Small Molecule Chaperones for the Treatment of Gaucher Disease and GBA1-Associated Parkinson Disease

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Parkinson disease, the second most common movement disorder, is a complex neurodegenerative disorder hallmarkled by the accumulation of alpha-synuclein, a neural-specific small protein associated with neuronal synapses. Mutations in the glucocerebrosidase gene (GBA1), implicated in the rare, autosomal recessive lysosomal disorder Gaucher disease, are the most common known genetic risk factor for Parkinson disease. Insights into the inverse relationship between glucocerebrosidase and alpha-synuclein have led to new therapeutic approaches for the treatment of Gaucher disease and GBA1-associated Parkinson disease. Unlike the current drugs used to treat Gaucher disease, which are highly expensive and do not cross the blood-brain-barrier, new small molecules therapies, including competitive and non-competitive chaperones that enhance glucocerebrosidase levels are being developed to overcome these limitations. Some of these include iminosugars, ambroxol, other competitive glucocerebrosidase inhibitors, and non-inhibitory chaperones or activators that do not compete for the active site. These drugs, which have been shown in different disease models to increase glucocerebrosidase activity, could have potential as a therapy for Gaucher disease and GBA1-associated Parkinson disease. Some have been demonstrated to reduce α-synuclein levels in pre-clinical studies using cell-based or animal models of GBA1-associated Parkinson disease, and may also have utility for idiopathic Parkinson disease.

Keywords: gaucher disease, Parkinson disease, lysosome, glucocerebrosidase, GBA1, small molecule chaperones

INTRODUCTION

Gaucher disease (GD), first described in 1882 by Dr. Phillipe Gaucher, is an inborn error of metabolism due to mutations in the gene GBA1, encoding the lysosomal enzyme glucocerebrosidase (GCase) (Sidransky, 2012). GCase, a lysosomal hydrolase, cleaves both glucosylceramide (GlcCer) to ceramide and glucose, and glucosylsphingosine (GlcSph) into sphingosine and glucose. This rare autosomal recessive inherited disorder occurs in approximately 1:40,000–60,000 live births in the general population, whereas among Ashkenazi Jews the frequency increases to around 1:850 (Horowitz et al., 1998). In patients with GD, “Gaucher cells,” macrophages engorged with lysosomal GlcCer, present in the spleen, liver, and bone marrow, lead to organomegaly and inflammation (Pastores et al., 2000; Beutler and Grabowski, 2001; Sidransky, 2012). Clinically, there are three types of GD. Type 1 or non-neuronopathic GD presents with organomegaly, bone manifestations, anemia, and thrombocytopenia (Pastores et al., 2000;
Acute neuronopathic GD2, the rarest form with devastating and progressive neurodegeneration including bulbar findings, seizures, and opisthotonos, results in death during infancy or early childhood (Beutler and Grabowski, 2001; Sidransky, 2004; Weiss et al., 2015). Chronic neuronopathic GD3 is characterized by a slower progression of neurological symptoms, with a later onset. These patients have extensive visceral and skeletal involvement, but also may exhibit myoclonic epilepsy, ataxia, impaired eye movements, developmental delay, or intellectual deterioration (Sidransky, 2004, 2012; Gupta et al., 2011; Sidransky and Lopez, 2012). There is considerable phenotypic variation within each type of GD, including rare patients who develop Parkinson disease (PD).

Parkinson disease, first described in 1817 by James Parkinson, is found in 2–3% of individuals over age 65 (Poewe et al., 2017). This disease is characterized by the loss of dopaminergic (DA) neurons in the substantia nigra, as well as by Lewy bodies containing alpha-synuclein (α-Syn) (Spillantini et al., 1997; Surmeier et al., 2017), which ultimately result in the classically associated symptoms, tremor, postural instability, bradykinesia, and rigidity (Poewe et al., 2017). Currently, mutations in GBA1 are one of the most common genetic risk factors for PD, as well as dementia with Lewy bodies (DLB). Mutations in GBA1 were found in 5–20% of sporadic PD cases with an estimated odds ratio of 5.4. In DLB the odds ratio was 8.3 (Sidransky et al., 2009; Nalls et al., 2013; Mullin et al., 2019). Patients with GBA1-associated PD (GBA1-PD) resemble those with sporadic PD but exhibit an earlier disease onset. The lysosome, the central player uniting GD and PD, is the main organelle responsible for α-Syn degradation (Sala et al., 2016). While the mechanism underlying the association between GD and PD still remains controversial, one hypothesis proposed posits that elevated substrate levels resulting from diminished GCase could stabilize soluble oligomeric α-Syn intermediates, enhancing oligomerization and accumulation of α-Syn within lysosomes (Yap et al., 2011; Taguchi et al., 2017). Increased levels of α-Syn in DA neurons could also inhibit the translocation of GCase from the endoplasmic reticulum (ER) to lysosomes which could lead to a gradual increase of the lipid substrates within the lysosome (Mazzulli et al., 2016a). This positive feedback loop with the combination of inhibited GCase trafficking and α-Syn accumulation may result in neurodegeneration (Balestrino and Schapira, 2018; Stojkovska et al., 2018). Furthermore, even patients with PD without GBA1 mutations have lower levels of GCase, suggesting that in idiopathic PD, reduced GCase activity may also contribute to disease progression (Gegg et al., 2012; Murphy et al., 2014).

Due to this apparent reciprocal association between GCase and α-Syn, attention is being directed toward designing therapeutics for GD with implications for PD. Currently, patients with type 1 GD are commonly treated with enzyme replacement therapy (ERT) (Jung et al., 2016) which alleviates the hematological, visceral, and sometimes skeletal manifestations, but does not cross the blood-brain-barrier, and thus does not impact neurological involvement or PD manifestations (Valayannopoulos, 2013). Substrate reduction therapy (SRT), a second approach for treating GD, focuses on upstream targets, ultimately inhibiting the accumulation of GlcCer (Lukina et al., 2019). Two SRT drugs approved by FDA have shown efficacy in type 1 GD (Schiffmann et al., 2008; Bennett and Turcotte, 2015) and some cross the blood-brain-barrier. However, the current SRTs have not been efficacious for neuronopathic GD (nGD) (Bennett and Turcotte, 2015; Mistry et al., 2018; Zimran et al., 2018) and there are several pharmacokinetic limitations (Henley et al., 2014).

Pharmacological chaperones, small molecules designed to bind to a specific target protein and assist in the folding of the protein, are also being developed as an alternative treatment approach for GD or GD-associated PD. They further function by stabilizing the enzyme to prevent misfolding and to enhance accurate translocation of the protein from the ER to the Golgi and lysosome (Compain et al., 2006; Lieberman et al., 2007). Since most mutations encountered in GD or GBA1-PD are missense mutations leading to misfolded enzyme, pharmacological chaperones which cross the blood-brain-barrier provide an attractive therapeutic strategy. Here we review studies of chaperones as a treatment for GD and GBA1-PD, focusing on preclinical developments.

**IMINOSUGARS**

The first chaperone considered for GCase was an iminosugar, N-(α-nonyl) deoxynojirimycin (NN-DNJ). Sawkar et al. (2002) reported that the addition of NN-DNJ to fibroblast culture medium increased the activity of GCase in N370S mutant and wild-type but not L444P mutant cells. However, NN-DNJ also inhibits α-glucosidase and GlcCer synthase, rendering it difficult to use as a target-specific drug. Another iminosugar, α-1-C-Nonyl-DIX that specifically inhibits GCase, but not α-glucosidase, doubled the residual cellular activity of GCase in N370S/N370S fibroblasts (Compain et al., 2006). Bicyclic nojirimycin (NJ) analogs with a sp2-iminosugar structure were found to be stronger chaperones as well as selective and competitive inhibitors of lysosomal GCase (Luan et al., 2009, 2010; Tiscornia et al., 2013). Some of the new sp2-iminosugar derivatives inhibited enzyme activity by 10-fold at pH 5 (Mena-Barragan et al., 2016).

Another type of iminosugar, isofagomine (IFG), was reported to bind to the GCase active site, increase GCase activity in cell lysates and restore lysosomal trafficking of GCase in N370S cells (Lieberman et al., 2007). Incubation of GD patient-derived lymphoblastoid or fibroblast lines with IFG increased GCase activity 3.5- and 1.3-fold and reduced endogenous GlcCer levels (Khanna et al., 2010). Testing the effects of IFG on GCase activity in a complex nGD mouse model, 4L.C* (V394L/V394L + saposin C−/−) showed that IFG administration extended lifespan and increased GCase activity and protein levels in the brain and visceral tissue, with attenuation of proinflammatory responses (Sun et al., 2011).
Further, in vivo tests of IFG in homozygous V394L, D409H, and D409V mice, which are nGD models demonstrated increased GCase activity in visceral tissues and brain extracts (Sun et al., 2012). Whether the effect of IFG on wild-type GCase could be beneficial in synucleinopathies was evaluated using mice overexpressing human wild-type α-Syn (Thy1-α-Syn). Treating with IFG (AT2101) orally for 4 months improved motor function, diminished microglial inflammatory response in the substantia nigra, reduced α-Syn immunoreactivity in nigral DA neurons, and reduced small α-Syn aggregates (Richter et al., 2016). Another study in 4L:C7 mice reported that IFG did not alter the GlcCer and Gsph accumulation, but attenuated disease progression and altered global expression profiles of brain mRNA and miRNAs (Dasgupta et al., 2015). Treating Drosophila manipulated to express human wild-type, N370S and L444P GBA1, with IFG resulted in decreased ER stress and preserved motor function, suggesting that IFG might have potential as a PD therapy (Sanchez-Martinez et al., 2016).

De La Mata et al. reported that a distinct iminosugar, NAdBT-AIJ restored mitochondrial dysfunction and GCase activity in L444P mice in combination with coenzyme Q10 (De La Mata et al., 2015). Another pyrrolidine-based iminosugar, α-1-C-tridecyl-DAB (5j) was shown to function as a chaperone inhibitor of GCase enhancing enzyme activity at concentrations 10 times lower than IFG (Kato et al., 2016).

AMBROXOL

Ambroxol (ABX), a drug used to treat airway mucus hypersecretion and hyaline membrane disease in newborns, was found to be a pH-dependent, mixed-type inhibitor of GCase, and thus a potential therapy for GD. With its inhibitory activity maximal at neutral pH, ABX is found in the ER, and is undetectable at the acidic pH of lysosomes (Maegawa et al., 2009). GCase activity was enhanced in ABX-treated GD fibroblasts and lymphoblasts. Furthermore, modeling studies indicated that ABX interacts with both active site and non-active site residues of GCase (Maegawa et al., 2009). The tolerability and efficacy of ABX was evaluated in 12 patients with type 1 GD not receiving ERT. While only three patients who continued on ABX for a year had improved platelet counts and decreased organ volumes, the others remained stable, supporting the need for a larger clinical trial (Zimran et al., 2012). The chaperone activity and cytotoxicity of ABX was tested in vitro, demonstrating low cytotoxicity and significantly increased GCase activity in GD and GD+PD fibroblasts with different GD mutations, without any serious adverse effects (Bendikov Bar et al., 2013; Luan et al., 2013; McNeill et al., 2014). Another study in GBA1-mutant fibroblasts showed that ABX enhanced GCase activity by increasing Sap C and LIMP-2 protein levels (Ambrosi et al., 2015). Testing in vivo efficacy of 12 days of ABX in wild-type, L444P carrier and transgenic mice overexpressing human α-Syn demonstrated increased brain GCase activity and decreased total and phosphorylated α-Syn levels (Migdalska-Richards et al., 2016). In nonhuman primates, daily administration of ABX increased brain GCase activity, supporting clinical testing in humans (Migdalska-Richards et al., 2017a). In Drosophila melanogaster with a mutated GBA1b ortholog, treatment with ABX did not rescue GCase activity, but did ameliorate the unfolded protein response, inflammation and neuroinflammation, and enhance the life span (Cabasso et al., 2019). Several pilot clinical studies of ABX were performed in patients with nGD (Narita et al., 2016; Pawlinski et al., 2018; Kim et al., 2019). One study in five patients with GD3 showed that high-dose oral ABX had good safety and tolerability, significantly increased lymphocyte GCase activity, permeated the blood–brain barrier, and decreased Glcph levels in the cerebrospinal fluid. The investigators reported that myoclonus, seizures, and pupillary light reflex dysfunction improved, leading to the recovery of gross motor function in two patients (Narita et al., 2016). Another study of the long-term safety and efficacy of combined high-dose ABX up to 21 mg/kg/day) and ERT in GD3 showed that during the first 2 years seizure frequency and neurocognitive function worsened, but after the ABX dosage was increased to 27 mg/kg/day, seizure frequency markedly decreased from the baseline, neurocognitive function improved and the drug was tolerated without severe adverse events (Kim et al., 2019). However, these are primarily antidotal reports, and a double-blind placebo-control study is needed. ABX is currently being tested under a single-center phase II clinical trial in 75 subjects with mild to moderate PD by randomizing participants into ABX high-dose (1,050 mg/day), low-dose (525 mg/day), or placebo group (Silveira et al., 2019), and reasonable cerebrospinal fluid levels were attained (Mullin et al., 2020).

NON-INHIBITORY CHAPERONES

A major limitation of inhibitory chaperones is that the chaperone activity must be balanced against the functional inhibition of GCase. In contrast, non-inhibitory chaperones can assist in the folding of mutant GCase in the ER and the translocation to lysosomes without interfering with the active site of the enzyme, and thus can restore enzyme activity in the lysosome. Several non-inhibitory compounds were identified by a high throughput screening (HTS) assay using an extract of spleen from a patient with GD as the source of mutant enzyme (Goldin et al., 2012). In this screen, saposin C and other potential cofactors were present in the extract, likely enhancing the detection of non-inhibitory chaperones. The activities of the compounds were confirmed in subsequent cell-based assays using patient-derived fibroblasts. The screen yielded novel pyrazolopyrimidine-based non-inhibitory pharmacological chaperones (Patnaik et al., 2012). Aflaki et al. (2014) assessed the efficacy of one non-inhibitory chaperone identified by HTS as a drug candidate for GD or GBA1-PD. Since fibroblasts do not store GlcCer, monocyte-derived macrophages from 20 patients with GD and GD iPSC-differentiated macrophages were examined. The non-inhibitory chaperone, NCGC717, enhanced GCase activity, reduced glycolipid storage, and normalized chemotaxis and the production of reactive oxygen species in...
TABLE 1 | Small molecules considered as therapeutics for GD and GBA1-associated PD.

| Name of small molecules | Disease modeled | GBA1 mutations tested | Model organisms | Effect of small molecules | References |
|-------------------------|-----------------|-----------------------|-----------------|--------------------------|------------|
| **Inhibitory chaperones** |                 |                       |                 |                          |            |
| NN-DNJ                  | GD1             | N370S                 | Fibroblasts     | Increased GCase activity | Sawkar et al., 2002 |
| α-1-C-Nonyl-DIX         | GD1             | N370S                 | Fibroblasts     | Increased GCase activity | Compain et al., 2006 |
| sp2-iminosugar          | nGD             | F213I, N370S          | DA neurons from iPSCs | Increased GCase activity | Luan et al., 2009 |
| Isofagomine             | GD1             | N370S                 | Fibroblasts     | Increased GCase activity | Lieberman et al., 2007 |
| nGD                    | L444P           | Fibroblasts and lymphoblasts | Increased GCase activity | Reduced GlcCer levels | Khanna et al., 2010 |
| nGD                    | V394L/V394L + saposin C−/− | Mouse | Increased GCase activity in relevant tissues | Khanna et al., 2010 |
| PD                     | V394L, D409H, and D409V | Mouse | Increased GCase activity in relevant tissues | Sun et al., 2012 |
| nGD                    | V394L/V394L + saposin C−/− | Mouse | Improved motor function | Richter et al., 2014 |
| GBA1-PD                | N370S, L444P    | Drosophila            | Decreased ERS stress | Sanchez-Martinez et al., 2016 |
| NAdBT-AIJ + coQ         | nGD             | L444P                 | Mouse           | Restored mitochondrial function | De La Mata et al., 2015 |
| α-1-C-tridecyl-DAB      | GD1             | N370S                 | Fibroblasts     | Increased GCase activity | Kato et al., 2016 |
| Ambroxol               | GD1             | N370S, F213I          | Fibroblasts and lymphoblasts | Increased GCase activity and protein levels | Maegawa et al., 2009 |
| GD1,2&3                | N370S, F213I, N188S, G193W, R120W | Fibroblasts | Increased GCase activity | Luan et al., 2013 |
| GD1,2&3                | N370S, V394L, R120W, R415R, R131C | Fibroblasts | Increased GCase activity | Bendikov Bar et al., 2013 |

(Continued)
| Name of small molecules | Disease modeled | GBA1 mutations tested | Model organisms | Effect of small molecules | References |
|-------------------------|-----------------|----------------------|-----------------|--------------------------|------------|
| GD1 and GBA1-PD         | PD              | N370S, etc.          | Fibroblasts     | Increased GCase activity and protein levels | McNeill et al., 2014 |
| GBA1-PD and non-GBA1-PD | PD              | N370S, L444P         | Fibroblasts, drosophila | Increased GCase activity | Ambrosi et al., 2015 |
|                         | L444P, α-Syn overexpression | Mouse | Increased GCase activity in brains | Migdalska-Richards et al., 2016 |
| PD                      | wildtype        | Non-human primate    | Drosophila      | Increased GCase activity in brains | Migdalska-Richards et al., 2017a |
| nGD                     | C-terminal 133aa deletion in GBAb | Drosophila | Reduced unfolded protein response | Cabasso et al., 2019 |

**Non-inhibitory Chaperones**

| Name of small molecules | Disease modeled | GBA1 mutations tested | Model organisms | Effect of small molecules | References |
|-------------------------|-----------------|----------------------|-----------------|--------------------------|------------|
| NCGC758                 | GD1             | N370S, L444P, c.84dupG, IVS2+1 | Macrophages from iPSCs | Increased GCase activity | Aflaki et al., 2014 |
|                         | GD2             | N370S, L444P, c.84dupG, IVS2+1 | Macrophages from iPSCs | Reduced GlcCer levels | Aflaki et al., 2014 |
|                         |                 |                      |                 | Recovered ROS*** production | Aflaki et al., 2014 |
|                         |                 |                      |                 | Improved chemotaxis | Aflaki et al., 2014 |
| NCGC758                 | GD1             | N370S                 | Macrophages from iPSCs | Induced autophagy and Reduced IL-1β secretion | Aflaki et al., 2016a |
|                         | nGD             | N370S, c.84dupG       | DA neurons from iPSCs | Increased GCase activity | Mazzulli et al., 2016b |
|                         |                 |                      |                 | Reduced α-Syn levels | Mazzulli et al., 2016b |
|                         |                 |                      |                 | Improved lysosomal function | Mazzulli et al., 2016b |
| NCGC607                 | GD, GD with parkinsonism | N370S, c.84dupG, IVS2+1, L444P | DA neurons from iPSCs | Increased GCase activity | Aflaki et al., 2016a |
|                         |                 |                      |                 | Reduced GlcCer and GlcSph levels | Aflaki et al., 2016a |
|                         |                 |                      |                 | Reduced α-Syn levels | Aflaki et al., 2016a |

**Others**

| Name of small molecules | Disease modeled | GBA1 mutations tested | Model organisms | Effect of small molecules | References |
|-------------------------|-----------------|----------------------|-----------------|--------------------------|------------|
| LB-250                  | GD              | N370S, L444P         | Fibroblasts     | Inhibit histone deacetylase activity | Lu et al., 2011 |
|                         |                 |                      |                 | Increased GCase activity and protein levels | Lu et al., 2011 |
| S-181                   | GBA1-PD and non-GBA1-PD | c.84dupG, wildtype | DA neurons from iPSCs | Increased GCase activity | Burbulla et al., 2019 |
|                         |                 |                      |                 | Reduced GlcCer levels | Burbulla et al., 2019 |
|                         |                 |                      |                 | Reduced the oxidized dopamine and α-Syn | Burbulla et al., 2019 |
| S-181                   | GBA1-PD         | D409V                | Mouse           | Reduced GlcCer and GlcSph levels in brain | Burbulla et al., 2019 |
|                         |                 |                      |                 | Reduced α-Syn levels in brain | Burbulla et al., 2019 |

*Differentially expressed brain mRNAs. **Endoplasmic reticulum. ***Reactive oxygen species.
the macrophages. NCGC758 also reversed inflammatory defects in GD macrophages by inducing autophagy and reducing IL-1β secretion (Aflaki et al., 2016b). Another lead non-inhibitory small molecule, NCGC607, was tested in dopaminergic (DA) neurons differentiated from type 1 and 2 GD and GD+PD iPSCs (Aflaki et al., 2016a). NCGC607 restored GCase activity and protein levels, and reduced glycolipid storage in GD DA neurons, indicating its therapeutic potential for GD. In addition, NCGC607 reduced α-Syn levels in DA neurons from patients with parkinsonism, suggesting that NCGC607 or a derivative of this lead compound may have efficacy as a treatment for PD (Aflaki et al., 2016a). NCGC758 also reduced α-Syn levels and improved lysosomal function in iPSC-derived DA neurons differentiated from patients with GD and PD (Mazzulli et al., 2016b).

**OTHER SMALL MOLECULES**

Histone deacetylase inhibitors (HDACi) have also been explored for the treatment of GD by modulating a GCase-associated ubiquitin–proteasome pathway (Lu et al., 2011). It was shown that a known HDACi (SAHA) and a unique small-molecule HDACi (LB-205) rescued GCase levels and increased enzymatic activity in fibroblasts derived from patients with GD1 and GD2. It was further shown that HDACi inhibits the deacetylation of heat shock protein (HSP90), resulting in impaired recognition of the mutant peptide by HSP90, thus protecting GCase from degradation (Yang et al., 2013).

Recently, S-181, a new small-molecule capable of stabilizing wild-type GCase was developed. S-181 increased GCase activity in iPSC-derived DA neurons from patients with idiopathic PD, as well as in patients with PD carrying GBA1 mutation c.84dupG and mutations in other PD genes (Burbulla et al., 2019). S-181 treatment in these DA neurons reversed pathogenic phenotypes including the reduced accumulation of oxidized dopamine. It was also shown in Table 1 that treating wild-type and heterozygous D409V mice with S-181 increased GCase activity in both, resulting in reduction of the lipid substrates and α-Syn in brain (Burbulla et al., 2019).

**DISCUSSION**

There are several FDA-approved ERT (Cerezyme, etc.) and SRT (Zaversca, etc.) drugs to treat GD. However, ERT does not have utility for GBA1-PD since it does not cross the blood-brain-barrier and since substrate accumulation in GBA1-PD is minimal, it is unlikely that SRT will be efficacious (Sidransky et al., 2019). Small molecule pharmacological chaperones have been developed to overcome these limitations. The first iminosugar developed, NN-DNJ, increased GCase activity in cells from patients with GD (Sawkar et al., 2002) by strong binding to GCase (Thirumal Kumar et al., 2019). However, NN-DNJ only increases enzymatic activity in lines with specific GBA1 mutations (Sawkar et al., 2002; Thirumal Kumar et al., 2019) and also inhibits GlcCer synthase activity in a dose-dependent manner (Compain et al., 2006). Isofagomine (IFG) appears to have more target-specific inhibition and broader pre-clinical efficacy in both in vitro and in vivo models of different types of GD and PD. However, like other inhibitory small molecule chaperones, IFG also causes dosage-dependent inhibition of GCase, limiting its clinical utility. A phase II clinical trial of IFG failed to improve clinical symptoms in patients with GD, likely due to dosing challenges. Ambroxol (ABX) has been evaluated as an inhibitory chaperone with fewer side effects and better efficacy, because its inhibitory effect does not occur at the low pH present in lysosomes. A recent comparative molecular docking analysis showed that ABX has more broad binding affinity toward GCase than NN-DNJ and other SRT drugs (Thirumal Kumar et al., 2019). A phase II clinical study of ABX for PD is currently underway (Silveira et al., 2019).

Non-inhibitory chaperones are highly attractive candidate drugs for the treatment of GD or GBA1-PD because they avoid the primary problem associated with inhibitory chaperones, the inhibitory competition with substrates of GCase in the lysosome. A few non-inhibitory chaperones identified by HTS were shown to have disease-reversing effects in cellular models of GD or GBA1-PD (Aflaki et al., 2014, 2016a). However, there are also difficulties in developing non-inhibitory chaperones. The binding of non-inhibitory chaperones to sites other than the active site makes it difficult to perform structure-guided optimization of drug efficacy and challenging to evaluate the potential activity of candidate molecules from HTS. To resolve this problem, visualizing the activity of endogenous levels of GCase in live cells is required. This might be achieved through the development of a fluorescence-based substrate probe representing GCase activity in lysosomes of live cells (Jung et al., 2016).

To increase the likelihood of success in clinical trials, a properly designed pre-clinical drug testing strategy is essential. Thus, appropriate in vitro and in vivo disease models which mimic key features of the disease are necessary. DA neurons differentiated from patient iPSCs accumulate α-Syn and oxidized dopamine, and thus can be used to test the efficacy of small molecules for PD (Aflaki et al., 2016a; Burbulla et al., 2019). However, mouse models accurately replicating PD phenotypes are still needed. A previous study showed that mice carrying the L444P mutation had increased stability of over-expressed human wild-type and A53T mutant α-Syn (Fishbein et al., 2014). However, the L444P mice did not demonstrate accumulation of α-Syn and there was no PD phenotypes in the L444P carrier mice (Migdalska-Richards et al., 2017b). While heterozygous L444P mice overexpressing mutant α-Syn had exacerbated motor deficits (Fishbein et al., 2014) and enhanced dopaminergic neurodegeneration (Migdalska-Richards et al., 2017b), it is not clear whether this phenotype can be used to establish drug efficacy, especially as in patients, the α-Syn levels are not as high, and the protein is not mutated. To identify and optimize candidate non-inhibitory chaperones, new methods for evaluating GCase activity in live cells and more appropriate GBA1-PD animal models need to be developed. Three dimensional in-vitro cell culture systems can be also considered for drug screening and preclinical validation of candidate drugs, because they more
closely resembles the in-vivo cell environment than routine in-vitro cell culture (Langhans, 2018).

**AUTHOR CONTRIBUTIONS**

T-UH contributed to the conceptual idea, reviewed, analyzed the literature, and wrote the manuscript and table. RS assisted in compiling background material and in writing the manuscript. ES provided the conceptual idea, reviewed, and edited the manuscript.

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Funding

This work was supported by the Intramural Research Programs of the National Human Genome Research Institute and the National Institutes of Health.

**FUNDING**

This work was supported by the Intramural Research Programs of the National Human Genome Research Institute and the National Institutes of Health.
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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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