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New Era in disease modification in Parkinson's disease: Review of genetically targeted therapeutics

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ABSTRACT

Disease modification remains a major unmet need in Parkinson's disease (PD) therapeutics. Despite multiple attempts, not a single study has yet been successful, perhaps due to our incomplete understanding of the underlying disease mechanisms. Genetic and epidemiologic studies of the last decade have substantially increased our comprehension of the etiology of PD. Once considered a pure sporadic disease, the discovery of familial mutations provided the initial paradigm shift and it is now widely accepted that PD has a substantial genetic component. These genetic discoveries have allowed the development of novel therapeutics aimed at halting or slowing the underlying disease process, rather than just ameliorating symptoms. Here, we discuss the latest advances in therapeutics based on three genetic discoveries (*SNCA, LRRK2 and GBA*) that are currently reaching the clinical arena and outline the challenges of therapeutic development of genetically targeted therapeutics.

1. Introduction

Disease modification remains a major unmet need in Parkinson's disease (PD) therapeutics. Despite multiple attempts, not a single Phase III study has been successful so far [1]. While there are multiple reasons for failure of the previously completed trials, one of the key reasons is our incomplete understanding of the disease biology. However, genetic and epidemiologic studies of the last decade have substantially increased our understanding of the etiology of PD. Once considered a pure sporadic disease, the discovery of familial mutations provided the initial paradigm shift and it is now widely accepted that PD has a substantial genetic component [2]. The α-synuclein (α-syn) gene (*SNCA*) was the first to be unequivocally associated to PD [3]. Since then, many other genes have been associated with PD, either as causative genes and/or increasing the risk for developing the disease [4]. These genetic discoveries have increased our understanding of the underlying disease pathogenesis and allowed for the development of novel therapeutics aimed at halting or slowing the underlying disease process, rather than just ameliorating symptoms. Here, we discuss the latest advances in therapeutics based on three genetic discoveries (*SNCA, LRRK2 and GBA*) that are currently reaching the clinical arena and outline the challenges of therapeutic development of genetically targeted therapeutics (Table 1).

2. Reduction of α-synuclein levels or transmission

The development of a-syn targeted therapeutics is founded on three major pillars: the discovery of SNCA mutations and duplications as causative of familial forms of PD [3]; the identification of aggregated α-syn as the major protein constituent of Lewy bodies and Lewy neurites [5]; and numerous experimental findings linking a-syn and neurodegeneration [6]. Numerous scientific studies have implicated α-syn as an important therapeutic target in PD. Examination of animal models of disease have demonstrated that simple overexpression of α-syn in neurons is associated with an increased risk for α-syn aggregation and neurodegeneration. The observation of Lewy bodies in grafted neurons in subjects with PD who underwent fetal transplants suggested host-to-graft α-syn propagation in a prion-like fashion [7-9]. This notion of prion-like propagation of α-syn pathology is also supported by the distinct spread of Lewy pathology in PD, which follows a pattern involving peripheral nerves and brain regions that are anatomically interconnected. The cell-to-cell transmission of aberrantly folded α-syn was later demonstrated by injecting fibrillar α-syn aggregates into animals, which triggers the misfolding of endogenous α-syn with the spreading of α-syn pathology throughout the brain and progressive loss of specific neurons depending on the initial site of injection [10]. These results validate the pivotal role of α-syn in the development of experimental neuropathology that mimics PD [11].

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Table 1
Disease modifying genetic-based targeted therapies in active PD clinical trials*.

Gene	Targeting mechanism	Mechanism of action	Drug	Therapeutic modality	Status	Target population (n)	Placebo	Primary Outcome	Enrolling Countries	ClinicalTrials.gov ID[a]	Sponsor
SNCA	Decrease α-synuclein aggregation	Inhibition of α-synuclein misfolding	NPT200-11	Small molecule	Phase I	HV (55)	Yes	Safety, Tolerability and PK/PD	USA	NCT02606682	Neuropore Therapies and UCB Pharma
		Reduction of α-synuclein aggregation	NPT088	Biologic	Phase I	AD (66) [b]	Yes	Safety	USA	NCT03008161	Proclara
	Increase α-synuclein degradation	Inhibition of c-Abl	Nilotinib	Small molecule	Phase II	Advanced PD (75)	Yes	Safety	USA	NCT02954978	Georgetown University
					Phase II	Early and moderate PD (135)	Yes	Incidence of TEAE	USA	NCT03205488	Northwestern University, MJFF, Cure Parkinson's Trust and Van Andel Institute
	Decrease extracellular α-synuclein	Passive immunization	RO7046015	Biologic	Phase II	Early PD (300)	Yes	Change in total MDS-UPDRS (I, II & III)	Austria, France, Germany, Spain, USA	NCT03100149	Prothena and Roche
			BIIB054	Biologic	Phase II	Early PD (311)	Yes	Safety, PK/PD	USA	NCT03318523	Biogen
			MEDI1341	Biologic	Phase I	HV (48)	Yes	Safety, Tolerability, PK/PD	United States	NCT03272165	AstraZeneca and Takeda
		Active immunization	PD01A, PD03A	Biologic	Phase I	Early PD (36)	Yes	Safety and Tolerability	Austria (completed)	NCT02267434 2014-000568-16	AFFITOPE
GBA	Increase in GCase	GCase activation	Ambroxol	Small molecule	Phase II	GBA-PD (10) PD (10)	No	Safety, Tolerability and PK/PD	UK	NCT02941822	UCL and Cure Parkinson's Trust
					Phase II	PDD (75)	Yes	Changes in ADAS-cog and CGIC	Canada	NCT02914366	Lawson Health Research Institute and Weston Foundation
			LTI291	Small molecule	Phase I	PD and GBA-PD	Yes	Safety, Tolerability and PK/PD	Netherlands	NTR6960[c]	LTI, Allergan
	Reduction of *GBA*-related GSLs	Glucosylceramide synthase inhibitor	Venglustat	Small molecule	Phase II	GBA-PD (243)	Yes	Change in MDS-UPDRS parts II & III	Austria, Canada, France, Germany, Israel, Italy, Japan, Norway, Portugal, Singapore, Spain, Sweden, Taiwan, UK, USA	NCT02906020	Sanofi
LRRK2	LRRK2 kinase inhibition	Kinase inhibitor	DNL201	Small molecule	Phase I	HV	Yes	Safety, Tolerability and PK/PD	USA	N/A[d]	Denali

GCase: glucocerebrosidase; GSLs: glycosphingolipids
HV: healthy volunteer; AD: Alzheimer's disease; PDD: PD dementia; GBA-PD; PK/PD: Pharmacokinetics, and Pharmacodynamics; TEAE: Treatment-Emergent Adverse Events; MDS-UPDRS: Movement Disorder Society-Unified Parkinson's Disease Rating Scale; ADAS-cog: Alzheimer's Disease Assessment Scale-cognitive subscale; CGIC: ADCS-Clinician's Global Impression of Change.

[a] From ClinicalTrials.gov unless noted otherwise. Accessed September 2018.
[b] Phase I in AD patients might support advancement into Phase 2/3 for PD.
[c] From http://www.trialregister.nl.
[d] LRRK2 program from: https://www.denalitherapeutics.com/press.

A multitude of α-syn-directed therapeutics is now entering the clinical arena. The majority of these efforts focus on reducing α-syn levels and/or its propensity to propagate. The observation that α-synuclein gene multiplications lead to familial α-synucleinopathies in humans is the premise for creating therapies that reduce α-syn levels. More recently, the finding of PD-associated polymorphisms adjacent to the *SNCA* locus that are hypothesized to increase neuronal α-syn levels provide additional support to expand α-syn-directed therapies to non-familial cases [12].

One active α-syn-targeting program is aimed at direct α-syn reduction through antisense oligonucleotides. This program is founded on the successful distribution of oligonucleotides in a phase III trial in spinal muscular atrophy [13] and the evidence of target engagement to decrease wild-type and mutant huntingtin in Huntington disease [14]. Another interesting approach to reduce α-syn expression is through β2-adrenergic agonists. This approach is based on a recent epidemiological and mechanistic report showing reduced PD risk in individuals using β2-adrenergic agonists for asthma and a novel epigenetic mechanism by which β2-adrenergic agonists reduce α-syn levels [15]. Importantly, a recent epidemiological study in United States Medicare beneficiaries including more than 48,000 incident PD cases and controls, failed to replicate the association between β2-adrenergic agonists or antagonists and PD risk [16]. These findings warrant further studies in additional population cohorts and in animal models of α-synucleinopathies.

Another fundamentally different therapeutic approach targeting α-syn is the use of passive or active immunization to reduce its extracellular levels. These approaches are founded on the notion that α-syn could act as a prion-like protein where intraneuronal α-syn aggregates are secreted into the extracellular space, taken up by neighboring neurons, seed aggregation of endogenous α-syn in the cells that they enter, and are transported between brain regions along axons [6]. The programs targeting pathogenic α-syn using antibodies postulate that capturing pathogenic α-syn in the extracellular compartment would reduce spread of pathology, thus slowing down or halting disease progression. There are now several clinical programs that use either active (immunizing patients with modified α-syn to generate endogenous protective antibodies) or passive (injecting antibodies targeting α-syn) immunotherapy approaches (ClinicalTrials.gov Identifiers: NCT03100149, NCT03318523, NCT03272165, NCT02267434). These molecules target different conformers of α-syn aggregates and have been carefully described elsewhere [17]. Of note, the first report of safety and target engagement using PRX002, a passive immunotherapy approach, in patients with PD was recently published [18]. Single and multiple doses of PRX002 were generally safe and well tolerated and resulted in robust reduction of free serum α-syn and dose-dependent increases of the antibody in cerebrospinal fluid (CSF), (0.3% relative to serum) [18].

Increasing the degradation of α-syn is another tactic to lower the toxic effects attributed to this protein. A major pathway for cellular α-syn degradation involves different forms of autophagy including lysosomal proteolysis. Numerous studies have demonstrated that disruption of autophagy by mutations or pharmacological inhibition can lead to accumulation and aggregation of α-syn. Conversely, genetic modifications and autophagy enhancers can decrease α-syn pathology. Recently, inhibitors of the tyrosine kinase c-Abl, initially developed as therapeutics in oncology, have attracted the attention of movement disorder researchers. Nilotinib, inhibitor of c-Abl, reportedly enhanced autophagy and reduced α-syn pathology in experimental models [19,20]. These studies prompted a small safety trial with Nilotinib in 12 patients with PD dementia and dementia with Lewy bodies (DLB) [21]. This trial was not designed or powered to detect efficacy, despite highly debated anecdotal reports suggesting that patients improved dramatically [22]. One of the major obstacles to development of nilotinib is its safety profile and question regarding sufficient central nervous system (CNS) penetration. Larger, randomized, double blind, placebo-controlled Phase IIa trials are now underway to evaluate safety, tolerability, clinical and biological activity of chronic administration of Nilotinib in PD (ClinicalTrials.gov Identifiers: NCT03205488, NCT02954978).

Reduction of α-syn aggregation ability is an alternative approach currently entering clinical testing. These programs are founded on the premise that aggregated α-syn is the pathological hallmark of synucleinopathies. The recent observation of abnormal α-syn seeding activity in patient CSF provides additional support to these approaches [23,24]. The small molecule NPT200-11 interferes with the formation of toxic α-syn oligomeric aggregates and has exhibited promise in animal studies. A small phase I safety clinical trial was recently completed (ClinicalTrials.gov Identifier: NCT02606682).

A common limitation of all α-syn-directed trials is the lack of biomarkers of α-syn related pathology for patient selection and stratification, target engagement and proof of biological activity. This is a research priority and several concerted efforts have been deployed to develop biomarkers of α-syn. These include positron emission tomography (PET) tracers of pathology, biochemical assays to evaluate α-syn in native, oligomeric and aggregated states in biofluids and tissues, assays for specific α-syn post-translational modifications, and tests to evaluate α-syn prion-like seeding activity [23-28]. Importantly, these assays need independent replication with blinded samples and analytical validation of the methods before widespread application. Development of such biomarkers will be essential for the ultimate advancement of the α-syn experimental therapies and successful translation.

The human testing of the α-syn-centric hypothesis for PD has been awaited with great anticipation and support. However, numerous challenges are foreseen, as our understanding of the underlying biological mechanisms of PD is incomplete, and the path to approval for disease-modifying therapies is still being charted. Abundant evidence from experimental animal models suggests that α-syn aggregates trigger neuronal dysfunction and death. However, it remains unclear which molecular species of α-syn is the appropriate target and which is the "toxic" species for this protein. In addition, the presence of α-syn pathology in most PD cases does not prove that Lewy pathology is *per se* toxic, there remains the possibility that these aggregates represent an innocuous epiphenomenon or perhaps a protective response whereby the cell sequesters toxic misfolded protein [29,30].

3. Reduction in LRRK2 levels or kinase activity

Leucine–rich repeat kinase 2 gene *(LRRK2)* mutations were initially described in a series of families with parkinsonism and diverse ethnic origin [31-33]. In addition to causing autosomal dominant PD, *LRRK2* mutations were also found to increase the risk of developing the disease [34]. Interestingly, genome-wide association studies implicated *LRRK2* as a major susceptibility gene in Crohn's disease, a chronic inflammatory bowel disease [35], which prompted the suggestion of a central role for LRRK2 in the regulation of chronic inflammatory responses in PD [36].

The LRRK2 protein is a large (2527 amino acids) kinase containing several conserved regions including an armadillo repeat (ARM) region, an ankyrin repeat (ANK) region, a leucine-rich repeat (LRR) domain, a kinase domain, a RAS domain, a GTPase domain, and a WD40 domain. LRRK2 interacts with many key proteins implicated in PD, suggesting that LRRK2 may be a central player in the pathways underlying disease pathogenesis [37]. The most frequent *LRRK2* mutations that segregate with familial PD map to its catalytic, GTPase, and kinase domains. Multiple lines of evidence suggest that LRRK2 regulates intracellular vesicular traffic and that mutant LRRK2-associated trafficking defects contribute to PD pathogenesis via increased kinase activity [37,38].

Accumulating evidence suggests that LRRK2 may play a role in the more common, sporadic form of the disease, independent of the *LRRK2* mutation status [39]. For example, genetic ablation of endogenous, wild-type *LRRK2* or pharmacological inhibition of its kinase activity protects the nigrostriatal system from neurodegeneration caused by the

toxin rotenone or viral-mediated α-syn overexpression [39,40]. Recently, a novel assay was developed to evaluate LRRK2's activation status at a cellular level by assessing LRRK2 Ser1292 phosphorylation or the dissociation of 14-3-3 proteins from LRRK2 as a surrogate. Using this novel assay, the authors reported a marked activation of LRRK2 in the substantia nigra from patients with sporadic PD compared to healthy controls [39]. Thus cumulative evidence indicates that wild-type, endogenous LRRK2 can be over-activated by oxidative or alternative noxious stimuli in idiopathic PD, and that this process might be exacerbated in the presence of *LRRK2* mutations. Hence, the beneficial effects of LRRK2 inhibition might extend beyond the *LRRK2*-mutation carriers with PD to patients with sporadic PD. Such approach will likely require selection of participants with higher levels of LRRK2 activity.

Several companies are pursuing LRRK2 inhibitors for PD, and highly potent, selective, and brain penetrant LRRK2 inhibitors are being evaluated preclinically [41]. To date, only one LRRK2 inhibitor has been studied in healthy volunteers. According to the company release, DNL201 was generally well tolerated with no serious adverse events at doses that achieved high levels of CSF exposure and direct target engagement as measured by two blood-based biomarkers of LRRK2 activity (https://www.denalitherapeutics.com/press).

The development of LRRK2 inhibitors faces some unique challenges that are being addressed. It is fairly well established that increased kinase activity by *LRRK2* mutations are responsible for some PD cases. However, it remains unclear whether elevated LRRK2 kinase activity in neurons (where it is expressed at low levels) or in immune cells is the key driver of PD pathogenesis [36]. This knowledge will inform the levels of brain penetrance required for potentially effective therapies. Secondly, it has been suggested that *LRRK2* mutations (leading to increased enzymatic activity) may have evolved for protection against opportunistic pathogenic infections [42]. Thus, current and future trials will need to carefully monitor the risk of opportunistic infections. In addition, a study describing the development of lung morphological changes after LRRK2 inhibition in nonhuman primates raised additional potential safety concerns [43]. These abnormalities were replicated at high doses using compounds with three different chemical scaffolds, strongly suggesting a LRRK2 “on-target” effect. Importantly, there were no deficits in measures of lung function at the highest doses and the morphological aberrations were reversible after a 2-week off dose period [44]. It will therefore be critical to understand the level of pathway inhibition required to achieve a therapeutic effect and avoid possible chronic toxic effects. The use of antisense oligonucleotides to reduce the total levels of LRRK2 represents an alternative strategy to directly reduce the LRRK2 activity in the CNS and bypass the peripheral adverse effects [45]. However, this approach will likely require direct CNS infusions, which might limit their usage.

4. Modulation of glucocerebrosidase activity and related glycosphingolipids

The initial observation of increased frequency of parkinsonism in family members of Gaucher patients suggested a role for *GBA* mutations in PD. This association was validated by a collaborative group that analyzed *GBA* mutations in a large cohort by sequencing the entire coding region [46]. Numerous genetic studies have substantiated the increased frequency of mutations in *GBA* in patients with PD and DLB [47]. Heterozygous carriers of *GBA* mutations have an increased frequency of PD; however, most *GBA* mutation carriers will not develop the disease, suggesting the presence of additional genetic modifiers [48].

The *GBA* gene encodes a lysosomal hydrolase, glucocerebrosidase (GCase) that hydrolyzes glucosylceramide into ceramide and glucose. Glucocerebrosidase deficiency by homozygous or compound heterozygous mutations causes Gaucher disease via the accumulation of undegraded substrates. Partial enzymatic deficiency, due to heterozygous *GBA* mutations increases the risk of developing PD and other synucleinopathies. Importantly, the presence of *GBA* mutations accelarates the disease course and conveys a higher risk for non-motor symptoms [49-51]. *GBA* mutation carriers exhibit an earlier and more rapid cognitive decline compared to non-carriers and the risk for dementia is strongly modulated by type of mutation and its residual enzymatic activity. Severe *GBA* mutations yielding significantly reduced residual enzymatic activity correlated with a more severe phenotype [50,51].

The main challenge for the development of *GBA*-associated therapeutics is the incomplete understanding of the underlying mechanisms governing the role/s of *GBA* mutations in the development of synucleinopathies. The inverse relationship between glucocerebrosidase activity and oligomeric α-syn led to the proposal of a pathogenic feedback loop [52,53] which is supported by a growing body of epidemiological, clinical, and basic science studies [54]. Decreased enzymatic activity by heterozygous *GBA* mutations can alter the membrane glycosphingolipid homeostasis and result in compromised cellular function, including vesicular transport, lysosomal/endosomal dysfunction, α-syn aggregation, and selective neuronal susceptibility [54-57] The postulated feedback loop allows for multiple avenues for therapeutic intervention.

The availability of successful therapies for Gaucher disease provided a springboard to accelerate the development of therapeutics for *GBA*-related PD. However, novel therapeutics had to be developed as the current approved therapies for Gaucher disease do not affect glycosphingolipid accumulation in the CNS. The leading hypothesis posits that *GBA*-mediated loss of function causes an abnormal glycosphingolipid environment, leading to cellular protein mishandling (proteinopathy) and neuronal dysfunction in synucleinopathies [54] Increasing glucocerebrosidase activity via gene therapy or small molecule chaperones as well as modulating the glycosphingolipid landscape via inhibitors of glucosylceramide synthase (GCS) have proven efficacious in correcting behavioral and pathological abnormalities in models of disease [58-60].

The initial support for rescuing brain GCase activity as a therapeutic strategy for *GBA*-associated synucleinopathies was obtained via gene therapy delivery of exogenous enzyme [61]. Due to the limited distribution of the gene-therapy approaches, the use of brain-penetrant small molecules was put forward. Two small molecular chaperones that increase GCase activity in the CNS are in early phase clinical testing for PD and related synucleinopathies. The mucolytic ambroxol approved by the European Medicines Agency (EMA) was reported to improve GCase lysosomal transport and reduce total and S129-phosphorylated α-syn levels in models of synucleinopathy [62]. In addition, an open-label study conducted to investigate the safety, tolerability, and efficacy of high-dose ambroxol in 5 patients with neuropathic Gaucher disease reported anecdotal improvements in neurological function accompanied by decreased lipid substrates in CSF [63]. These results have prompted the initiation of 2 clinical trials to evaluate the safety, tolerability, and efficacy of ambroxol in PD (ClinicalTrials.gov identifiers: NCT02941822 and NCT02914366).

Another approach is development of noninhibitory chaperones of GCase that bind to GCase away from the active site and induce a conformational change that increases its catalytic activity and/or extends its half-life. These noninhibitory chaperones reduced glycosphingolipid substrate accumulation, increased lysosomal activity, enhanced GCase translocation to lysosomes, and reversed α-syn accumulation in iPSC-derived dopaminergic neurons from patients with Gaucher disease and PD [64] [65]. Medicinal chemistry optimization of these chaperones have led to LTI-291 which is being developed as a potential treatment for *GBA*-associated PD and has initiated human clinical testing in the Netherlands (Nederlands Trial Register: NTR6960).

An alternative therapeutic strategy to reduce the glycosphingolipid buildup in Gaucher disease is to target glucosylceramide synthase (GCS), the enzyme that catalyzes the first step in the biosynthesis of glycosphingolipids. This approach is commonly referred to as substrate

reduction therapy (SRT). Antagonists of GCS can effectively treat the visceral and hematological manifestations of Gaucher disease; however, the current approved inhibitors do not achieve effective CNS concentrations [66]. SRT does not target the mutant enzyme, but prevents substrate accumulation. Treatment of cellular models of disease with GCS inhibitors can prevent the conversion of α-syn to toxic entities and restore physiological α-syn conformers [56,57]. A novel GCS inhibitor, Genz-667161, shows a good brain penetration profile, improved α-syn processing, and improved behavioral outcomes in animal models of *GBA*-related synucleinopathy and α-syn overexpression [59]. Clinical testing of venglustat, a highly potent, selective, and brain-penetrant analog of Genz-667161 has begun (ClinicalTrials.gov identifier: NCT02906020). This Phase 2 double-blind, placebo-controlled study aims to assess early efficacy and safety of venglustat in PD patients carrying a *GBA* mutation (ClinicalTrials.gov identifier: NCT02906020).

There are specific challenges to the development of *GBA*-targeting therapeutics. Importantly, it is unclear what level of brain GCase activation and/or lipid modulation would be required for an effective therapy. Along these lines, accumulation of glucosylceramide has not been observed in whole brains from GBA-PD patients. Instead, glycosphingolipid aberrations have been described in isolated neurons, which raises the possibility that the more numerous glia masks the neuronal lipid buildup when whole brains are analyzed [67,68]. There are several ongoing efforts to determine the lipid profiles in biospecimens of PD patients carrying wild-type and mutant *GBA* alleles. These biomarkers (or biomarker signatures) will help corroborate brain target engagement and, conceivably, aid in the identification of patient subgroups that might present a glycosphingolipid dysregulation despite carrying wild-type alleles thus potentially opening venues for *GBA*-associated therapeutics in patients with sporadic PD.

5. Challenges in translation of genetically targeted drug discoveries into clinical trials

While above discussed targets represent major advancements in our understanding of PD biology and have already entered the human trials, a number of challenges in translation remain [1]. Obviously the primary one is proof of principle in PD population. That proof will require confirmation that the targeted biology is indeed relevant to progression of PD in patients, which has not been a case with the previously completed studies despite solid underlying pathogenic rationale. However, there are challenges and limitations in the current status of the design and implementation of clinical trials testing putative disease modifying interventions to be highlighted.

Design of the clinical trials: there is no “go-to” standard design of the trials to prove disease modifying benefit of intervention in PD. One of the major challenges is separation of the disease modifying benefit of intervention (change in the course of the disease) from the symptomatic effect (treatment of the symptoms that does not impact the natural history of the disease). This challenge is specifically relevant for PD, compared to other neurodegenerative disease due to availability of a large armamentarium of highly effective symptomatic therapies (ST). Historically, trials have enrolled newly diagnosed untreated PD patients into disease modifying trials, to allow a “clean” read out not “contaminated” by the effect of concomitant ST [69]. However, on average 50% of the participants in such studies will initiate ST within the first year, thus reducing the observation period and ability to conduct longer studies which would be more meaningful to define long term effect of intervention [70,71]. Alternatively, studies would define the primary outcome by the ability of the intervention to delay time to initiation of ST, however such approach is subject to significant variability based on the investigator and participant preference. Alternative approaches included delayed start design and prolonged wash out though both approaches have their particular limitations [69]. Ideally an outcome measure should be independent of the impact of ST, be clinically meaningful and correlate with the progression of the underlying disease biology. Such outcome does not exist currently in PD. Traditionally, the majority of previously completed studies used change of the validated clinical scales, predominantly Unified Parkinson Disease Rating Scale (UPDRS) or more recently revised version, Movement Disorders Society (MDS- UPDRS) [72,73]over a period of one-two years to determine efficacy of intervention compared to placebo. While the scale has been validated, correlates with the progression of disability and has established data on the minimally significant change, it is intrinsically sensitive to the effect of ST. Once ST is initiated. UPDRS is completed in the defined medications OFF state (12 h after the last dose of ST) to allow wash out of the effect of ST [74]. However, it is well established that even levodopa has a long duration effect that exceeds its short half-life. The issue becomes even more challenging with long acting dopamine agonists and other classes of PD STs. Ultimately the only way to overcome this limitation is development of disease progression biomarkers. Such efforts are underway with the largest initiative lead by the Michael J Fox Foundation-sponsored Parkinson Progression Markers Initiative (PPMI) [75]. Development of digital outcome measures that would allow reliable observer independent ascertainment of function in real life is another important goal with multiple initiatives under way [76].

Recruitment of participants into genetically targeted therapeutic studies: recruitment of participants into clinical trials is number one challenge in timely completion of any clinical trial [77]. The challenge increases exponentially when the studies target a very narrow subset of participants like *GBA* and *LRRK2* mutation carriers. Prevalence of these genetic mutations in general PD population is estimated to be below 5% though with substantially higher numbers in selected ethnic groups and in familial cases [4]. If the studies follow the current paradigm of targeting newly diagnosed participants, which is most likely, the potentially eligible population will represent minute proportion of PD patients. In order to assure feasibility of testing these exciting targets, PD clinical community has to be prepared to launch wide base genetic testing of at least patients from ethnic background with higher prevalence of these genetic mutations. Such effort will require paradigm shift with education of the patient and physician community on the value of genetic testing and establishing an infrastructure for testing and genetic counseling. These initiatives will require substantial resources, collaboration and coordination of efforts between patient advocacy organizations, industry, and governments and will have to be done on the global multinational level. In order to be successful such programs have to be launched now to establish sufficient pools of potentially studies ready participants.

Because of the significant challenges of recruiting these rare populations, these therapeutic approaches potentially might be tested in larger PD populations irrespective of their genetic status. As mentioned above, accumulating evidence indicates that some of the underlying pathogenic mechanisms are implicated in non-genetic forms of the disease. These trials should incorporate alternative biomarkers (biochemical and/or imaging) to better define appropriate subpopulations and reduce the risk of diluting a beneficial effect in a larger non-responsive population. However, proof of principal will have to be determined in the targeted genetic population.

In conclusion, tremendous progress has been made in understanding of genetic contribution to PD pathogenesis that is rapidly translating into exciting therapeutic targets. Clinical trials methodology has to catch up with the rapid scientific discoveries.

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