Disease Modification in Parkinson's Disease:

Current Approaches, Challenges and Future Considerations.

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Abstract

The greatest unmet therapeutic need in Parkinson's disease is the development of treatment that slows the relentless progression of the neurodegenerative process. The concept of "disease modification" encompasses intervention types ranging from those designed to slow the underlying degeneration to treatments directed at regenerating or replacing lost neurons. To date all attempts to develop effective disease modifying therapy have failed. Many reasons have been proposed for these failures including our rudimentary understanding of disease pathogenesis and the assumption that each targeted mechanisms of disease apply to most subjects with the same clinical diagnosis. Here we review all aspects of this broad field including general concepts and past challenges followed by a discussion of treatment approaches under four categories: 1) α-synuclein, 2) pathogenic mechanisms distinct from α-synuclein (most also potentially triggered by α-synuclein toxicity), 3) non-*SNCA* genetic subtypes of "PD", and 4) possible "disease modifying" interventions not directly influencing the underlying PD pathobiology. We emphasize treatments that are currently under active clinical development and highlight a wide range of important outstanding questions and concerns that will need to be considered to advance the field of disease modification in PD. Critically, it is unknown whether the dysfunctional molecular pathways/organelles amenable to modification occur in a sequential fashion across most clinically affected individuals or manifest differentially in independent molecular subtypes of PD. It is possible that there is no "order of disruption" applicable to most patients but rather "type of disruption" applicable to subtypes dependent on unknown factors, including genetic variability and other causes for heterogeneity in PD. Knowing *when* (early vs late), *which* (e.g., synaptic transmission, endosomal sorting and maturation, lysosomal degradation, mitochondrial biogenesis) and in *whom* (PD subtype) specific disrupted cell pathways are truly pathogenic versus compensatory or even protective, will be important in considering the use of single or combined ("cocktails") of putative disease modifying therapies to selectively target these processes. Beyond the current Phase 2 or 3 studies underway evaluating treatments directed at oxidative stress (inosine), cytosolic Ca^{2+} (isradipine), iron (deferiprone) and extracellular α-synuclein (passive immunization), and upcoming trials of interventions affecting c-Abl, GLP-1, and GCase, it might be argued that further trials in populations not enriched for the targeted pathogenic process are doomed to repeat the failures of the past.

Introduction

Although textbook chapters and many papers dealing with Parkinson's disease (PD) continue to describe the disorder simply as a disease of substantia nigra dopamine neurons, it is now widely appreciated to

be far more complex. PD likely encompasses many genetic-molecular entities unified under a multi-systems disorder affecting both central and peripheral nervous systems, resulting in a broad spectrum of motor and non-motor features, of which dopamine deficiency is only one of several common denominators (1),(2),(3). The disorder is relentlessly progressive and within 15-20 years of disease onset the expected mortality is 2-3 times that of the general population. Surviving patients experience disability from a variety of treatment-resistant problems including postural instability and falls, speech and swallowing dysfunction, autonomic failure, psychosis and dementia(4). Recognizing this inexorable progression and resulting disability, it is widely believed that the most fundamental unmet therapeutic need is treatment that can effectively change the course of the disease by slowing and, ideally, halting its progression. Unfortunately, all attempts to obtain "disease modification" to date have failed. In this paper, we will review the concept of "disease modification", the reasons for past failures, the approaches that are currently being explored or that will be explored in patients in the near future and finally how the field should proceed over the short- and medium-term future. Our purpose is not to exhaustively review pathogenic disease mechanisms or basic preclinical studies justifying therapeutic interventions. These will be briefly summarized in order to provide the reader an accurate overview of the current landscape of this challenging field.

Concepts of Disease Modification

The term "disease modification" in the broadest sense refers to an intervention that modifies the natural clinical course of the disease. In PD and other neurodegenerative disorders a principal unmet need is the development of treatment that has a direct impact on the underlying disease pathogenesis which prevents further neuronal cell death and thus slows or halts disease progression. This concept describes true "*neuroprotection*", which is the primary goal of most putative disease modifying therapies (i.e., the "Holy Grail"). However, disease modification encompasses other potential strategies (5) including 1) bolstering or supporting failing compensatory mechanisms for dopamine deficiency or other degenerative changes (i.e. *compensation*(6)); 2) salvaging dying neurons either by reversing established metabolic abnormalities or providing failing trophic support (i.e. *neurorescue*); and 3) providing cell-based therapies designed to replace degenerating neurons (i.e. *neurorestoration*).

Proving true neuroprotection in PD is a challenge that is currently not possible since it requires some method of reliably measuring the effect of the intervention on the underlying disease process. At

present, we are limited to assaying the consequences of the disease in the form of clinical (motor and non-motor) markers, which occur as a consequence of neuronal degeneration, or selected surrogate markers of these degenerative changes (e.g., functional imaging). However, these "markers" are remote from the disease process and may be poorly reflective of or dissociated from its status, progression, or changes in response to interventions. These clinical and nonclinical markers of disease may also be influenced by a number of factors unrelated to the underlying primary neurodegenerative process, including, most prominently, symptomatic effects of the experimental intervention that could be misinterpreted as disease modifying. Furthermore, patients involved in clinical trials are inevitably treated with dopaminergic medications with potent symptomatic effects that can mask disease modifying effects of a concurrent intervention. These issues have served as major challenges to the design of studies attempting to demonstrate disease modification (7),(8). Other factors that need to be considered in interpreting the results of these trials include the impact of the drug on compensatory CNS processes or concurrent/co-pathologies (e.g., concurrent Alzheimer's disease or cerebrovascular disease)(9) rather than the primary degenerative process of interest (see later). In the case of certain imaging markers (e.g., ([^{123}I]-FP-CIT SPECT), there is the potential for dynamic drug effects to influence the affinity of the ligand for the target unrelated to the severity of the neurodegeneration. Finally, it should be acknowledged that our current "markers" of disease are heavily weighted towards the nigrostriatal dopamine system which may have little to do with the many treatment-resistant motor and non-motor symptoms that result in morbidity and mortality in the later stages of the disease(4). In fact, it is this late-stage debilitating disease that represents the primary incentive for the development of effective disease modifying therapy and it will be critical to demonstrate that a treatment that successfully protects or slows the progression of dopaminergic cell loss in patients with the earliest clinical manifestations also has a positive influence on late-stage, currently untreatable manifestations.

History to Date: Reasons for Failure

A large number of trials have attempted to evaluate the potential for disease modification using treatments with a broad spectrum of mechanisms of action that have shown promise in various models of PD(10),(11). Unfortunately, all of these have either failed to demonstrate the hoped-for benefit or have given inconclusive results, largely due to possible confounding symptomatic effects. This latter

issue began with the earliest drug to be studied for disease modification, selegiline, and has continued up to the most recently reported agent proposed to have disease modifying effects, exenatide. Initial positive results believed to indicate a neuroprotective effect of selegiline(12) (13), were subsequently thought to be entirely due to dopaminergic symptomatic benefit. In the case of exenatide(14), as discussed later, the difference between the active treatment and placebo groups was largely indicative of an early but sustained symptomatic effect. Finally, special mention should be made of the most potent symptomatic therapy for PD, levodopa(15, 16). In the ELLDOPA trial (15), where 3 doses of levodopa were compared to placebo over 9 months, UPDRS scores in the levodopa treated groups never declined to the level seen in the placebo group after up to 4 weeks of drug washout. Although it is generally believed that this could simply relate to a very long-duration symptomatic effect of levodopa, the difference between the two groups was greater than has been seen in any other similar trial in early PD. This encouraged the conduct of the delayed-start LEAP-study which will report results sometime later this year(16).

There are a wide variety of reasons that could explain the past failures of disease modifying therapy trials(10),(11). Table 1 summarizes the major obstacles in this field and provides some additional commentary on each of these issues. As we have discussed elsewhere, one important reason for past treatment failures has been the regular assumption in clinical trials that enrolled patients all suffer from the same uniform disorder and that this disorder, while clinically heterogeneous, has a dominant contribution from the pathogenic mechanism influenced by the drug being studied (17),(18). We will return to this and other challenges to the future of disease modification in the final sections of this review.

Targets for Disease Modification

It is not our intention to review every possible cellular mechanism, target and therapeutic intervention proposed for disease modification in PD(10),(11). Instead, we will discuss potential therapeutic targets under four categories: 1) α-synuclein, 2) pathogenic mechanisms distinct from α-synuclein (although most also potentially triggered by α-synuclein toxicity), 3) non-*SNCA* genetic subtypes of "PD", particularly *LRRK2*-related PD, and 4) interventions with possible "disease modifying" effects but not specifically influencing the underlying PD pathobiology. In each section, we will emphasize those therapies that are either under active study, about to be studied, or show considerable promise for

clinical evaluation in the near future. We will also discuss the critical challenges and unanswered questions that may confound each of these therapeutic approaches. The first section on α-synuclein will cover topics relevant to subsequent sections (e.g., disease pathogenesis and selective neuronal vulnerability). In light of the importance of α-synuclein to the pathogenesis of the disease and the widespread interest in the future potential of synuclein-targeted therapeutics, challenges and caveats to these approaches will be discussed in greater detail.

1. α-synuclein a. The role of α-synuclein in disease pathogenesis (Figure 1)

Remarkably, it has been 20 years since Polymeropoulos and colleagues first reported a mutation in the gene *SNCA* for the synaptic protein α-synuclein as a cause of autosomal dominant PD (19) and Spillantini and her colleagues discovered that α-synuclein is a major component of the key pathological hallmark of PD, the Lewy body(20) . The subsequent development of immunohistochemical staining using antibodies to α-synuclein has demonstrated extensive neuritic involvement and the combination of Lewy bodies and Lewy neurites is now typically referred to as Lewy pathology (LP). An overwhelming amount of evidence has accumulated over the past 20 years to support a principal role of α-synuclein in the pathogenesis of PD. Although there is some evidence that the loss of normal α-synuclein function contributes to the disease(21), most animal data supports a toxic process. However, the exact nature of the toxic species (e.g. oligomers versus fibrils) remains uncertain. Numerous cellular pathways have been implicated in α-synuclein toxicity(21), including dysfunction of a variety of organelles as well as disturbances of inter-organelle contacts (e.g. the mitochondria-associated ER membrane (MAM)) and misregulation of organelle dynamics (e.g. axonal transport). Disturbances of synaptic-vesicle trafficking and the autophagy and lysosomal degradation pathways, as well as dysfunction of endoplasmic reticulum (ER) and Golgi, mitochondria, and nuclear processes have all been demonstrated in various cellular and animal models of α-synuclein toxicity. Not only is it possible that these disturbances are caused by α-synuclein but pre-existing organelle dysfunction (i.e., due to other primary genetic or environmental causative factors) may contribute to α-synuclein toxicity (e.g., disturbances of protein degradation and clearance) and thus a vicious cycle of α-synuclein accumulation and further organelle dysfunction may arise (Figure 1). An example of this reverberating process that may be an important therapeutic target is the reciprocal relationship between glucocerebrosidase and α-synuclein and their impact on the autophagy lysosomal pathway(22). Mutations in the glucocerebrosidase gene (*GBA*) are the most common genetic risk factor for developing PD and dementia with Lewy bodies (DLB)(23),(24), and patients with PD without *GBA* mutations can exhibit lower enzymatic levels of glucocerebrosidase

(GCase) in the CNS, possibly as a consequence of α-synuclein toxicity. Furthermore, dopamine may interact with and amplify α-synuclein toxicity, potentially contributing to the vulnerability of SNc neurons (see (21)).

Importantly, it is unknown whether there is a sequential or parallel order in which these pathways/organelles become dysfunctional or their differential manifestation in molecular subtypes of PD. It is possible that some are disrupted early in the disease and others later while it is also possible that there is no "order of disruption" applicable to everyone but rather "type of disruption" applicable to subtypes and dependent on a variety of unknown factors including genetic variability and other causes for heterogeneity in PD. In some situations these abnormalities may be a consequence of α-synuclein accumulation (possibly combined with genetic predisposition) while in others, selected abnormalities may be a protective compensatory response (e.g., decreased mitochondrial biogenesis compensating for inefficient lysosomal degradation(21)). Knowing *when* (early vs late), *which* (synaptic transmission, endosomal sorting and maturation, lysosomal degradation and mitochondrial biogenesis (25), (21)) and *in whom* (PD subtype) specific disrupted cell pathways are truly pathogenic versus compensatory or even protective, will be important in considering the use of putative disease modifying therapies that selectively target each of these processes.

Another important factor in considering a pathogenetic role of α-synuclein in PD is the potential for cell-to-cell transmission, possibly through secretion via exosome release and uptake via endocytosis, the latter process in part mediated by a potential future therapeutic target, the transmembrane protein lymphocyte-activation gene 3 (LAG 3)(26). This concept of cell-to-cell transmission, combined with the demonstrated ability of toxic conformations of α-synuclein to induce seeding, recruitment and permissive templating of normal α-synuclein, have encouraged the concept that LP in PD (and many other neurodegenerative disorders) progresses and spreads through the nervous system in a prion-like fashion. It has been proposed that this spread could begin outside the brain, reaching the CNS via olfactory and enteric routes, the latter involving vagal nerve brainstem connections (27). This model of cell-to-cell spread of pathology in PD has been supported by a large number of cellular studies, in vivo animal work, including recent experiments involving intracerebral injections of α-synuclein preformed fibrils (PFFs)(28), and the discovery of Lewy pathology in fetal nigral dopaminergic cells transplanted into humans with PD 10 or more years earlier(29, 30). Another feature of this pathogenic process that further likens PD to prion diseases is evidence demonstrating that variants of α-synuclein have distinct differences in structure, toxicity, seeding and propagational properties supportive of the concept that

different strains of α-synuclein could account for variations in cellular dysfunction between PD patients, different clinical phenotypes of PD or different pathologies associated with α-synuclein aggregation (e.g., PD vs DLB vs multiple system atrophy (MSA)). Despite considerable enthusiasm and support for the role of cell-to-cell prion-like spread of α-synuclein it should be emphasized that this mechanism remains to be proven in patients with PD.

Finally, there is considerable evidence implicating the activation of both the innate and adaptive immune systems in PD and that the related inflammatory response plays an important role in the ongoing neurodegenerative process(31), including a role in the propagation and spread of α-synuclein pathology (32). This is supported by epidemiological evidence for a reduced incidence of PD in individuals receiving certain nonsteroidal anti-inflammatory agents(33). There is basic science evidence that α-synuclein itself (aggregated and especially nitrated) induces and maintains inflammation and that the inflammatory response involves the central nervous system (microglia and CD4+ T cells) but also peripheral immune cells (M1 macrophages and CD4+ T cells). With respect to the latter, there is increasing interest in the potential role of the gut microbiome as a peripheral drive of chronic pro-inflammatory immune activity in PD (34),(35). Independent of the initial trigger, subsequent activation of cytokines and chemokines, phagocytosis and the production of complement continue to stoke the inflammatory process suggesting that it is an important downstream mechanism that could continue to drive neurodegeneration beyond the activation of many of the processes outlined above. Although there is pathological evidence that inflammation occurs relatively early in the disease (36), theoretically, treatments targeting this feature independently, or as an adjunct to other interventions, might also be effective later in the disease at a time when interventions directed at early propagation and aggregation of α-synuclein, lysosomal or mitochondrial dysfunction, etc, may be too late.

b. Selective neuronal vulnerability

Any consideration of disease pathogenesis and the critical targets for disease modifying therapy must take into consideration the fact that the neurodegeneration associated with LP, although widespread at the end stages, still only affects very selected regions of the central and peripheral nervous systems. The exact reasons for this selective neuronal vulnerability have been widely discussed but remain uncertain. All neurons contain α-synuclein as well as all of the cell processes and pathways outlined above, known to be dysfunctional. Despite the increasing evidence for cell-to-cell transmission as a potential mechanism for spread of the pathology, it is clear that the number and strength of synaptic connections (i.e. the connectome) between nuclei prominently affected by LP and other brain regions cannot explain

the distribution of the pathology in PD(37). Thus, specific cell-autonomous features must either protect the majority of neurons from LP and neurodegeneration or, more likely, predispose specific types of neurons to the degenerative process. Neuronal vulnerability likely reflects shared cellular and molecular phenotypic characteristics that result from important transcriptional factor programs active during development of the nervous system (38). Braak and his colleagues(27) have emphasized that neurons predisposed to the pathology share the common feature of long and thin unmyelinated or partially myelinated axons that require prodigious expenditures of energy, increasing their mitochondrial oxidative stress. Indeed, it is estimated that the axons of human SNc neurons are approximately 4.5 m in length with as many as 16,000 branches forming around 2.5 million striatal synapses(39). Surmeier and colleagues have further pointed out that these and other predisposed cells are autonomous pacemakers with large fluctuations in poorly buffered cytosolic Ca^{2+} that drives mitochondrial oxidant stress. There may be additional interplay between these anatomical and cellular predisposing factors with elevated α-synuclein expression due to the long and highly branched axons, elevated cytosolic Ca^{2+}, and reactive oxygen and nitrogen species promoting the further formation of intracellular α-synuclein aggregates and vice versa(37). Sustained mitochondrial oxidant stress may further increase α-synuclein aggregates and disturb proteostatic protective mechanisms. In addition to the selective vulnerability to neurodegeneration and LP demonstrated by a number of regions, other factors may contribute to the progressive neuronal loss independent of α-synuclein. For example, it is also likely that dopamine itself further heightens the vulnerability of SNc neurons. It is clear that dopamine metabolism contributes considerably to oxidative stress (40),(41),(42) and evidence from mouse models suggest that dopamine and α-synuclein conspire and interact to enhance degeneration of dopaminergic neurons (43),(44). As will be discussed later, these issues need to be considered in determining when selected disease modifying approaches might be effective and particularly why combinations of therapy (“cocktails”) may be more likely to succeed than treatments directed at single pathogenic mechanisms.

c. Potential therapeutic strategies

Theoretically there are a number of processes that one could target in an attempt to directly reduce α-synuclein toxicity ranging from protein synthesis, misfolding, fibril formation and aggregation, degradation, and cell-to-cell transmission. Most of these processes involve the affected neurons, however, recent work suggests that another alternative could be the enhancement of astrocytic trapping and degradation of α-synuclein fibrils(45). Many of the proposed therapeutic approaches remain at the theoretical or planning stages(37), (46),(47). Attempts to reduce α-synuclein synthesis

using siRNAs and antisense oligonucleotides may not be far from study in humans. The recent discovery that the β2-adrenoreceptor (β2AR) is a regulator of the α-synuclein gene and β2AR agonists are associated with a lower incidence of PD(48) may support the early repurposing of safe and available β2AR agonists as a method of reducing α-synuclein gene transcription. Table 2 lists the therapies targeting α-synuclein currently in or close to human trials(37),(49), (50),(51),(52),(47). The approaches pursued on the largest scale currently involve active (PD01A, PD03A) (49), (53) and particularly passive (PRX002/RO7046015, BIIB054, BAN0805, MEDI1341) immunization(51),(47). The proposed mechanisms of action of anti-synuclein antibodies are numerous including intra- and extracellular effects in enhancing the clearance of the protein and / or blocking its putative adverse cellular effects on neuronal processes and the resulting inflammatory response(51), theoretically resulting in both reduced cellular toxicity and spread of the pathology. Extensive preclinical studies have assessed the effects of a variety of monoclonal antibodies generated to different parts of the α-synuclein protein (N-, mid-, C-terminal or full length peptide) and evidence for safety and target engagement (reduction in serum free α-synuclein levels and increased free plus antibody bound levels (54)) have encouraged progress to Phase 2 randomized controlled clinical trials (RO7046015, the PASADENA trial; BIIB054, the SPARK trial).

Other approaches being explored include a stabilizing small molecule designed to block misfolding of α-synuclein (NPT200-11) (in Phase 1 testing), treatments directed at inhibiting α-synuclein aggregation (NPT088, glycerol phenylbutyrate, squalamine, and nilotinib) and oligomer formation (epigallocatechin gallate (EGCG))(49),(47, 55), (56). There has been a great deal of publicity and enthusiasm generated by claims originating from a small open-label trial of nilotinib in late stage patients, many with dementia(57). Nilotinib is a kinase inhibitor approved for the treatment of chronic myelogenous leukemia. It has been shown to inhibit c-Abl and potentially protect against neuronal death in PD by reducing pathologic phosphorylation of both parkin and α-synuclein(58),(59). Unfortunately, the quality of the reported trial was poor and there is strong possibly that a placebo effect accounted for most if not all of the clinical efficacy. The Michael J. Fox Foundation is in the process of conducting a more definitive trial of nilotinib and other companies are actively developing more potent and selective c-Abl inhibitors.

Another important potential target relates to the mentioned inverse relationship between α-synuclein levels and toxicity and GCase activity. Although patients with *GBA* mutations are the logical greatest beneficiaries of drugs directed at this association, there are reasons to believe that patients with sporadic PD, particularly those confirmed to have low GCase, could also benefit. Methods of reducing α-

synuclein toxicity by targeting this relationship potentially could include enzyme replacement therapy, drugs that modulate the activity of GCase, and glucosylceramide synthase inhibitors which block the formation of glucosylceramide (GL-1) and reduce α-synuclein aggregates in a transgenic mouse model(60). Ambroxol, a secretolytic agent licensed for respiratory diseases to reduce mucous production, increases glucosylceramidase activity with effects in preclinical models(61). A small Phase 2 study is underway (NCT02941822) with the possibility of repurposing this agent for PD (with and without *GBA* mutations). Studies using a glucosylceramide synthase inhibitor (GZ/SAR40261) are also about to begin, initially in PD patients with *GBA* mutations.

d. Challenges/caveats

Although these are exciting times, with two large clinical trials of anti-synuclein monoclonal antibodies currently underway, there a number of unanswered questions or concerns that need to be considered as these therapies move forward (Table 3). In contrast to specific genetic forms of PD due to mutations in *SNCA* or associated with excess levels of α-synuclein (duplications/triplications), where this protein is clearly a critical initiating factor for disease pathogenesis, in sporadic PD the timing of α-synuclein accumulation vs possible initiating upstream factors (e.g., lysosomal dysfunction, impaired synaptic transmission, endosomal disruption) is unknown. Therefore, in the earliest stages of disease (i.e., pre-clinical disease (62)), antibodies directed at α-synuclein might not have sufficient impact and it has been argued that the development of neuroprotective strategies emphasizing Lewy body pathology focuses on end-stage disease rather than on early pathophysiological events(25). Indeed, it is known that LBs do not correlate with symptoms (it is unclear whether this also applies to other aspects of LP) and nigral cell loss has been shown to occur in advance of the presence of α-synuclein aggregates(63). Furthermore, it is well recognized that although most PD patients with *LRRK2* mutations demonstrate LP at autopsy, some have typical PD (possibly with less dementia and autonomic failure(64)) in the absence of any evidence of α-synuclein aggregation and most patients with homozygous or compound heterozygous *parkin* mutations completely lack α-synuclein deposition. On the other hand, it is plausible that by the time patients with sporadic PD present clinically the disease process is well established and both intra- and extra-cellular α-synuclein may be playing an active role in the progressive neurodegenerative process.

Although early studies have shown evidence for blood brain barrier penetration by α-synuclein monoclonal antibodies, it is not known with certainty whether sufficient antigen is accessible in an extracellular phase to allow impactful clearance, particularly of oligomeric or fibrillar α-synuclein, and it is not known how effectively these antibodies will influence intracellular processes. Nor is it known whether antibodies directed at the C-, N- or mid-terminus of the protein would be more effective in selected patient populations or at different stages of the disease.

The finding of LBs in transplanted neurons in patients with PD has been a critical driving force for the belief that cell-to-cell transmission of α-synuclein underlies disease progression and that this process could be influenced by treatment with immunization therapies. However, the transplant experience might also be informative of the expected timelines that could influence the likelihood of response to antibody therapy. Although changes in transplanted fetal dopaminergic cells are evident in earlier years, Lewy pathology, at least in these very young dopaminergic neurons, requires 10 or more years to develop. This, as well as the very slow progressive natural history of PD (apart from the loss of DA neurons which, as discussed below, might not be purely related to α-synuclein toxicity) suggests an extremely long timeline for the prion-like cell-to-cell spread, seeding and permissive templating and subsequent pathogenic changes that might not be evident or significantly influenced after only 1-2 years of immunization therapy.

Finally, another important factor that needs to be considered in evaluating the impact of α-synuclein-targeted therapies in early disease is the basis of the clinical changes that are taken as evidence for disease progression. Much of the clinical change documented in the early years of PD relates to progressive decline of the dopamine system (as evidenced by changes in presynaptic dopamine imaging and clinical responses to dopamine replacement therapy). It is possible that these changes are only partially explained by the direct effects of progressive α-synuclein spread and “toxicity”. It is possible that once the degenerative process affects nigral dopaminergic cells additional biochemical factors more specific to these cells than to other areas affected by the neurodegeneration (e.g., oxidative stress, mitochondrial dysfunction, calcium channel pacemaking) are triggered and overwhelm cellular protective mechanisms. This could result in an accelerated phase of the neurodegenerative process specific to this cellular region that is not exclusively related to the direct effects of α-synuclein(65). This possibility and other caveats outlined above provide further arguments for considering the use of combined therapies or cocktails in disease modifying efforts that may include but do not exclusively depend upon treatments targeting α-synuclein.

2. Pathogenic mechanisms distinct from α-synuclein

Many molecular pathways have been proposed to be involved in the neurodegenerative process underlying PD. According to a convergent model of disease, dysfunction in one pathway may trigger abnormalities in others with the potential for a variety of self-sustaining vicious pathogenic cycles (Figure 1). A broad spectrum of abnormalities are described in synaptic transmission, vesicle trafficking and protein sorting, proteostatic clearance by autophagy and the ubiquitin-proteosome system (UPS), ER stress and the unfolded protein response (UPR), mitochondrial homeostasis including mitophagy, oxidant stress, and sustained elevated cytosolic Ca^{2+}, and finally activation of both the innate and adaptive arms of the immune system. As outlined in the previous section, some of these can be directly attributable to assumed toxic effects of α-synuclein, while others may be factors that contribute to the selective vulnerability of specific neurons affected in PD, related to genetic risk or vulnerability of selected patient populations or representing a downstream response to a variety of upstream stressors. Here we will discuss putative disease modifying therapies currently under study based on the proposed mechanism of action or selected molecular pathways of interest. The challenges outlined in Table 1 need to be kept in mind when considering these studies, especially the lack of markers for patients enriched for dysfunction in the target pathogenic mechanism.

a. Trials/Drugs in Advanced Stages of Study

Four drugs, each addressing distinctly different molecular pathways/mechanisms, are in advanced stages of testing in patients with PD. *Isradipine* is a dihydropyridine calcium channel blocker with a relatively high affinity for Cav1.3 channels currently approved for the treatment of hypertension. *Inosine* is a urate precursor that increases plasma urate, the main antioxidant found in plasma. *Deferiprone* is a potent iron chelator. Finally, *exenatide*, a synthetic version of exendin-4, is a naturally occurring analogue of human glucagon-like peptide-1 (GLP-1) approved for the treatment of type 2 diabetes. Table 4 summarizes the reasons for studying these agents as potential disease modifying therapy and provides details on the studies currently underway or recently completed.

b. Other treatment categories

i. Anti-inflammatory Therapy

There is considerable interest in the potential for anti-inflammatory therapies to have disease modifying effects in PD. Numerous studies of a wide variety of therapies claimed to have anti-inflammatory properties (often combined with other effects (e.g., pro-apoptotic)) have been conducted in various animal models. To date there have been no large clinical trials designed to address this issue in patients with PD. A number of drugs believed to modulate the immune system have undergone preliminary studies. AZD3241 is a selective and irreversible inhibitor of myeloperoxidase, a reactive oxygen generating enzyme expressed by microglia. It is hypothesized that this effect will lead to a sustained reduction of neuroinflammation. A recent PET study demonstrated a reduction of (11)C-PBR28 binding to translocator protein in the brain of PD patients after 6 weeks of treatment with AZD3241 (66). These results provide support for the proposed mechanism of action of AZD3241 on microglia and encourage further studies evaluating its potential to modify the disease course of PD as well as other neurodegenerative disorders. A small double-blind trial of sargramostim, a recombinant granulocyte macrophage colony stimulating factor (GM-CSF) demonstrated modest improvements in clinical, MEG-recorded cortical activities and regulatory T cell number and function compared to placebo or pretreatment states (67). Review of clinicaltrials.gov shows a number of other terminated or completed but unreported studies of treatments directed at neuroinflammation. ViNeuro, a compound said to have a variety of immunomodulating functions (increases the activities of T-cells, B-cells and NK cells, enhances mitochondrial antioxidant status) in various tissues including brain, was studied in a triple-blind RTC for safety and efficacy (NCT00517842, completed in Sept. 2008). Stromal stem cells are being studied in neurodegenerative diseases. Although these have some regenerative capacity, perhaps more importantly they release a wide array of soluble factors that have immunosuppressive, anti-inflammatory, and trophic effects. An ongoing trial is studying intra-arterial (via the vertebral artery) and intravenous delivery of autologous adipose-derived stromal stem cells in PD in a phase 1/2 trial (NCT01453803).

ii. Exercise and Physical Therapy

A large number of different types of exercise and physical therapy have been proposed to be beneficial for patients with PD. Obvious benefits can accrue from improvement in muscle weakness, increased aerobic capacity, and reduction in gait and balance dysfunction and resultant falls. Distinct from these physical benefits, it has been claimed that exercise may induce central neuroplasticity changes that could positively affect neurodegenerative disease processes. Proposed mechanisms demonstrated in preclinical models include anti-inflammatory, antioxidant, pro-mitochondrial, trophic and anti-synuclein

effects and small studies in PD patients have shown increased cortical motor excitability, elevated striatal dopamine D2 receptor binding, increased serum levels of brain-derived neurotrophic factor (BDNF) and improvements in gray matter volumes (see (68) for review). Whether these findings truly represent central neuroplastic changes that will have a long-term impact on the natural history of the neurodegenerative process or simply central consequences of improved fitness and physical training will only be established when reliable biomarkers for the disease status become available. On the other hand, even in the absence of direct effects on the neurodegeneration, it is clear that the peripheral muscular, cardiovascular and practice (i.e. gait and balance) effects resulting from exercise and physical therapies can have a positive influence on the patient's tolerance of progressive debility and the concurrent aging process.

iii. Other approaches

A loss of the endogenous antioxidant glutathione (GSH) within the brain has been implicated in PD. A number of treatments have been directed at this finding including peripheral GSH infusions (not proven to cross the blood brain barrier). In a recent study, supplementation with N-acetylcysteine, a GSH precursor, significantly increased peripheral antioxidant measures (catalase and GSH/GSSG) but failed to increase brain GSH (using proton magnetic resonance spectroscopy (^{1}H-MRS) at 3 and 7 tesla), possibly related to low oral NAC bioavailability(69). EPI-589 (also known as (R)-troloxamide quinone in amyotrophic lateral sclerosis research), a drug claimed to catalytically increase GSH in cells, is being evaluated in a phase 2a safety and biomarker study in mitochondrial genetic subtypes as well as sporadic PD (NCT02462603).

The negative association between smoking and PD has been repeatedly demonstrated in epidemiological studies. It remains unclear whether this is related to a protective effect of some component of cigarette smoke, including nicotine, or whether this is a consequence of the underlying disease or factors predisposing to it. For example, a recent study proposed that patients destined to develop PD have significantly less difficulty giving up the smoking habit than those who don't develop PD, suggesting that ease of smoking cessation is an aspect of premanifest PD similar to olfactory dysfunction, REM sleep disorder, depression or constipation(70). A recent SPECT study suggested that current but not past smoking has a direct effect on increasing dopamine transporter activity in the putamen, without impacting on clinical findings(71) . Despite the conclusions of the authors, this interesting finding cannot be accepted as proof that smoking "protects dopamine neuronal degeneration in the sensorimotor striatum" but may instead have a direct effect on the transporter or

the binding of the ligand. The results of a phase 2 RCT investigating transdermal nicotine in early untreated PD (160 subjects) are currently under review (NIC-PD; NCT01560754).

The influence of statins on PD is controversial. Although preclinical studies suggest the potential for neuroprotection, epidemiological studies have reported protective(72), facilitating(73) and no effects(74) on PD risk, likely confounded by background levels of HDL, LDL and possibly other variables, often not available in large retrospective epidemiologic studies. The use of statins in preventing cerebrovascular co-pathology is discussed further below.

Other available therapies that might be "repurposed" for disease modification based on either epidemiological or basic science studies include caffeine(75), and antidepressants including tricyclics(76),(77), fluoxetine(78) (79) and trazodone. The latter has the novel effect of potentially supressing the overactive unfolded protein response by inhibition of eIF2α-P activity(80). However, as with other interventions under development, challenges with assessing the potential of repurposing these therapies include the absence of biomarkers of disease and treatment response and the lack of pharmacogenomic data to predict which individuals may benefit or be harmed with these interventions.

3. Treatments directed at specific subtypes of Parkinson's disease: LRRK2

The term "subtypes" is applied to PD in a variety of ways. For example, there have been many attempts to distinguish different clinical phenotypes that could have etiologic or prognostic implications(81). However, there are a variety of problems with these approaches and none show promise for directing disease modification therapies in the near future. Etiological subtyping based on monogenetic causes will likely have the greatest impact on future disease modifying approaches. The pursuit of the pathogenic mechanisms discussed in previous sections has been especially driven by studies in several autosomal dominant monogenetic forms including *SNCA*, *GBA*, several of the late-onset familial forms (e.g., *LRRK2*, *VPS35*, *DNAJC13*)(25) as well as the younger-onset autosomal recessive forms (e.g., *parkin*, *PINK1*, *DJ-1*). In the future, disease modifying therapies directed at the specific pathogenic mechanisms involved will likely be applied to patients with these genetic subtypes (both symptomatic and presymptomatic). An obvious hope is that the mechanisms involved will be more generalizable and that developed treatments will be successfully applied to PD patients lacking an obvious genetic cause. However, as summarized in the Reasons for Failure section above, assuming that therapies based on mechanism-specific forms of PD may offer opportunities for treatment of "sporadic" PD patients lacking

evidence for dysfunction in these mechanisms may continue to result in negative studies and the premature rejection of treatments that may be disease modifying in smaller but molecularly suitable subtypes.

Separate mention should be made of PD caused by mutations in *LRRK2*, the commonest autosomal dominant genetic subtype. *LRRK2* (leucine-rich kinase 2) codes for a large multi-functional protein; despite extensive studies it is still unknown which of its neuron-specific functions are most relevant in causing PD(82). Indeed, many *LRRK2* interactors have been identified with potential impact on autophagic, mitochondrial signaling, and oxidative pathways. Further challenge to our understanding comes from the intriguing observation that, although most patients with mutations in *LRRK2* demonstrate typical LP, as mentioned earlier, some have no evidence of α-synuclein aggregation and there may be clinical differences between patients manifesting these different pathologies(64). Still other *LRRK2* patients demonstrate primary tau pathology similar to progressive supranuclear palsy(83) or corticobasal degeneration(84) and studies have shown *LRRK2* effects on tau and microtubules (see (85)). The weight of evidence supports the likelihood that *LRRK2*-PD is due to a gain of function rather than a loss of function of the gene. Pathogenic mutations, especially in the GTPase (ROC: Ras-of-complex) and COR (C-terminal of Roc) domains, increase kinase function and inhibition of *LRRK2* kinase activity provides neuroprotection in a variety of models(86). Programs are underway attempting to develop effective and safe small-molecule *LRRK2* kinase inhibitors(86),(85). Denali Therapeutics has very recently announced successful results of a Phase 1 study in which DNL201, which was measured in the CSF (i.e., demonstrating CNS penetration), significantly reduced LRRK2 kinase activity in a small number of healthy volunteers. Safety of *LRRK2* inhibition has been an important hurdle with earlier agents resulting in major kidney and pulmonary toxicity, presumably due to altered endolysosomal and degradation pathways. Safety issues will remain an ongoing concern especially for the long-term chronic therapy that will be required for disease modification in PD. Furthermore, increasing evidence supports the possibility that non-kinase *LRRK2* activity may be involved in the pathogenesis of *LRRK2*-PD(85). These issues have encouraged consideration of other approaches such as the use of *LRRK2* antisense oligonucleotides (87).

4. "Disease modifying" interventions not specifically influencing the underlying PD pathobiology

A number of therapeutic approaches could be considered disease modifying without having impact on the multi-systems progressive neurodegenerative processes. For example, if it were possible to reinstate or bolster the compensatory mechanisms(6) that presumably fail as clinical symptoms develop, it might be possible to "turn back the clock" on some features without changing the underlying disease. To date, there have been no treatments developed with this goal in mind. A variety of surgical approaches have been directed at reinnervating the severely depleted nigrostriatal dopaminergic system. If successful, these regenerative/restorative therapies would have major impact on the dopaminergic motor features of the disease (including the complications of dopamine replacement therapy) without changing the overall disease process or without affecting the non-dopaminergic features of the disease. As a consequence, the later levodopa-resistant motor and non-motor features will probably develop even in those patients who initially respond very well to these therapies(29), comparable to the experience of patients successfully treated with deep brain stimulation (DBS) of the subthalamic nucleus (STN) (88). Table 5 summarizes the various surgical therapies that have been applied to date. In the future, "hybrid stereotactic therapies" using DBS lead implantation to complement and accentuate the impact of cell- or gene-based therapies will almost certainly be explored (89).

As in other neurodegenerative diseases of the elderly, postmortem studies in patients with PD have demonstrated the presence of a variety of other concurrent pathologies, particularly Alzheimer's disease and cerebral white matter rarefaction(9). Addressing these co-pathologies could have "disease modifying" effects without impacting upon PD-related neurodegeneration per se. In some cases, these co-pathologies may simply be coincidental given their common presence in the aging population. In others these may represent distinct subtypes of PD which combine additional pathogenic mechanisms involving, for example beta amyloid or tau. A recent report showing Alzheimer pathology in 77% of patients with synucleinopathies manifesting dementia strongly favors this possibility (90).

There is considerable interest in the potential for lifestyle modifications and aggressive management of vascular risk factors to prevent cognitive impairment in the elderly. To date, 3 important trials, the Multidomain Alzheimer Preventive Trial (MAPT) (91), the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER) (92) and the Dutch Prevention of Dementia by Intensive Vascular Care (PreDIVA) trial (93), have shown somewhat variable results but suggest that targeting interventions to individuals at increased risk for dementia might be effective (94). The evidence that patients with PD have a higher incidence of vascular risk factors and cerebrovascular disease is limited but there is reason to believe that those patients with this combination have a greater occurrence of

certain levodopa-resistant features such as cognitive decline and axial motor dysfunction even in the early clinical stages of the disease (95). Furthermore, statin therapy was found to be underutilized in those with high or medium cardiovascular risk (60% of recent-onset patients in one study from Scotland) (96), thus not only increasing the threat of vascular morbidity and mortality but also potentially accentuating cognitive and motor features associated with their disease. These studies emphasize an important potential for "disease modification" unrelated to the primary neurodegenerative process by careful attendance to vascular risk factors in this elderly population.

Finally, a discussion of disease modification is incomplete without mention of the poorly understood role of aging on PD(97). Age is the most important risk factor for the development of PD. Age (chronological age and age of disease onset) also has significant impact on clinical features, with older patients having a faster rate of progression, more axial features such as gait dysfunction and postural instability, poorer response to levodopa and greater cognitive decline. If disease modifying therapies are to have an important impact on the later-stage levodopa-resistant features of the disease, the role of aging will need to be better understood and accounted for in future clinical trials.

Questions and Challenges ahead

For the sake of simplicity, we have subdivided these questions under the general headings of: Why, Who, When, What and How. Many of the questions outlined below overlap with or relate to more than one of these issues.

a. Why

As outlined in the Introduction, the question of "why" we should pursue disease modification is probably the only simple and easily answered question related to this topic. A far more difficult "why" question asks "Why at this time?". In other words, can we justify further large and expensive trials of single putative disease modifying therapies in our current state of ignorance. Considering the many potential explanations for past failures (Table 1) it is very difficult to justify continuing to utilize existing disease modifying treatment paradigms in the absence of major advances in a number of areas particularly biomarkers, especially those that successfully subtype patients into categories that predict response to specific mechanistic interventions. A set of biomarkers of general "disease pathogenesis" (a goal of many current programs) will probably be too broad to be applicable to specific subtypes or to

predict the likelihood of one subtype to respond to a specific treatment. Instead, "biomarker fingerprints", characterizing *who* might respond to a particular therapy and *when* such therapy would have its greatest impact, should be a priority for development. In the absence of these advances, therapeutic targets need to be broadly generalizable and treatments should be given to patients at a time in the disease course when successfully addressing the proposed pathogenic mechanism(s) would have a reasonable chance of demonstrating impact.

b. Who

"Who"-related questions address the specific patient populations to be studied in disease modifying trials. They also relate to some of the "when" questions below. The obvious first "who" study groups will be individuals carrying the more common monogenetic causative or risk factor genes (e.g., *LRRK2* and *GBA*). An important challenge to this approach is the limited number of candidates for such trials making them a very coveted testing ground for novel therapeutics. The field may have to develop some method of prioritizing the most promising therapies with the greatest likelihood of benefit rather than leaving this to the marketplace or to a "first out of the gate" approach, even if more efficient clinical trial designs, such as basket and umbrella designs employed in precision medicine treatments in oncology, were to be adapted to neurodegenerative disorders. In the future, it is likely that clinical trials will be conducted in other "enriched" patient populations distinguished by selected markers that would predict a greater potential for benefit from a therapy targeting a specific pathogenic mechanism. As already emphasized, in the absence of such biomarkers it is important to question whether it is appropriate to continue to lump all patients with "early PD" for the purposes of trial recruitment, treating the disorder as a uniform, homogeneous pathogenic condition.

c. When

"When" over the course of the disease should putative disease modifying therapies be tested needs to be addressed in future trials. Treatment in the "preclinical" stages of the disease will be possible in non-manifesting carriers of monogenetic forms of PD. There is also considerable interest in treating patients with a high likelihood of having "prodromal" disease, for example as defined by the MDS research criteria for prodromal PD (98). There are major challenges to both of these approaches. The limited number of non-manifesting monogenetic PD gene carriers has already been emphasized. The commonest genetic cause, *LRRK2*, has a relatively low penetrance; without a better understanding of which gene carriers are most likely to phenoconvert, many individuals would need to be treated, most

never destined to develop the disease. This also assumes that "phenoconversion" would continue to be defined as the time point when a feature of classic parkinsonism, such as tremor or bradykinesia, emerges. Given the multiple other disease phenotypes associated with *LRRK2*, this motor-centered threshold may need to be reconsidered. Certain other genetic subtypes (e.g. homozygous or compound heterozygous *parkin* mutation carriers) have a very high penetrance but disease modification trials will be challenged by their exceedingly slow rate of progression. Treating individuals fulfilling criteria for "prodromal PD" will also involve exposing many who will never develop the disease to long-term, potentially toxic therapy and the time required for those who are destined to become clinically symptomatic may be so long as to preclude obtaining an answer in the absence of disease biomarkers. Furthermore, if clinical outcomes (e.g., the development of manifest parkinsonism) are to be used in any of these trials, careful and reliable definitions of phenoconversion will be required (part of the "how" questions below), particularly acknowledging the low diagnostic accuracy rate in individuals diagnosed as having early possible PD (99). A common argument for introducing disease modifying therapies at these earlier stages rather than the current model of recruiting early symptomatic patients has been that this early clinical state actually represents advanced established disease that may be more resistant to these treatments. On the other hand, the disease certainly continues to progress over many years and so we need effective therapies that will modify the course in the later stages as well.

d. What

What target or targets to prioritize has been the emphasis of much of this review. There are almost an infinite number of targets and interventions currently being explored in preclinical models. The "infinite" set of targets available can be explained by the many molecularly characterized animal and genetic human PD models, where a given finding is taken as a "piece of the puzzle" that helps explains the whole of PD. Given the track record of failures and the uncommon matching of therapies tested to disease pathogenesis in the targeted populations, the bar for many of these to reach the stage of clinical testing or succeed into Phase 3 studies in unselected groups is high. Our goal here is to consider the short- and medium-term landscape and to discuss how the field might achieve the goal of some degree of successful disease modification sooner rather than later. Currently, larger Phase 2 or 3 studies are underway evaluating treatments directed at oxidative stress (inosine), cytosolic Ca^{2+} (isradipine), iron (deferiprone) and extracellular α-synuclein (passive immunization). Other targets actively being pursued in PD patients include c-Abl, GLP-1, and GCase. The results of the nicotine trial (NIC-PD) and the large delayed-start levodopa trial (LEAP-study (16)) will be available over the next few months. Beyond these

it might be argued that further trials in patients not enriched for the targeted pathogenic process are doomed to repeat the failures of the past.

Lacking biological markers that can distinguish patient subtypes or knowledge of the order in which different cellular mechanisms contribute to neuronal loss (if the cellular mechanisms thus far reported are placed in series for a single disease rather than in parallel, in different subtypes), success may only be possible with the use of rational combinations of therapies directed at different components of the neurodegenerative process. Even when we have a better understanding of disease pathogenesis, cocktails of differently acting agents may be necessary for effective disease modification, as frequently applied in the management of many cancers, AIDS, tuberculosis, and autoimmune disorders. This need is further enhanced by potential redundancies or compensatory mechanisms that could reduce the potential success of even the most promising single target-directed therapy. Rather than discarding drugs that are found to be safe but "ineffective" when given as monotherapy, it might be appropriate to combine these with agents that target different but potentially complementary biological mechanisms. Combination therapy could include a variety of approaches, for example targeting α-synuclein itself, cellular mechanisms that either fail as a consequence of the toxic protein or contribute to its accumulation, and further downstream factors such as inflammation that perpetuate the neurodegenerative process. Combinations of different anti-synuclein therapies (alone or in combination with other agents) could be directed at distinct processes, such as transcription and aggregation or extracellular transmission, or different forms of α-synuclein such as soluble oligomers and aggregated fibrils. One important consideration in choosing the components of drug cocktails is the selective neuronal vulnerability discussed earlier. Again, this might be a two-fold vulnerability combining specific physical neuronal characteristics (e.g., that could be directly influenced by drugs directed at fluctuations in cytosolic Ca^{2+}) (37) and factors (many of them overlapping with the former) directly related to dopamine metabolism in SNc neurons that could further accelerate neuronal loss in this region (65).

e. How

How will we know disease modification when we see it? "How" questions relate to the complex issues of clinical trial study designs which are beyond the scope of this review (7),(100),(101),(102). If the populations targeted are subtyped based on the mechanism of action of the tested therapies, design issues may be less challenging, as the signal-to-noise ratio for the scope of these therapies would increase. As previously emphasized, the early evaluation of future therapies will require effective assessments of target engagement. These studies will also be greatly influenced by the development of

reliable disease-related biomarkers. Furthermore, unique challenges in PD that have plagued the field from the outset are the confounding impact of symptomatic benefits induced by some drugs that could also have disease modifying effects as well as the potent symptomatic effects of concurrent dopaminergic therapy that risk masking any disease modifying effects of study interventions. A related important issue mentioned above concerns the long-term effect of levodopa documented in the ELLDOPA trial (15) and the major challenge to the design of future trials if the LEAP-study (16) were to show that initial benefits from early introduction of levodopa are not recouped in the delayed start group. The adoption of non-dopaminergic disease milestones, such as dementia and falls, would obviously circumvent this pitfall but would require long study durations to assess adequately.

The need for studies of combination therapies raises a new level of complexity and challenge to trial design. Initially, combinations of 2 agents could return to the 2x2 factorial design used originally in the first "neuroprotection" study in PD, DATATOP ((103). As in DATATOP this might be more easily accomplished if one of the study drugs is already approved and being repurposed for use in PD. A study combining a promising drug that "failed" but was shown to be safe in a monotherapy trial could be evaluated in a 3 arm trial with a novel, differently acting agent (i.e., agent X with placebo, agent X combined with the "failed but safe agent", and dual placebo). For example, this could apply to drugs such as isradipine or inosine if the current phase 3 trials fail to meet their endpoints. The study of 3 or more drugs will require more complicated adaptive designs, as have been applied in the I-SPY2 program for breast cancer (104). However, there are multiple challenges to adopting this approach to PD, particularly the absence of biomarkers that predict clinical responses over short timelines. Finally, the field needs to strongly consider the development of large public-private partnerships similar to the European Prevention of Alzheimer's Dementia (EPAD) project (105), (106) if these efforts are ever going to be advanced successfully. Indeed, such success will require that the Parkinson's community emulate similar efforts well advanced in the Alzheimer's field which have recognized the need for and have begun to tackle the hurdles for successful collaboration between industry, academia and regulatory agencies in developing effective combination therapies in AD(107),(108).

Conclusions

The answer as to "which therapeutic developments are more likely to modify progression in PD?" may depend, at a basic level, on the understanding of what "PD" means from the standpoint of therapeutic development. We would argue that our emphasis should not be about the *tools* of warfare (the design of clinical trials, the sensitivity of the endpoints, the therapeutic interventions) but about the

reconfiguration of *warfare itself*. The *Who/What* we are fighting (based on disease model of pathogenicity) is as important as how we fight. The face of our "enemy" has undergone relatively few changes ever since we evaluated whether vitamin E and selegiline could have neuroprotective effects using a 2x2 factorial design. Over the space of the subsequent three decades, we have tested ever more interesting potential treatments but have used the same enemy, "early, drug naïve PD", with features only cosmetically more attractive than those originally described 200 years ago. Our overall tools have been refined, but the *Who/What* targets with those tools have not. We have excused ourselves for therapeutic failures with a variety of cogent reasons (Table 1) but have only tangentially included in the list our definition of the target as a problem.

Most ongoing studies are examining interventions with attractive molecular mechanisms in clinical trials encompassing early PD patients without biomarkers of disease pathogenesis that would confirm their suitability to theoretically benefit from the therapies tested. Choosing patients for inclusion in clinical trials for more "precise warfare" will require moving beyond simply requiring a clinical definition for enrollment; this will demand a "tectonic" change in biomarker development (17). While a "cocktail" or drug combination approach is likely in future therapeutic development, regardless of our refinement in molecular fingerprinting of disease, subtyping PD for trial populations will become critical irrespective of progress in (factorial or adaptive) clinical trial designs. Disease mechanisms affected by a drug must be recognized through an appropriate biomarker if the odds of success are to improve. Trials with smaller but more molecularly suitable populations are bound to replace large generic trials in "early PD" that disregard molecular subtyping.

Figure legend

Figure 1. Proposed mechanisms resulting in cell death in PD (diagram not meant to imply a necessary temporal sequence or a process of events in series). Although alpha synuclein (αSYN) is depicted as a principal player, Cell Death can occur in its absence, induced by any of the failed processes outlined (as seen on the left side of the figure). Dysfunction in these processes may precede and cause or contribute to αSYN dysfunction or follow as a consequence of αSYN toxicity and in turn feedback and enhance this toxicity. Note that convergence to Cell Death does not mean a single disease. Each of the mechanisms likely represent a separate molecularly-targetable disease subtype.

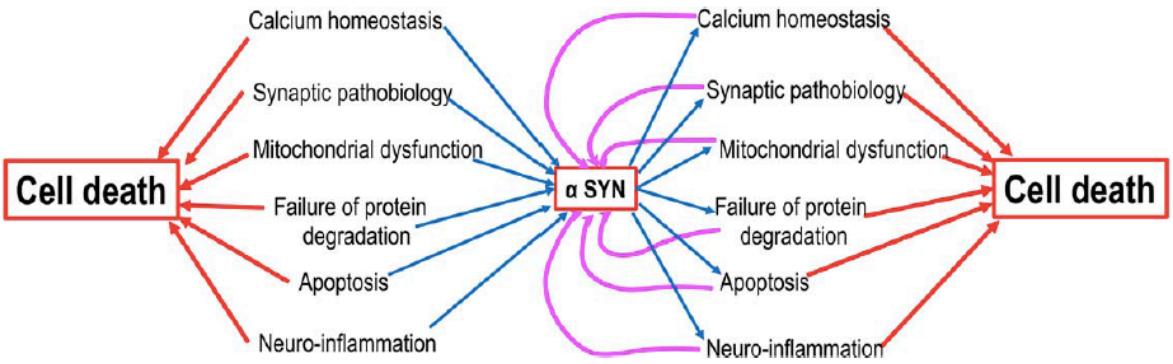

Table 1. Reasons for Past Failures of Neuroprotection Trials in PD		
Obstacle	Consequence / Issue	Additional Comments
Disease pathogenesis	Precise cause(s) and pathogenesis unknown; studies treat PD as a single disorder	Phenotypic and genotypic heterogeneity of the populations on which new drugs are tested dilutes the power to find which patients would respond
Preclinical models	Preclinical cellular and animal models used are probably poorly reflective of the pathogenesis of human PD (also typically assume a single predominant pathogenic mechanism); Toxin-based models might be better representative of certain autosomal recessive forms of "PD", now known to have disturbances of mitochondrial function, clearance etc. (109)	Not yet known whether aSYN based models (110) will be better predictive of outcomes in patients with PD - still many concerns about the relevance of these models to the human disease (e.g., inordinately high levels of aSYN involved compared to those found in humans).
Drug dose and Target engagement	Not known whether the doses of the drugs studied were adequate to accomplish their goal in humans or whether the intended target of the drug was actually engaged	Inability to predict doses necessary for disease modification; lack of biomarkers of target engagement (see below).
Outcome measures and Trial designs	Not clear whether the outcome measures or the clinical trial designs used were ideal for assessing disease modification(7, 8, 100)	Endpoints may be insensitive to capturing disease modification; symptomatic effects of intervention confound outcomes; concurrent symptomatic therapies (e.g., L-dopa) may mask evidence of disease modification; treatment could influence surrogate marker (e.g., imaging) and not disease state; treatment could influence a compensatory factor or concurrent pathology rather than the intended disease mechanism
Biomarkers	The complete lack of reliable biomarkers that reflect disease presence and severity, as well as target engagement and impact of the therapy	The lack of these various types of biomarkers is a major limitation in this field (see above). Biomarker development efforts currently remain anchored on clinically defined cohorts.

Enrolled patients with “early disease” already have extensive neurodegeneration	Disease typically affects lower brainstem structures and the olfactory system well in advance of nigral cell loss(27),(63),(111); estimated 30 to 50% loss of SNc dopaminergic neurons and a 60% reduction in nigrostriatal dopamine at the time of clinical presentation and almost complete loss of dopamine terminals in the dorsal putamen within 4 years of diagnosis (112)	This raises the important concern that the disease may be too far advanced even in the earliest symptomatic stages of motor PD for us to be able to discern an impact of putative disease modifying therapies, particularly with treatments designed to influence only one pathogenic mechanism (see text).

αSYN: α-synuclein

Table 2. Treatments targeting α-synuclein active in patients with PD		
Drug	Mechanism of Action	Status
RO7046015	Passive immunization	Phase II
BIIB054	Passive immunization	Phase II
PD01A, PD03A	Active immunization	Phase I
Nilotinib	C-Abl inhibition	Phase II
NPT200-11	Inhibition of α-synuclein misfolding	Phase I
Ambroxol	Increases glucosylceramidase activity	Phase II
SAR40261	Glucosylceramide synthase inhibitor	Phase II

Table 3. Unanswered questions / concerns related to α-synuclein-directed therapies
Timing in disease course of α-synuclein influencing cell death is not known; other mechanisms may precede and/or trigger α-synuclein toxicity
α-synuclein toxicity only proven in animal models; not necessary for neurodegeneration
α-synuclein aggregation not necessary for neurodegeneration (e.g. *LRRK2*, *parkin*)
α-synuclein aggregation not proven to correlate with neurodegeneration
Unclear if sufficient α-synuclein accessible in extracellular phase for monoclonal antibodies to influence disease progression
Uncertain if current actively studied anti-α-synuclein antibodies sufficient (alternative targets: different sites on protein, specific species (e.g., oligomers), or strains)
Slow development of Lewy bodies in fetal transplant cases suggest extremely slow cell-to-cell transmission and pathogenic process
Clinical features typically assessed in early PD largely relate to SNc dopaminergic cell loss - other important pathogenic processes may not directly involve α-synuclein

Table 4. Medical Therapies in Advanced Stages of Study							
Drug	Mechanism of Action	Preclinical / Epidemiological Evidence	Trial Status	Marker for Patient Selection	Endpoints	Expected Completion Date	Comments
Isradipine	Dihydropyridine calcium channel blocker; relatively high affinity for Cav1.3 channels	Inhibition of Cav1 channels in PD in order to lower cytosolic Ca^{2+} levels, mitochondrial oxidant stress and sensitivity to toxins in neurons at risk of LP or cell death in PD (see (37)); Epidemiological evidence suggests that CaV1 calcium channel blockers are associated with a lower incidence of PD	Ongoing: 36-month phase 3 RTC (STEADY-PD III), 336 (early, initially untreated) PD patients; conducted by the PSG	No	Primary outcome measure: change in Part I-III UPDRS score in the practically defined ON state; study design should allow determination of longer term benefits including impact on dopaminergic drug use, motor complications and non-motor features(113)	Early 2019	Trial assumes fluctuations in cytosolic Ca^{2+} are critical in all PD subtypes
Inosine	Urate precursor that increases plasma urate (the end product of	6-OHDA rodents: elevation of urate has neuroprotective effects; Humans:	Ongoing: 2-year phase 3 RTC study (SURE-PD3); 270 patients with	Yes, serum urate <5.7 mg/dl. Dosed to moderately elevate serum	Primary outcome measure: rate of change in MDS-UPDRS I-	February 2020	Previous clinical trials of antioxidants have failed to

	purine metabolism in humans): main antioxidant found in plasma	large prospective epidemiological and clinical studies - higher urate levels in plasma or cerebrospinal fluid associated with both a lower risk of developing PD and a slower rate of its subsequent progression(114).	early (initially untreated) PD; conducted by the PSG	urate (to 7.1–8.0mg/dl).	III total score over 24 months; multiple secondary measures.		demonstrate disease modification in PD; however, oxidative stress remains a compelling target and is magnified in the recruited subgroup of low-urate early PD patients.
Deferiprone	Iron chelator; crosses BBB; delivers chelated iron to extracellular apotransferrin (less risk of systemic Fe loss)	Iron plays important role in oxidative stress; levels of iron elevated in the SNc of patients with PD (115),(116)	Ongoing: At least 2 studies evaluating potential disease modifying effects. SKY: 140 early (< 3 years since diagnosis) stable treated PD patients; 4 doses (600-2400 mg/d) vs placebo; FAIRPARKII: 338 early untreated PD patients; 30 mg/kg/d vs placebo.	No	SKY -Primary outcome measure: change in Part III MDS-UPDRS score at 9 mo; multiple secondary measures. FAIRPARKII - Primary outcome measure: total MDS-UPDRS at 36 mos; multiple secondary measures.	SKY: July 2018. FARIPARKII: December 2018	FAIRPARKI: Small randomized double-blind delayed start (and delayed cessation) trial of 30mg/kg/d in treated patients showed promising effects(117); better responses in patients with lower ceruloplasmin-

							ferroxidase activity(118)
Exenatide	Synthetic version of exendin-4; a naturally occurring analogue of human GLP-1	Proposed to favorably modulate several relevant cellular processes including disturbances in protein synthesis, apoptosis, autophagy, mitochondrial biogenesis and inflammation(119)	Completed: double-blind trial in 60 patients experiencing motor fluctuations (14).	No	Primary outcome measure: off-medication motor UPDRS score at 60 weeks	Completed: positive effects on the practically defined off-medication state were maintained after a 12 week washout	The difference between the active and placebo arms at 60 weeks was the same motor UPDRS reduction at 12 weeks, suggesting a sustained symptomatic effect and not true disease modification.

6-OHDA: 6 hydroxydopamine; GLP-1: glucagon-like peptide-1; PSG: Parkinson Study Group; RTC: randomized controlled clinical trial; UPDRS: Unified Parkinson Disease Rating Scale

Table 5. Regenerative / Restorative Therapies				
Treatment Modality	Proposed Mechanism of Action	Experience to Date	Ongoing / Recent Studies	Comments
Transplantation of fetal mesencephalic cells into striatum	Replacement of dopaminergic neurons and reinnervation of striatum	2 NIH-funded double blind trials failed to show significant benefit; some patients from these studies did respond(120); a small number of other patients obtained prolonged benefit (including able withdraw from dopaminergic medications)(121); transplant-induced dyskinesias a major concern(120)	TRANSEURO program (NCT01898390)(122) attempting to define the optimal transplantation methods using fetal tissue grafts before possibly proceeding to dopaminergic cells derived from stem cells(123).	One very well studied patient with the largest number of surviving dopamine neurons and the densest and most widespread graft-mediated striatal dopamine reinnervation (associated with profound improvement in F-dopa PET scans) failed to obtain any clinical benefit(124).
Trophic factors	Reinnervation of striatum by surviving host dopaminergic cells	Largely unsuccessful(125) including a large double-blind trial of intraputamenal infusion of GDNF (126); trial of combined intrastriatal and intra-nigral AAV-neurturin failed to demonstrate any clinical benefit, in contrast to earlier open-label studies (127).	A 12 month double-blind trial with a further 12 month open-label extension of GDNF therapy using a novel convection enhanced delivery system failed to meet its primary endpoint.	Failures of trophic factor therapy may relate to the already profound extent of striatal dopamine terminal degeneration within 4 years from clinical disease onset, prior to the intervention (112).
Gene therapy with enzymes involved in	Increase and enhance striatal dopamine	Safety and preliminary evidence of efficacy reported from small open label studies using bilateral	Safety and Efficacy Study of intraputamenal VY-AADC01 is currently recruiting for	Well-designed randomized sham surgery controlled double-blind trials will be necessary to confirm efficacy - many double-blind

dopamine synthesis		intraputamenal infusions of AAV vector-mediated gene delivery of AADC (128) and a lentiviral vector-based triple gene therapy (Prosavin) of AADC, TH and GTP-cyclohydrolase 1 (129).	Advanced PD (NCT03065192)	trials of surgical therapies have failed to confirm important clinical benefits reported in earlier open-label trials.
STN DBS	Proposed to have disease modifying effects distinct from its profound symptomatic benefit: reducing excitotoxic drive from the STN glutamatergic input to the SNc (130); BDNF signaling through the trkB receptor in SNc neurons (131); direct effects on α-synuclein toxicity in a AAV1/2-A53T-aSyn rat model (132).	A group from Vanderbilt University actively pursuing the potential disease modifying effects of STN DBS in very early-stage PD (133) (134).	In planning stages	Challenging population to recruit given mild motor disability at a stage in which surgical intervention is proposed. Lessebo effect in the group allocated to "standard medical care" may further affect validity of the results of subsequent studies if comparison does not include sham surgical arm.

AADC: aromatic L-amino acid decarboxylase; AAV: adeno-associated virus; AAV1/2-A53T-aSyn: an adeno-associated virus 1/2-driven human mutated A53T α-synuclein overexpressing rat model; BDNF: brain derived neurotrophic factor; DBS: deep brain stimulation; GDNF: glial derived neurotrophic factor; PET: positron emission tomography; SNc: substantia nigra pars compacta; STN: subthalamic nucleus; TH: tyrosine hydroxylase; trkB: cognate tropomyosin receptor kinase type B receptor

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