recruiting PD subjects is now underway (clinicaltrials.gov/ct2/show/NCT02157714) (Table 2). If safety of α-Syn–targeted immunotherapies can be well established, further development to more specifically target the toxic forms of α-Syn is expected.

Caffeine

Coffee drinking is associated with a reduced risk of PD,[19] and caffeine may have symptomatic benefits in PD. A pilot study investigating symptomatic effects of caffeine (200-400 mg/d for 6 weeks) in PD subjects with excessive daytime somnolence found no clear benefit on daytime sleepiness, but improvement in motor symptoms was observed (mean improvement of UPDRS part 3 score greater than placebo by 3.15 points).[20] Based on this positive finding, a long-term phase 3 RCT investigating caffeine treatment for PD has been initiated (clinicaltrials.gov/ct2/show/NCT01738178) (Table 2). The bulk of the study will examine effects of caffeine on motor symptoms (primary outcome) as well as on non-motor features, quality of life, and use of other PD medications (secondary outcomes). The final stage of the study includes a delayed start of caffeine treatment in the placebo group to assess for potential disease-modifying properties of caffeine.

GDNF

Glial cell-line derived neurotrophic factor (GDNF) is a known neurotrophic factor for dopaminergic neurons that continues to be developed as a potential disease-modifying target for PD. The first double-blind RCT of GDNF for PD used intraventricular delivery of recombinant GDNF and demonstrated no clinical benefit,[21] so this method was abandoned, and direct infusion of GDNF into bilateral putamen was pursued. However, a RCT testing intraputaminal GDNF infusion also showed no clinical benefit at 6 months, and a few of the treated subjects developed neutralizing antibodies to recombinant GDNF.[22] Currently, a National Institutes of Health (NIH)/ National Institute of Neurological Disorders and Stroke (NINDS)-sponsored, open-label study is using a gene therapy approach with AAV2-GDNF and a convection enhanced delivery system for intraputaminal GDNF in advanced PD (clinicaltrials.gov/ct2/show/NCT01621581) (Table 2). Although neurotrophic factors were initially hailed as promising therapeutics for neuroprotection in PD, clinical studies have not yet demonstrated their efficacy. The reasons are likely multifactorial, including technical issues (eg, limited tissue coverage) and unanticipated biological complexities (eg, PD pathology mitigating the activity of neurotrophic factors[23] or preventing effective retrograde transfer from striatal terminals to nigral cell bodies).[5] Despite these disappointments, clinical trials with GDNF and neurturin established the safety of direct intraparenchymal injection or viral-mediated gene therapy strategies, which may be translated into development of other biological agents for disease-modifying treatments for PD.

Inosine

Urate possesses antioxidant properties *in vitro*, and elevation of urate levels in rodent models can protect SNpc dopaminergic neurons from 6-OHDA toxicity.[24,25] Epidemiological studies showed that higher serum urate levels are associated with reduced risk of developing PD. Furthermore, early PD patients with higher plasma urate levels have slower disease progression.[26] Inosine is a urate precursor that, when taken orally, can elevate serum urate. Safety of Urate Elevation in PD (SURE-PD) was a multi-center, double-blind, phase 2 RCT that enrolled 75 subjects with early PD to receive inosine (0.5-3 g/d) or placebo.[27] Subjects with baseline serum urate levels higher than the population median (>6 mg/dL or 360 μM) or with increased risk of gout or urolithiasis (i.e., history of gout or urolithiasis, urine pH $\leq$ 5.0) were excluded. Titrated administration of oral inosine resulted in urate elevations in serum and cerebrospinal fluid to levels previously associated with slower disease progression. No subject developed gout during the study, but symptomatic urolithiasis occurred in three treated participants. Treatment for 8 to 24 months was not associated with increased frequency of cardiovascular events. SURE-PD demonstrated that oral inosine can sufficiently elevate urate levels and is safe in a select population of early PD patients, supporting further investigation of inosine as a potential disease-modifying therapy.

Isradipine

Experimental evidence suggests that dopaminergic neurons in the SNpc as well as other selectively vulnerable neurons in PD have spontaneous pacemaking properties that rely on L-type Cav1.3 calcium channels.[28] Use of calcium channel blockers is associated with reduced risk of developing PD.[19] Isradipine is a dihydropyridine calcium channel blocker with relatively high affinity for Cav1.3 channels that is approved for treatment of hypertension. Preclinical studies have demonstrated that isradipine can protect SNpc neurons from 6-OHDA toxicity in a rodent model.[29] Safety, Tolerability, and Efficacy Assessment of Dynacirc CR in PD (STEADY-PD) was a multicenter, phase 2 RCT that enrolled 99 subjects with early PD to receive isradipine controlled-release (5, 10, or 20 mg/day) or placebo.[30] The primary outcome measure was tolerability, defined as the proportion of subjects able to complete the 12-month study on the originally assigned dosage with tolerability threshold set as 30% or less difference between active and placebo groups. Isradipine controlled-release 10 mg/d was found to be the maximal tolerable dosage in this study. The most common adverse events were peripheral edema and dizziness not associated with changes in blood pressure. STEADY-PD laid the groundwork for STEADY-PD III, which is a NIH-sponsored phase 3 RCT to assess efficacy of isradipine as a disease-modifying therapy in PD (clinicaltrials.gov/ct2/show/NCT02168842) (Table 2).

Nicotine

Epidemiological studies identified tobacco smoking to be associated with decreased risk of developing PD.[19] This finding led to the proposal that nicotine might have neuroprotective properties. A phase 2 RCT investigating transdermal nicotine is currently recruiting subjects (clinicaltrials.gov/ct2/show/NCT01560754) (Table 2). The study is proposed to include 12 months of treatment followed by a 2-month washout period to assess for disease-modifying potential. If the results are negative, the hypothesis that nicotine protects against PD will need to be revisited. Based on findings of a recent large case-control study showing that PD patients are able to quit smoking more easily than controls, an alternative hypothesis to explain the negative association between smoking and PD is that patients in the prodromal phase of PD might have a decreased "responsiveness" to nicotine that results in less long-term use and addiction.[31]

Others

Exenatide is a synthetic agonist for the glucagon-like peptide-1 (GLP-1) receptor that is currently prescribed for management of glucose control in type II diabetes. Stimulation of GLP-1 receptors in the gastrointestinal tract is associated with pancreatic islet β-cell proliferation, increased insulin production, decreased gluconeogenesis, and weight loss. The GLP-1 receptors are also expressed in the brain, but effects of their stimulation are less well defined. Exenatide has been found to have neuroprotective and possibly neurorestorative effects in rodent PD models, although its mechanism of action is unclear.[32] A single-center, randomized, single-blind, proof-of-concept trial enrolled 45 subjects with moderate PD to receive subcutaneous injections of exenatide for 12 months or to act as controls without placebo.[33] Weight loss was the most common adverse event among subjects treated with exenatide and resulted in withdrawal of one subject. Another subject withdrew because of recurrent levodopa dose failures possibly attributable to reduction in gastric emptying caused by exenatide. After a 2-month washout period, subjects treated with exenatide had a mean improvement of Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part 3 "off"-state score that was 4.4 points greater than that of controls. Limited conclusions can be drawn from this study because of its single-blind design and lack of placebo, but exenatide did demonstrate reasonable safety and thus may be considered for further investigation in PD.

Glutathione (GSH) is an endogenous antioxidant within the brain, and loss of GSH has been implicated in PD.[34] Currently, a phase 2 RCT is recruiting subjects with PD to examine intranasal GSH (clinicaltrials.gov/ct2/show/NCT02424708) (Table 2). Intranasal administration for delivery to the central nervous system bypasses some obstacles associated with oral delivery (eg, first-pass effect). A phase 1/2 study is also underway using proton magnetic resonance spectroscopy to determine whether levels of GSH are decreased in PD subjects and whether GSH levels increase after supplementation with N-acetylcysteine, a GSH precursor (clinicaltrials.gov/ct2/show/NCT01470027) (Table 2).

GM1 ganglioside is a glycosphingolipid in the brain that is abundant in plasma membrane of neurons and is essential in membrane signaling pathways. In PD, GM1 ganglioside may be reduced in SNpc dopaminergic neurons.[35] Mice deficient in GM1 ganglioside have reduced numbers of SNpc neurons and increased α-Syn aggregation that can be partially attenuated by treatment with a membrane-permeable analog of GM1 ganglioside.[36] A single-center, delayed-start RCT enrolled 77 subjects with PD to receive GM1 ganglioside (intravenous loading dose followed by subcutaneous injections) for 120 weeks (early-start group) or placebo for 24 weeks followed by GM1 ganglioside for 96 weeks (delayed-start group).[37] At 120 weeks, mean reduction in UPDRS part 3 "on"-state score was 4.3 points greater in early-start compared with delayed-start groups. GM1 ganglioside has limited ability to penetrate the blood–brain barrier, so development of membrane-permeable analogs may be necessary if GM1 ganglioside is to be further investigated as a disease-modifying therapy for PD.

Growing evidence suggests a role of the immune system in PD pathogenesis, although the precise pathways upstream and downstream of neurodegeneration remain to be elucidated. Ongoing trials aimed at developing treatments that may enhance immunity in PD include a phase 1 RCT to study safety of sagramostim, a granulocyte-macrophage colony-stimulating factor that has been safely used for decades to mobilize myeloid progenitor cells in immunosuppressed patients after chemotherapy or transplantation (clinicaltrials.gov/ct2/show/NCT01882010). Another trial is studying intraarterial and intravenous delivery of autologous adipose-derived stromal stem cells in PD in a phase 1/2 trial (clinicaltrials.gov/ct2/show/NCT01453803). Stromal stem cells have some regenerative capacity, but, perhaps more importantly as a cell-based therapy for neurodegeneration, release a wide array of soluble factors that have immunosuppressive, anti-inflammatory, and trophic effects. If successful, the studies described could provide justification for larger controlled clinical trials to further investigate immune system modulators in PD.

Future Directions

Specific Therapeutic Targets

α-Synuclein has become an increasingly attractive therapeutic target as our understanding of its contributions to the pathogenesis, not just the pathology, of PD has expanded. Scientists are still faced with the challenge of determining which α-Syn aggregate species are most toxic to neurons in PD. These specific forms of α-Syn will be ideal targets for positively impacting the disease process. Conformational-specific antibodies that recognize oligomeric or transmissible forms of α-Syn have shown promise in animal models,[38,39] and this approach is anticipated to lead the next wave of α-Syn–based immunotherapies. Strategies using small molecules to disrupt or inhibit α-Syn aggregation are an active area of investigation[40] but come with the challenges of delivery to the central nervous system. As with immunotherapies, the ideal small molecules will specifically act on the pathogenic α-Syn aggregates or alternatively stabilize nonpathogenic forms.[41] α-Synuclein is a remarkably abundant protein in the brain and blood, and its normal physiological functions are still poorly understood. Therefore,

caution must be taken when considering approaches that nonspecifically lower α-Syn levels; for example, with RNA interference strategies.[42] Instead of directly targeting α-Syn, upregulating intrinsic cellular systems that neutralize or dispose of toxic α-Syn, such as the autophagy–lysosomal pathway, is an increasingly feasible approach as the molecular mechanisms underlying these systems are better deciphered.[43,44]

Discoveries in the genetics of PD have led to identification of key molecular pathways important to neurodegeneration in familial PD.[2] If these same pathways are also relevant to the pathogenesis of sporadic PD, then these discoveries have provided many novel targets for potential disease-modifying treatments. After α-Syn, the protein encoded by a PD-related gene that seems to be the most attractive therapeutic target is leucine-rich repeat kinase 2 (LRRK2). Mutations in *LRRK2* are the most common known genetic cause of PD but *LRRK2* also appears to be closely linked to sporadic PD. Specifically, polymorphic variants in *LRRK2* modulate risk of sporadic PD,[45] and the clinical phenotype of *LRRK2*-related PD very much resembles sporadic PD.[46] Because the most frequent pathogenic *LRRK2* mutation causes an amino-acid substitution (G2019S) that enhances kinase function of the protein, LRRK2 inhibitors are being actively investigated by various academic institutions and pharmaceutical companies.[47,48] Toxicity attributable to kinase inhibition has been a challenge in the cancer field, where many kinase inhibitors have been developed as chemotherapies.[49] A recent preclinical study found pulmonary toxicity in wild-type nonhuman primates treated with small molecule LRRK2 inhibitors.[50] Similar toxicity might not be observed in patients with *LRRK2*-related PD associated with the G2019S mutation who presumably have systemic expression of overactive LRRK2. For treatment of sporadic PD patients, who theoretically have LRRK2 overactivity limited to susceptible neurons, this finding will need to be carefully considered with further development of LRRK2-targeted therapeutics.

Mutations in lysosomal-related genes, including *GBA* and *ATP13A2*, also have been implicated in familial PD, raising the possibility of a pathogenic role for lysosomal dysfunction in sporadic PD. The lysosome–autophagy system is responsible for degradation and recycling of intracellular components, such as proteins and organelles (eg, mitochondria), via macroautophagy, microautophagy, or chaperone-mediated autophagy. Constitutive activity of the lysosome–autophagy system is essential for neuronal survival. Interestingly, recent evidence suggests a mechanistic link between lysosomal dysfunction and both α-Syn and LRRK2, further supporting involvement of the lysosome-autophagy system in PD. Specifically, α-Syn accumulation can occur when the lysosome–autophagy system is impaired but can also directly inhibit lysosomal function.[51] Emerging data indicate that LRRK2 may upregulate macroautophagy.[52] Small molecules that enhance the lysosome–autophagy system are being explored as potential treatments for PD.[53] Rapamycin (or sirolimus) is known to induce macroautophagy, but its potential use in PD is limited by its safety profile. Challenges in the development of other chemical inducers of autophagy include lack of specificity with respect to the substrates of lysosomal-mediated degradation and the potential negative consequences of excessive autophagy. Alternative approaches, such as peptide-based strategies,[54] may circumvent these challenges by allowing for specific substrates, such as α-Syn, to be targeted for lysosomal-mediated degradation.

Drug Screens and Repurposing Strategies

The process of discovering and developing therapies aimed at a specific target can be costly and slow. With cuts in the budgets of academic funding agencies and reductions in investment by pharmaceutical companies for Research and Development, alternative strategies are required to make the necessary breakthroughs in PD research. Unbiased compound screening of small molecules previously proven to be safe in humans and approved by agencies such as the United States Food and Drug Administration (FDA) could identify novel candidates for potential disease modification for PD. Another repurposing or repositioning approach is to explore whether a specific drug currently used to treat another disease could have disease-modifying properties in PD based on its proposed mechanism of action or early preclinical experiments. For instance, the commonly used cephalosporin antibiotic ceftriaxone has been found to bind to α-Syn and block its *in vitro* aggregation,[55] as well as to reduce dopaminergic neuron loss in MPTP and 6-OHDA rodent models.[56,57] A meta-analysis using data from prior clinical drug trials for early PD found that tricyclic antidepressant use is associated with a delay in requiring initiation of dopaminergic therapy, suggesting either disease-modifying or symptomatic effect.[58] In support of the disease-modifying possibility, the tricyclic antidepressant amitriptyline was found to increase levels of neurotrophic factors (GDNF and brain-derived neurotrophic factor [BDNF]) in the SNpc as well as stop progressive degeneration of dopaminergic neurons in a 6-OHDA rat model.[58,59] Drug screens and repurposing strategies will be needed in parallel with rational therapeutic targeting to enhance and expedite the identification of novel agents for future clinical trials.

Ongoing Challenges

Researchers have made progress with some of the longstanding challenges in our quest for effective

disease-modifying therapies for PD. Many early attempts at translating preclinical findings to positive clinical trial outcomes were based only on toxin-based animal models. Genetic models for preclinical investigation and, very recently, animal models of the prion-like spreading of α-Syn have been developed.[60-63] This selection of models currently allows for a potential therapy to be tested against a specific pathogenic mechanism (eg, mitochondrial dysfunction with MPTP models, LRRK2 overactivity with G2019S transgenic rodent models, cell-to-cell transmission of α-Syn with intracerebral inoculation models). Multiple models can also be used to assess whether an agent is capable of impacting several different mechanisms involved in PD pathogenesis. The hope is that these developments in preclinical models will better predict promising agents for clinical trials.

Research into biomarkers for early diagnosis is very active, and advances in this area will have significant impact on enrollment of subjects into clinical trials, as we believe the notion of "the earlier the better" for disease-modifying treatment in PD still holds true. In addition to establishing the diagnosis at the earliest stages, biomarkers also will be needed to characterize distinct disease subtypes that will respond to very different disease-modifying approaches. Characterizing patients based on genomic, proteomic, metabolomic, and other factors may be necessary in designing future clinical trials, enriching patient samples with those more likely to respond to a specific drug mechanism of action. Finally, discovery of biomarkers of disease progression also continues to be important for clinical trials to help define disease modification.

We continue to be humbled by the challenges of finding a disease-modifying therapy to help our patients with PD. It is seemingly less and less likely that we will discover a therapy that is "the magic bullet" for PD as we appreciate the many genotypes and multiple molecular mechanisms that underlie the varied phenotype of PD. We anticipate that not one but several disease-modifying therapies need to be discovered and developed. Multiple therapies are frequently combined to achieve adequate symptomatic management of PD. Similarly, we expect that combinations of drugs with different mechanisms of action (ie, cocktails), as used in oncology, may be necessary to successfully impact on disease progression. ●

References

1. AlDakheel A, Kalia LV, Lang AE. Pathogenesis-targeted, disease-modifying therapies in Parkinson disease. Neurotherapeutics 2014; 11:6-23.
2. Kalia LV, Lang AE. Parkinson's disease. Lancet 2015; doi: 10.1016/S0140-6736(14)61393-3
3. Marks WJ, Ostrem JL, Verhagen L, et al. Safety and tolerability of intraputaminal delivery of CERE-120 (adeno-associated virus serotype 2-neurturin) to patients with idiopathic Parkinson's disease: an open-label, phase I trial. Lancet Neurol 2008;7:400-408.
4. Marks WJ, Bartus RT, Siffert J, et al. Gene delivery of AAV2-neurturin for Parkinson's disease: a double-blind, randomised, controlled trial. Lancet Neurol 2010;9:1164-1172.
5. Bartus RT, Herzog CD, Chu Y, et al. Bioactivity of AAV2-neurturin gene therapy (CERE-120): differences between Parkinson's disease and nonhuman primate brains. Mov Disord 2011;26:27-36.
6. Bartus RT, Baumann TL, Siffert J, et al. Safety/feasibility of targeting the substantia nigra with AAV2-neurturin in Parkinson patients. Neurology 2013;80:1698-1701.
7. Olanow CW, Bartus RT, Baumann T, et al. Gene delivery of neurturin to putamen and substantia nigra in Parkinson's disease: a double-blind, randomized, controlled trial. Ann Neurol 2015; doi: 10.1002/ana.24436.
8. Beal MF. Bioenergetic approaches for neuroprotection in Parkinson's disease. Ann Neurol 2003;53(Suppl 3):S39-S47.
9. Parkinson Study Group QE3 Investigators, Beal MF, Oakes D, Shoulson I, et al. A randomized clinical trial of high-dosage coenzyme Q10 in early Parkinson disease: no evidence of benefit. JAMA Neurol 2014;71:543-552.
10. Writing Group for the NINDS Exploratory Trials in Parkinson Disease (NET-PD) Investigators, Kieburtz K, Tilley BC, Elm JJ, et al. Effect of creatine monohydrate on clinical progression in patients with Parkinson disease: a randomized clinical trial. JAMA 2015;313:584-593.
11. Gu M, Iravani MM, Irvani M, et al. Pramipexole protects against apoptotic cell death by non-dopaminergic mechanisms. J Neurochem 2004;91:1075-1081.
12. Iravani MM, Haddon CO, Cooper JM, et al. Pramipexole protects against MPTP toxicity in non-human primates. J Neurochem 2006;96:1315-1321.
13. Iravani MM, Sadeghian M, Leung CCM, et al. Continuous subcutaneous infusion of pramipexole protects against lipopolysaccharide-induced dopaminergic cell death without affecting the inflammatory response. Exp Neurol 2008;212:522-531.
14. Schapira AH, McDermott MP, Barone P, et al. Pramipexole in patients with early Parkinson's disease (PROUD): a randomised delayed-start trial. Lancet Neurol 2013;12:747-755.
15. Fox SH, Katzenschlager R, Lim S-Y, et al. The Movement Disorder Society evidence-based medicine review update: treatments for the motor symptoms of Parkinson's disease. Mov Disord 2011; 26(Suppl 3):S2-S41.
16. NINDS Exploratory Trials in Parkinson Disease (NET-PD) FS-ZONE Investigators. Pioglitazone in early Parkinson's disease: a phase 2, multicentre, double-blind, randomised trial. Lancet Neurol. 2015; doi: 10.1016/S1474-4422(15)00144-1
17. Kalia LV, Kalia SK, McLean PJ, et al. α-Synuclein oligomers and clinical implications for Parkinson disease. Ann Neurol 2013;73:155-169.
18. Visanji NP, Brooks PL, Hazrati L-N, et al. The prion hypothesis in Parkinson's disease: Braak to the future. Acta Neuropathol Commun 2013;1:2.
19. Noyce AJ, Bestwick JP, Silveira-Moriyama L, et al. Meta-analysis of early nonmotor features and risk factors for Parkinson disease. Ann Neurol 2012;72:893-901.
20. Postuma RB, Lang AE, Munhoz RP, et al. Caffeine for treatment of Parkinson disease: a randomized controlled trial. Neurology 2012;79:651-658.
21. Nutt JG, Burchiel KJ, Comella CL, et al. Randomized, double-blind trial of glial cell line-derived neurotrophic factor (GDNF) in PD. Neurology 2003;60:69-73.
22. Lang AE, Gill S, Patel NK, et al. Randomized controlled trial of intraputamenal glial cell line-derived neurotrophic factor infusion in Parkinson disease. Ann Neurol 2006;59:459-466.
23. Decressac M, Kadkhodaei B, Mattsson B, et al. α-Synuclein-induced down-regulation of Nurr1 disrupts GDNF signaling in nigral dopamine neurons. Sci Transl Med 2012;4:163ra156.
24. Gong L, Zhang Q-L, Zhang N, et al. Neuroprotection by urate on 6-OHDA-lesioned rat model of Parkinson's disease: linking to Akt/GSK3β signaling pathway. J Neurochem 2012;123:876-885.
25. Chen X, Burdett TC, Desjardins CA, et al. Disrupted and transgenic urate oxidase alter urate and dopaminergic neurodegeneration. Proc Natl Acad Sci U S A 2013;110:300-305.

26. Chen X, Wu G, Schwarzschild MA. Urate in Parkinson's disease: more than a biomarker? Curr Neurol Neurosci Rep 2012;12: 367-375.
27. Schwarzschild MA, Ascherio A, Beal MF, et al. Inosine to increase serum and cerebrospinal fluid urate in Parkinson disease: a randomized clinical trial. JAMA Neurol 2014;71:141-150.
28. Surmeier DJ, Schumacker PT. Calcium, bioenergetics, and neuronal vulnerability in Parkinson's disease. J Biol Chem 2012;288: 10736-10741.
29. Ilijic E, Guzman JN, Surmeier DJ. The L-type channel antagonist isradipine is neuroprotective in a mouse model of Parkinson's disease. Neurobiol Dis 2011;43:364-371.
30. Parkinson Study Group. 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 2013;28:1823-1831.
31. Ritz B, Lee P-C, Lassen CF, et al. Parkinson disease and smoking revisited: ease of quitting is an early sign of the disease. Neurology 2014;83:1396-1402.
32. Aviles-Olmos I, Limousin P, Lees A, et al. Parkinson's disease, insulin resistance and novel agents of neuroprotection. Brain 2013; 136:374-384.
33. Aviles-Olmos I, Dickson J, Kefalopoulou Z, et al. Exenatide and the treatment of patients with Parkinson's disease. J Clin Invest 2013;123:2730-2736.
34. Martin HL, Teismann P. Glutathione: a review on its role and significance in Parkinson's disease. FASEB J 2009;23:3263-3272.
35. Wu G, Lu Z-H, Kulkarni N, et al. Deficiency of ganglioside GM1 correlates with Parkinson's disease in mice and humans. J Neurosci Res 2012;90:1997-2008.
36. Wu G, Lu Z-H, Kulkarni N, et al. Mice lacking major brain gangliosides develop parkinsonism. Neurochem Res 2011;36:1706-1714.
37. Schneider JS, Gollomp SM, Sendek S, et al. A randomized, controlled, delayed start trial of GM1 ganglioside in treated Parkinson's disease patients. J Neurol Sci 2013;324:140-148.
38. Lindström V, Fagerqvist T, Nordström E, et al. Immunotherapy targeting α-synuclein protofibrils reduced pathology in (Thy-1)-h[A30P] α-synuclein mice. Neurobiol Dis 2014;69:134-143.
39. Tran HT, Chung CH-Y, Iba M, et al. α-Synuclein immunotherapy blocks uptake and templated propagation of misfolded α-synuclein and neurodegeneration. Cell Rep 2014;7:2054-2065.
40. Wagner J, Ryazanov S, Leonov A, et al. Anle138b: a novel oligomer modulator for disease-modifying therapy of neurodegenerative diseases such as prion and Parkinson's disease. Acta Neuropathol 2013;125:795-813.
41. Fonseca-Ornelas L, Eisbach SE, Paulat M, et al. Small molecule-mediated stabilization of vesicle-associated helical α-synuclein inhibits pathogenic misfolding and aggregation. Nat Commun 2014;5:5857.
42. Cooper JM, Wiklander PBO, Nordin JZ, et al. Systemic exosomal siRNA delivery reduced α-synuclein aggregates in brains of transgenic mice. Mov Disord 2014;29:1476-1485.
43. Decressac M, Mattsson B, Weikop P, et al. TFEB-mediated autophagy rescues midbrain dopamine neurons from α-synuclein toxicity. Proc Natl Acad Sci 2013;110:E1817-E1826.
44. Steele JW, Ju S, Lachenmayer ML, et al. Latrepirdine stimulates autophagy and reduces accumulation of α-synuclein in cells and in mouse brain. Mol Psychiatry 2013;18:882-888.
45. Nalls MA, Pankratz N, Lill CM, et al. Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. Nat Genet 2014;46:989-993.
46. Marras C, Schüle B, Schuele B, et al. Phenotype in parkinsonian and nonparkinsonian LRRK2 G2019S mutation carriers. Neurology 2011;77:325-333.
47. Kethiri RR, Bakthavatchalam R. Leucine-rich repeat kinase 2 inhibitors: a review of recent patents (2011–2013). Expert Opin Ther Pat 2014;24:745-757.
48. West AB. Ten years and counting: moving leucine-rich repeat kinase 2 inhibitors to the clinic. Mov Disord 2015;30:180-189.
49. Zhang J, Yang PL, Gray NS. Targeting cancer with small molecule kinase inhibitors. Nat Rev Cancer 2009;9:28-39.
50. Fuji RN, Flagella M, Baca M, et al. Effect of selective LRRK2 kinase inhibition on nonhuman primate lung. Sci Transl Med 2015;7:273ra15.
51. Dehay B, Martinez-Vicente M, Caldwell GA, et al. Lysosomal impairment in Parkinson's disease. Mov Disord. 2013;28:725-732.
52. Schapansky J, Nardozzi JD, Felizia F, et al. Membrane recruitment of endogenous LRRK2 precedes its potent regulation of autophagy. Hum Mol Genet. 2014;23:4201-4214.
53. Renna M1, Jimenez-Sanchez M, Sarkar S, et al. Chemical inducers of autophagy that enhance the clearance of mutant proteins in neurodegenerative diseases. J Biol Chem 2010;285:11061-11067.
54. Fan X, Jin WY, Lu J, et al. Rapid and reversible knockdown of endogenous proteins by peptide-directed lysosomal degradation. Nat Neurosci. 2014;17:471-480.
55. Ruzza P, Siligardi G, Hussain R, et al. Ceftriaxone blocks the polymerization of α-synuclein and exerts neuroprotective effects in vitro. ACS Chem Neurosci 2014;5:30-38.
56. Chotibut T, Davis RW, Arnold JC, et al. Ceftriaxone increases glutamate uptake and reduces striatal tyrosine hydroxylase loss in 6-OHDA Parkinson's model. Mol Neurobiol 2014;49:1282-1292.
57. Ho S-C, Hsu C-C, Pawlak CR, et al. Effects of ceftriaxone on the behavioral and neuronal changes in an MPTP-induced Parkinson's disease rat model. Behav Brain Res 2014;268:177-184.
58. Paumier KL, Siderowf AD, Auinger P, et al. Tricyclic antidepressants delay the need for dopaminergic therapy in early Parkinson's disease. Mov Disord 2012;27:880-887.
59. Paumier KL, Sortwell CE, Madhavan L, et al. Tricyclic antidepressant treatment evokes regional changes in neurotrophic factors over time within the intact and degenerating nigrostriatal system. Exp Neurol 2015;266:11-21.
60. Luk KC, Kehm VM, Zhang B, et al. Intracerebral inoculation of pathological α-synuclein initiates a rapidly progressive neurodegenerative α-synucleinopathy in mice. J Exp Med 2012;209:975-986.
61. Luk KC, Kehm V, Carroll J, et al. Pathological α-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. Science 2012;338:949-953.
62. Sacino AN, Brooks M, McGarvey NH, et al. Induction of CNS α-synuclein pathology by fibrillar and non-amyloidogenic recombinant α-synuclein. Acta Neuropathol Commun 2013;1:38.
63. Recasens A, Dehay B, Bové J, et al. Lewy body extracts from Parkinson disease brains trigger α-synuclein pathology and neurodegeneration in mice and monkeys. Ann Neurol 2014;75: 351-362.