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Gaucher Disease: Progress and Ongoing Challenges

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Abstract

Over the past decades, tremendous progress has been made in the field of Gaucher disease, the inherited deficiency of the lysosomal enzyme glucocerebrosidase. Many of the colossal achievements took place during the course of the sixty-year tenure of Dr. Roscoe Brady at the National Institutes of Health. These include the recognition of the enzymatic defect involved, the isolation and characterization of the protein, the localization and characterization of the gene and its nearby pseudogene, as well as the identification of the first mutant alleles in patients. The first treatment for Gaucher disease, enzyme replacement therapy, was conceived of, developed and tested at the Clinical Center of the National Institutes of Health. Advances including recombinant production of the enzyme, the development of mouse models, pioneering gene therapy experiments, high throughput screens of small molecules and the generation of induced pluripotent stem cell models have all helped to catapult research in Gaucher disease into the twenty-first century. The appreciation that mutations in the glucocerebrosidase gene are an important risk factor for parkinsonism further expands the impact of this work. However, major challenges still remain, some of which are described here, that will provide opportunities, excitement and discovery for the next generations of Gaucher investigators.

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Keywords

Gaucher disease; glucocerebrosidase; lysosomal storage disorder; Enzyme Replacement Therapy; Dr. Roscoe Brady; parkinsonism

1 Introduction

With the recent passing of Roscoe Brady, an era has ended that laid the foundation for the contemporary field of lysosomal biology in health and disease. This is particularly true in the case of Gaucher disease (GD) where in the past decades much progress was achieved by Dr. Brady and other scientists at the National Institutes of Health (NIH) and around the world. It is appropriate to honor his lasting contributions to science and medicine through evoking the enduring lessons that his life's work has taught us. This chapter will discuss the major developments made in Gaucher disease research over the past century, focusing on the many contributions of the NIH (Table 1) and other research centers. It will highlight the development of therapies for this disorder, as well as research advancing our knowledge of disease pathogenesis and the role of the lysosome in neurodegeneration. Lastly, we will identify ongoing challenges in the field of Gaucher disease that merit increased attention from the research community in the coming years.

2. Progress in the field of Gaucher disease

2.1 The Historical Context

One hundred and thirty five years ago, Gaucher disease was first described by a French medical student Philippe Charles-Ernest Gaucher in his thesis entitled “De L'epithelioma de la Rate” [1]. In this work, Gaucher described the presence of unusual appearing cells in the spleen of a 34-year-old woman who presented with splenomegaly. The identification of similar patients with the same pathological findings in subsequent years lead to the disorder being referred to as “Gaucher disease” and the abnormal cells became known as “Gaucher cells”. It was only in 1901 that Brill [2] appreciated that Gaucher disease was an inherited disorder. Neuronopathic Gaucher disease was first recognized in 1927 [3]. However, the biochemical basis for the disorder described by Gaucher was not identified until 1934, 50 years after Gaucher's original description, when Aghion, also in Paris, determined that the distorted cells, sketched in the publication by Gaucher, resulted from the accumulation of the lipid glucocerebroside [4].

2.2 Dr. Brady Enters the Field

The reason for this lipid accumulation still remained a mystery until a young physician scientist, Dr. Roscoe Brady entered the scene. Dr. Brady had already committed to this career path during his postdoctoral fellowship, where he acquired laboratory skills at the University of Pennsylvania in the Department of Physiological Chemistry under Dr. Samuel Gurin, a pioneer in the study of lipid metabolism. Dr. Gurin had just arranged to receive radioactive carbon 14 from the Manhattan Project, the research and development project that developed the atomic bomb, and Dr. Brady began his work studying the metabolism of long chain fatty acids, lipids, and sterols using radiolabeled precursors. His early scientific work,

published in a series of elegant papers, helped form the basis of the biochemistry of fatty acid and cholesterol metabolism. For an investigator interested in studying lipid metabolism, the NIH provided a unique opportunity for expanding his research goals, where under Dr. Donald Fredrickson's leadership, a new field of 'lipidology' was taking shape. Instead of joining the efforts of these pioneering NIH investigators focused on disorders of cholesterol, triglyceride and lipoprotein metabolism, Dr. Brady chose to put to use his newly acquired skills with radioisotope tracer studies to investigate more challenging "familial lipodystrophic conditions" such as Gaucher, Niemann-Pick and Tay-Sachs disease characterized by intracellular accumulation of sphingolipids.

In 1956, noting that the anabolism of glucocerebroside was intact in spleen samples of patients with Gaucher disease [5], Dr. Brady turned his attention to catabolism of the lipid. The use of ^{14}C labeled glucocerebroside, which he synthesized together with Dr. Shapiro at the Weizmann Institute in Israel, was key in establishing the deficient enzyme responsible for the disease [6]. In 1965, Dr. Brady and coworkers in the United States [7] and subsequently Dr. Patrick in the United Kingdom [8] both clearly established that the metabolic effect in Gaucher disease was the inherited deficiency of the enzyme glucocerebrosidase (GCCase). This colossal finding started the NIH team on a quest to develop an effective treatment for this disorder. Immediately, Dr. Brady predicted that Gaucher disease could be amenable to treatment by administration of exogenous enzyme, and he proposed several approaches to target the therapeutic enzyme to the reticuloendothelial system [9]. This extraordinary pace of discoveries and conceptual advances in a space of a few years underscored the power of traditional isotope tracer studies and in fact, is reminiscent of the rate of discoveries in the genomic era [10].

It had long been appreciated that Gaucher disease was a diverse disorder, and the specific phenotypes associated with type 2GD [11] and type 3GD [12] had been noted. Now, deficient glucocerebrosidase was found to be responsible for all three types of Gaucher disease. Uncovering the deficient enzyme also enabled the Brady group to develop reliable diagnostic tests for Gaucher disease [13] using washed concentrated white blood cells, a method that is still in use today, albeit with fluorescent forms of the substrate and more modern technology. In addition, it also later led to prenatal diagnosis for this disease [14]. In 1968, Weinreb *et.al.* discovered that in rat liver, glucocerebrosidase, as well as other sphingolipid hydrolases, were localized to ultracentrifuge fractions identified with lysosomes [15]. As a result, these diseases became known as the lysosomal storage disorders.

2.3 Advances in Protein Chemistry

In the following decades, research at the National Institutes of Health performed by Dr. Brady and other investigators significantly impacted many different aspects of our understanding and treatment of Gaucher disease. Dr. Brady's mammoth contribution was the development and implementation of the first successful therapy for Gaucher disease, which later translated into enzyme replacement therapies for other lysosomal storage disorders. However, during the long and arduous years while the treatment was being developed, optimized and tested, other centennial contributions to this field continued to accumulate.

The road to successful enzyme replacement therapy was particularly challenging, and in order to move forward it was necessary to devise and develop new tools, assays and reagents to produce, assess, and measure parameters to follow. It quickly became clear that large quantities of purified enzyme were required. Achieving such quantities was problematic, as this was long before the era of recombinant production of proteins. The strong hydrophobicity of this membrane-associated enzyme necessitated arduous purification procedures. Eventually, Pentchev *et.al.* [16] developed a method of purifying glucocerebrosidase from placenta using affinity column chromatography, enabling the production of sufficient quantities to begin. Moreover, isolation of this purified enzyme facilitated its characterization [17], and allowed the production of the first antibodies to the protein, allowing scientists to determine the protein structure and location of carbohydrate chains.

It soon became clear that there were different isoenzymes of glucocerebrosidase. Isoelectric focusing of patient and control white blood cell samples indicated that the demonstrated differences in isozyme patterns from patients correlated somewhat with different types of Gaucher disease [18]. This finding was the first indication that different mutant alleles contributed to disease pathogenesis and phenotypic expression [19].

While the struggle to produce adequate quantities of purified protein for infusions was underway, investigators at NIH and Harvard Medical School performed the first bone marrow transplant on a critically ill child with type 3 GD in 1984 [20]. Although engraftment of the marrow was successful and some facets of the child's disease were reversed, he ultimately succumbed to sepsis thirteen months post-transplant. However, this "proof of concept" intervention later led to the consideration of alternative means to correct the bone marrow function using gene therapy strategies.

Advances in purification procedure translated into more availability of placental enzyme for evaluation and testing. The active placental enzyme was found to have a molecular mass of 67kd. The protein sequence and glycosylation sites were determined initially by direct enzymatic and chemical cleavage with subsequent amino acid sequencing [21]. Glucocerebrosidase is a membrane associated protein that is made up of 497 amino acids, with five glycosylation sites [22]. The location of the active site was eventually confirmed to be located at amino acid D443 in 1994 [23].

2.4 Molecular Biology: the Glucocerebrosidase Gene and Genotype-Phenotype Studies

While the protein sequence was being elucidated, newly available techniques in molecular biology enabled the characterization of the glucocerebrosidase gene (*GBA1*). The gene was localized to chromosome 1q21 by in-situ hybridization analysis [24, 25]. The *GBA1* cDNA served as a probe to identify and isolate clones from controls and patients. The gene was found to encompass 11 exons spanning around 7000 base pairs. Almost immediately, it was recognized that a highly homologous pseudogene was present in close proximity to *GBA1*. The elucidation of the full sequence of *GBA1* ultimately enabled the production of recombinant protein for therapeutic use [26].

The first mutation in the glucocerebrosidase gene identified was a C to T substitution in exon 10, resulting in the replacement of a proline for leucine at amino acid position 444 [L483P] [27]. Identification of the common N370S [N409S] mutation was later found in a patient with type 1 Gaucher disease [28]. To date more than 300 different *GBA1* mutations have been described [29]. The mutation nomenclature is at times confusing, as the numbering of the affected amino acid was eventually changed to include the 39 amino acid leader sequence [shown in brackets].

The ability to identify common *GBA1* mutations led investigators to explore the correlation between genotype and phenotype in this disorder. The first two identified mutations were reported as “type 2” and “type 1” mutations, respectively. However, as knowledge continued to accrue, it became clear that this was likely an oversimplification. Mutation L444P [L483P] was seen in patients with all three Gaucher disease types. Furthermore, patients sharing the same mutations, even siblings or twins, could have different phenotypes, complications and responses to therapy. Mutation L444P at times was encountered as an isolated point mutation, and in other instances was part of a recombinant allele resulting from recombination with the nearby and homologous pseudogene sequence [30, 31]. The N370S mutation is generally considered to be exclusively associated with non-neuronopathic forms of GD, and homozygosity for the mutation is often predictive of milder disease features and is frequent in asymptomatic individuals [32]. However, other efforts to correlate genotype with specific phenotype features have been incomplete, and suggest the existence of additional genetic modifiers [30, 33]. Some mutant *GBA1* alleles are common, while others are private, and they include point mutations, insertions, deletions, splice-site alterations and mutations that result from recombination with the nearby pseudogene.

2.5 Enzyme Replacement Therapy – From Concept to Treatment

The actual development of glucocerebrosidase as a therapy for patients took over two decades [10]. Dr. Brady was determined to find a human source of the enzyme to minimize sensitization and ultimately focused on human placental extracts. However, this required rigorous purification, which was accomplished in 1973 [16]. Two patients were infused with this material; both infusions were well tolerated without safety issues. Each patient underwent a pre-treatment liver biopsy for measurement of glucocerebroside concentration, which served as a pharmacodynamic biomarker. A second biopsy performed two days after the infusion demonstrated a 26% reduction in glucocerebroside in both patient samples [34]. These important observations set the course for subsequent investigations that ultimately culminated in demonstration of the clinical effectiveness of enzyme replacement therapy. Efforts were undertaken initially to scale up the purification procedure for the native placental enzyme so that larger quantities of material would be available for clinical study. However, enzyme purified by the new procedure produced inconsistent reductions in hepatic glucocerebroside, which was attributed to variable enzyme uptake by hepatocytes versus the desired target in macrophages. Since native placental glucocerebrosidase is a glycoprotein, modification of the carbohydrate structure of the purified enzyme was attempted in order to more selectively target it to macrophages. This was accomplished by the sequential enzymatic removal of *N*-acetylneuraminic acid, galactose and *N*-acetylglucosamine from the protein with exoglycosidases, yielding a mannose-terminal product [35]. With this

modification, a striking increase in delivery of glucocerebrosidase to hepatic macrophages was observed in laboratory models. Based on these findings, a clinical trial was initiated with intravenous infusion of the macrophage-targeted enzyme at a dose of 190 units weekly over a 6-month period in eight patients. Only the youngest and smallest subject, who received the highest dose per kilogram of body weight (10 U/kg), showed clinical benefit; his condition regressed when the infusions were discontinued [36]. Resumption of the infusions at a higher dose (30 U/kg body weight) resulted in correction of anemia, massive reduction in hepatosplenomegaly and clearance of storage cells from the bone marrow. This dramatic clinical response in a single child prompted the investigators to conduct a dose-response study in 23 Gaucher patients in order to more thoroughly define the pharmacodynamics of glucocerebrosidase clearance from the liver following a single 4-hour intravenous infusion of macrophage-targeted enzyme; a dose range of 0.6–234 U/kg of body weight was examined [10]. Enzyme doses above 30 U/kg consistently reduced hepatic glucocerebrosidase content. Ultimately, a dose of 60U/kg was chosen for a pivotal efficacy trial in twelve subjects who received biweekly 2-hour infusions for six months. All twelve patients showed striking clinical improvement in response to treatment, without safety concerns [37]. Macrophage-targeted glucocerebrosidase, alglucerase, was approved under the brand name of Ceredase (Genzyme, Cambridge MA) by the FDA for the treatment of non-neurological forms of Gaucher disease in April 1991. Twenty-six years had passed since the initial description of the enzyme deficiency characteristic of the disorder.

2.6 Neuronopathic Gaucher disease

Over the years, Dr. Brady and his group made many contributions to the diagnosis and treatment of patients with neuronopathic Gaucher disease. Efforts to distinguish neuronopathic Gaucher from non-neuronopathic phenotype initially centered on the higher molecular weight of glucocerebrosidase in the former, which combined with low residual activity suggested that the neuronopathic form involves the active site of the enzyme [19]. It was recognized however, that it is the phenotype alone that distinguishes the various form of neuronopathic Gaucher disease [38].

Moreover, each type of neuronopathic Gaucher disease is clinically heterogeneous. Gaucher disease type 3 in particular encompasses multiple phenotypes [39]. The most common form of Gaucher disease type 3, with impaired saccadic eye movements and extensive visceral involvement, was named type 3b, and is described in detail neurologically and systemically [40, 41]. A more rare form, referred to as type 3a, includes the development of myoclonic epilepsy, but likewise, this type does not have a unique genotype [42]. A third variant is so distinctive from type 1 (OMIM #230800), type 2(OMIM #230900) and type 3 (OMIM #231000) that it has its own OMIM designation (OMIM #231005) and is called type 3c. It probably represents the most consistent example of genotype/phenotype correlation involving cardiac valves, aortic calcification, coronary artery disease and at times hydrocephalus and dysmorphic changes associated with homozygosity for mutation D409H [D448H] [43].

Various forms of enzyme replacement therapy for neuronopathic Gaucher disease have been contemplated and one of the earliest was kidney transplantation performed with limited

success in type 2 Gaucher disease [44]. When alglucerase enzyme replacement therapy for Gaucher disease was approved in 1991 based on results from patients with type 1 Gaucher disease, it remained unclear whether the same intervention would be effective either on the systemic disease or the neurological deficits seen in patients with Gaucher disease type 3. An open label study in a small number of patients with Gaucher disease type 3 showed a marked improvement in hematological, visceral and even skeletal disease. Although the initial improvement in well-being in these patients led to a small cognitive improvement in the first six months of therapy, it became increasingly clear that systemically administered enzyme does not cross the blood-brain barrier and therefore does not truly modify the neurological outcome of these patients [45–47]. Dr. Brady and colleagues attempted to circumvent this difficulty and determined that the mannose receptor is expressed on the surface of neurons allowing the uptake of infused glucocerebrosidase directly into the brain [48]. However, enzyme directly infused into the cerebral spinal fluid did not impact the neurological deterioration in infants with type 2 Gaucher disease [49]. Using convection-enhanced delivery Dr. Brady's team succeeded technically in delivering the therapeutic enzyme to the brain of small and large animals and then into the brainstem of a patient with Gaucher type 2, albeit without clinical improvement [50]. To date there is no effective disease modifying therapy for neurological involvement of Gaucher disease [51]. A small molecule therapy approach for neuronopathic Gaucher disease was initiated at Dr. Brady's Branch with a randomized controlled trial of miglustat in patients with Gaucher type 3 [52]. Although this was a negative therapeutic trial, this study generated clinical outcome measures for future testing of better therapeutic agents. In recent years, Dr. Brady continued the effort to target the central nervous system in Gaucher disease and showed that small molecules that function as histone deacetylase inhibitors are able to increase the level of the endogenous glucocerebrosidase [53–55]. However, effective therapy for the various forms of neuronopathic Gaucher disease is still elusive. Systemically administered therapeutic agents in the form of pharmacological chaperones, enzyme replacement or gene therapy that can be widely distributed into the nervous system are needed, whether to increase glucocerebrosidase levels or to reduce substrate synthesis (discussed in Section 4). But even with effective widespread biochemical correction, the challenge of initiating therapy sufficiently early in Gaucher disease type 2 remains and neurological deficits also may be irreversible in patients with Gaucher disease type 3. Thus, a better understanding of the natural history and the pathogenesis of neuronopathic Gaucher disease is needed.

3. Recent Clinical Advances

3.1 Gaucher Disease: A Phenotypic Continuum

As our appreciation of the complexity of genotypes associated with Gaucher disease expanded, the recognition of the full spectrum of phenotypic manifestations also evolved [56]. Classically, it was appreciated that hepatosplenomegaly, cytopenia, abnormal coagulation and bone disease could all be features of Gaucher disease type 1. Acute neuronopathic Gaucher disease was described as a disorder of late infancy with opisthotonos, strabismus, seizures and neurodegeneration, while type 3 Gaucher disease was associated with abnormal eye movements and myoclonic epilepsy. However, newer disease presentations and organ involvement not previously associated with Gaucher disease, such

as the collodian baby phenotype, and patients with parkinsonism, gallstones and cardiac valvular calcification or fibrosis are now part of the phenotypic spectrum. Moreover, asymptomatic individuals are being discovered through genetic screening and whole exome sequencing. It also appears that the phenotypes encountered may be associated with ethnicity or genetic background. Patients from Africa and Asia appear to have more severe presentations, but it is not clear whether this is related to different distributions of mutant alleles in different populations, or the fact that in some regions only patients with more blatant manifestations come to diagnosis.

3.2 The Association of Gaucher Disease and Parkinsonism

Parkinson disease is a neurodegenerative disease that affects over 1% of people over age 60 [57]. This clinical diagnosis is made when patients develop bradykinesia (slowness of movement) and either a rest tremor, rigidity that tends to be initially unilateral, and/or postural instability. In addition, patients have a favorable response to dopaminergic therapy. Symptoms are secondary to loss of dopaminergic neurons in the substantia nigra pars compacta, although the involvement of non-dopaminergic pathways has been recognized due to development of non-motor manifestations, such as depression or anxiety, cognitive dysfunction, sleep disorders and olfactory dysfunction, many years prior to the development of motor symptoms [58].

The identification of specific genetic mutations associated with the development of Parkinson disease was first achieved in 1997, when Polymeropoulos *et.al.* demonstrated segregation of Parkinson disease in affected individuals carrying a mutation in the alpha-synuclein gene in two Italian and one Greek families [59]. Shortly thereafter, Spillantini's group demonstrated the presence of the alpha-synuclein protein in Lewy bodies, which are aggregate inclusions in neuronal bodies that are crucial pathological hallmark for a definitive diagnosis of Parkinson disease [60]. To date, many other genes, including some with highly penetrant monogenic mutations, have been identified [61]. Each gene discovery continues to significantly advance our knowledge of potential mechanisms contributing to neurodegeneration and the role of mitochondria, endoplasmic reticulum, and lysosomal pathways in neuronal cell death.

The description of occasional patients with Gaucher disease who also developed parkinsonian manifestations can be found in the early literature including a small cohort of patients affected with both diseases described in 1996 [62]. These patients were described as having parkinsonian symptoms similar to patients with sporadic Parkinson disease, but were thought to be refractory to dopaminergic therapy. Investigators at the NIH described individual patients with Gaucher disease and parkinsonism showing Lewy bodies that were in brain regions typically involved in neuropathological Gaucher disease [63, 64][65] These findings gave critical credibility to the possibility of a Parkinson-Gaucher link. A series of 18 patients with the two phenotypes was later published by Tayebi *et al* demonstrating a range of genotypes and disease manifestations [66]. Furthermore, about 25% of patients with Gaucher disease surveyed reported a first- or second-degree relative with parkinsonism, implicating heterozygous mutation carrier status as a genetic risk factor for the development of Parkinson disease [67]. Indeed two early studies screening subjects with Parkinson

disease for *GBA1* mutations performed in brain bank samples [68] and in a Parkinson disease clinic in Northern Israel identified a surprisingly high number of Gaucher heterozygotes [69]. In 2009, a large multicenter collaborative effort that included 5691 patients with Parkinson disease and 4898 healthy controls showed that among patients with Parkinson disease, the odds ratio of carrying a *GBA1* mutation was 5.43 (95% CI 3.89–7.57), confirming that mutations in this gene are a major genetic risk factor [70]. *GBA1* mutations have since been associated with other synucleinopathies [71] including Dementia with Lewy Bodies [72] and multiple system atrophy [73]. Furthermore, the role of lysosomal function integrity in protein aggregation and clearance has been pursued by studying mutations responsible for other lysosomal storage disorders, such as Niemann-Pick disease in cohorts with parkinsonism [74].

The clinical parkinsonian manifestations in patients with Gaucher disease can be variable, ranging from symptoms indistinguishable from sporadic Parkinson disease with good response to dopamine supplementation to patients with symptoms suggestive of Dementia with Lewy Bodies [75]. In addition, many patients develop parkinsonism at an earlier age at onset when compared to those without *GBA1* mutations, as well as cognitive and olfactory dysfunction [76, 77]. It has been suggested that such patients have a more aggressive disease course that may be associated with the severity of the *GBA1* mutation [78, 79]. Several studies emphasize accelerated cognitive decline in patients with *GBA1* associated parkinsonism [79–81]. However, alterations such as E326K and T369M, that do not cause Gaucher disease have also been identified at an increased frequency in subjects with parkinsonism [82].

Neuroimaging studies conducted at the NIH using ¹⁸F-dopa Positron Emission Tomography have shown that in patients with *GBA1*-associated parkinsonism the pattern of dopaminergic cell loss in the posterior putamen is indistinguishable from sporadic Parkinson disease. However, in this small study they had regional cerebral blood flow pattern deficits similar to those seen in patients with Dementia with Lewy Bodies that may correlate with the reported increased likelihood of cognitive dysfunction reported by patients and found in the literature [83].

The precise mechanism of neurodegeneration remains elusive at this time, although there are several theories proposed [71, 84–87]. It does appear that there is a reciprocal relationship between levels of glucocerebrosidase and alpha-synuclein, as even patients with idiopathic Parkinson disease are found to have lower levels of glucocerebrosidase activity in brain tissue [88, 89]. Elucidating the mechanisms involved will likely shed light on the pathogenesis of parkinsonism and the role of the lysosome.

3.3 The Association with malignancies

From the beginning, when Dr. Phillippe Gaucher misdiagnosed his patient with a type of splenic neoplasm, to this day, patients with Gaucher disease presenting with their protean manifestations are often given an initial diagnosis of malignancy as they frequently begin their long diagnostic odyssey [90]. However, there have been many sporadic reports of cancers in patients with Gaucher disease and it was a major contributor of premature death in patients in the pre-ERT era [91]. In recent years, investigators have attempted to identify

types of cancers and magnitude of risk of malignancy in Gaucher disease. All studies uniformly agree that there is an increased risk of multiple myeloma, and it appears that the risk of other hematological malignancies may also be increased, especially B cell lymphomas [92]. The glycosphingolipid pathway is intimately involved in cancer pathogenesis, prompting numerous investigations to exploit it as a therapeutic target via inhibitors of glucocerebrosidase synthase or by shifting the ceramide-sphingosine sphingolipid rheostat towards the pro-apoptotic and anti-cancer sphingolipid ceramide [93]. It appears that of all the glycosphingolipidoses, Gaucher disease is uniquely associated with increased cancer risk, suggesting Gaucher lipids may have a specific role in promoting malignancies.

The highest cancer risk in Gaucher disease is attributable to multiple myeloma with relative risk ranging from 5.9 in the International Gaucher registry to 37.5–51.5 from long-term observational cohorts from single centers [94–96]. The study from the International Gaucher registry may have under-estimated the risk because myeloma occurs in an older age group and the registry is not designed to capture cancer data. Polyclonal and monoclonal gammopathy, a finding often preceding multiple myeloma, is common in Gaucher disease. This may be suggestive of involvement of B cells as a key role in disease pathophysiology, a finding reinforced by the Proia group studies in their mouse model, which showed elevated serum IL6 and B cell lymphoproliferation [97].

Numerous clinical and animal model studies have clearly implicated a prominent role of immune activation in the pathophysiology of Gaucher disease [98]. Studies of immune activation in Gaucher disease have revealed broad involvement of innate and adaptive immune system [99] but the precise mechanism linking Gaucher lipids to immune activation is not understood. Genetic deficiency of glucocerebrosidase leads to accumulation of not only the primary lipid substrate, but also the downstream bioactive lipids glucosylsphingosine, sphingosine and sphingosine 1 phosphate [100, 101]. Recent studies have shown that Gaucher lipids, glucocerebrosidase and glucosylsphingosine are presented in CD1d-restricted fashion to type II NKT cells that exhibit markers of follicular helper T cells which then provide help to germinal center B cells to produce lipid-reactive antibodies [102]. In patients and mice with Gaucher disease that harbor monoclonal gammopathy, the monoclonal immunoglobulin was found to be reactive to Gaucher lipids [103]. In fact, in another mouse model of Gaucher disease that spontaneously develop myeloma and other B cell malignancies, therapeutic reduction of glucosylsphingosine with eliglustat tartrate substrate reduction therapy prevented development of these cancers [104]. Interestingly, in a subset of sporadic multiple myeloma patients, clonal immunoglobulin was also reactive to lysolipids, such as glucosylsphingosine and lysophosphatidylcholine. Clearly much work is needed to understand the basic mechanisms underlying the role of lipids in genetic mechanisms underlying myeloma, but this understanding could have benefit not only for patients with Gaucher disease but also patients with sporadic multiple myeloma, another great example where study of a rare disease provides unique insights into mechanisms in a more common disease. More than half-century ago Roscoe Brady was among the first to propose that glycolipids can act as antigens, and he demonstrated a role of anti-glycolipid antibodies in a human disease when he showed that monoclonal gammopathy associated with peripheral neuropathy was glycolipid reactive [105].

4. Current Therapies for Gaucher disease

4.1 Enzyme Replacement Therapy

Prior to the availability of enzyme replacement therapy (ERT), only symptomatic care was available in the form of blood transfusions, splenectomy for relief of pressure symptoms or severe cytopenia, and joint replacement surgery. Splenectomy was commonly performed for symptomatic relief, but this frequently resulted in accelerated disease and complications involving other organs. When patients became dependent on blood transfusions, the natural history accelerated alarmingly, with massive load of toxic glycosphingolipids from the transfused red and white blood cell membranes. The natural history of type 1 Gaucher disease, prior to the availability of ERT, was often one of devastating disease, including a high incidence of splenectomy, bleeding complications, bone marrow failure, liver failure, crippling skeletal disease, pulmonary hypertension, malignancies, and premature death. Yet at the other side of the spectrum, some patients were identified only later in life, when they were evaluated because of an affected relative, or were incidentally found to have splenomegaly during an evaluation for other reasons.

Striking and rapid reversal of many of the overt aspects of type 1 Gaucher disease were seen in pivotal clinical trials of alglucerase and later with the recombinant form of the enzyme, imiglucerase (Genzyme, Cambridge, MA, USA) and have been replicated again and again in small series of patients from single centers as well as a global, industry-sponsored multinational registry comprising almost 6,000 patients that collectively harbor ~54,000 patient-years of enzyme therapy experience [106]. Enzyme replacement therapy has proven highly effective in reversing hepatosplenomegaly, cytopenia, osteopenia, especially in children and young adults, and in reducing the risk of avascular osteonecrosis [107]. The new generation of patients who have access to enzyme replacement therapy hardly ever undergo splenectomy, and generally begin treatment before irreversible complications of the disease occur. As a result, certain phenotypes, such as pulmonary hypertension and cirrhosis/hepatopulmonary syndrome have virtually disappeared from modern-day clinics as hitherto unknown aspects of Gaucher disease have been unraveled [65, 108].

As with type 1 Gaucher disease, equally striking responses of visceral, hematological and skeletal responses have been described in patients with type 3 Gaucher disease [109]. In fact, early childhood lethality due to massive visceral and hematological disease have been averted, although eventually, these patients may succumb to progressive neurological and pulmonary disease. In some, this is aggravated by a disabling ‘gibbus’ spinal deformity, characteristic in certain populations of L444P homozygotes around the world. The success of enzyme replacement therapy in reversing visceral and hematological disease has brought into focus a major clinical dilemma when a symptomatic infant cannot be reliably assigned with a diagnosis of type 2 or type 3 disease. Since ERT does not cross the blood-brain barrier, progressive type 2 disease may then be unmasked. Clearly, much progress needs to occur in the areas pertaining to accurate predictive phenotyping based on clinical annotation, imaging, biomarkers and modifier genes. Two recent studies detailing the autopsy findings in treated patients with Gaucher disease have demonstrated variability in response, both in different patients and in different organs [110, 111].

For a protein therapeutic, safety track record of enzyme infusions has been remarkable. Anaphylaxis, neutralizing antibodies and anti-IgE antibodies have occurred in only a handful of patients around the world [112]. However, up to 15% of patients develop anti-drug antibodies that are non-neutralizing and appear to have no effect on efficacy of treatment. It has been argued that in fact, presence of non-neutralizing antibodies may in fact improve targeting to the macrophages via Fc receptors. The rarity of immune adverse effects may be related to the circulating half-life of the mannose-terminated enzyme, which is barely a few minutes.

From 1991 to 2009, the only approved ERTs for Gaucher disease were alglucerase and imiglucerase. In June 2009, vesivirus 2117 contaminated one of six bioreactors at the manufacturing facility which led to temporary suspension of enzyme manufacturing to clear the virus. This event triggered a severe global shortage of imiglucerase. At that time, two new enzyme preparations were in advanced stages of development: velaglucerase-alfa (h-GCB, Shire Human Genetic Therapies, MA, USA) and taliglucerase-alfa (pr-GCD, Protalix Biotherapeutics, Carmiel, Israel). Global shortage of imiglucerase expedited the approval process of velaglucerase-alfa and taliglucerase-alfa in 2010 and 2012, respectively. Velaglucerase-alfa is acid β -glucocerebrosidase produced by gene-activation in a human fibrosarcoma line (HT-1080) cultured in the presence of kifunensine, an inhibitor of mannosidase I, resulting in primary biosynthesis of recombinant wild type enzyme terminating in immature high mannose-type N-linked glycan chains. Taliglucerase-alfa is a variant human acid β -glucocerebrosidase manufactured in carrot root cells using a plant-specific C-terminal sorting signal which allows the nascent enzyme to target to storage vacuoles and moreover, display terminal mannose residues on its complex glycans. To facilitate secretion of the protein via the vacuolar pathway in plant cells, taliglucerase has unusual sugar sequences, including xylose. Taliglucerase has the same core amino acid sequence as imiglucerase, including the single amino acid substitution of histidine to arginine at position 495. Both velaglucerase and taliglucerase have higher mannose terminal residues, and it has been suggested that these may improve in vivo targeting to the macrophage system. Overall however, all three enzyme preparations have similar safety and efficacy with respect to visceral and hematological disease [113–115]. It is expected that similar efficacy with respect to marrow and skeletal disease will be seen with longer-term experience.

In 2016, additional ERTs may come to market, exemplified by development of Abcertin, claimed to utilize an identical system to that used for production of imiglucerase. However, clinical studies and trials are necessary to ensure that these agents are genuinely biosimilar or interchangeable. With the proliferation of ERTs, two important issues are coming into sharp focus. First, there is the need for standardization of antibody assays and secondly, a need for registries to capture safety and efficacy data in an unbiased and consistent manner. In the small number of patients who develop anti-drug antibodies, their safety depends on accurate evaluation of reliable outcome measures and biomarkers to discern true efficacy or lack thereof, especially when the patient has developed cross-reacting antibodies to multiple ERTs. Current assays are only offered by individual pharmaceutical companies posing its unique challenges to interpretation, and results are difficult to compare between formulations.

4.2 Substrate reduction therapy (SRT) for Gaucher disease

In mid-1990s, Fran Platt and Norman Radin proposed the concept of ‘substrate balance therapy’, via inhibition of ceramide glucosyltransferase (EC:2.4.1.80) using their respective candidate molecules, iminosugar (N-butyl-deoxynojirimycin, NB-DNJ) and a ceramide analog (D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol: PDMP), respectively [116, 117]. The development of NB-DNJ (miglustat, Actelion, Allschwil, Switzerland) and its introduction into the clinical setting was relatively rapid because there was abundant pre-existing toxicology and drug metabolism/pharmacokinetic data in multiple species as well as pre-existing clinical safety data at high dosage related to its use as a potential antiviral therapy for HIV. Moreover, there was almost one metric ton of the drug readily available from the HIV trials [118]. Therefore, drug development progressed rapidly from concept in 1994 to clinical trial in 2000 and its approval in 2003 [119]. The approval of miglustat for type 1 Gaucher disease was restricted to patients who could not take ERT and those with mild disease due to an unfavorable adverse risk/benefit profile. While NB-DNJ inhibits ceramide glucosyltransferase, its inhibitor constant is relatively high (IC_{50} : 50 μ M) and it has multiple off-target effects (IC_{50} for *GBA1* 520 μ M, *GBA2* 0.31 μ M). It also inhibited intestinal disaccharidases, sucrose, maltase and lactase as well as glucosidase I and II. Thus, the therapeutic effect of NB-DNJ may be more complex than simply substrate reduction, involving inhibition of *GBA2* as well as chaperoning effect. In an open label, non-inferiority design trial comparing miglustat with imiglucerase ERT, it met the primary end-point of reduction in liver volume but almost half of the patients on miglustat discontinued treatment due to adverse effects [120]. While the reversal of visceral disease with miglustat is similar to ERT, hematological responses are slower and of lesser magnitude compared to ERT. Therefore, very few patients are currently being treated with miglustat SRT.

In contrast to miglustat, the development path for eliglustat (Genzyme, Cambridge, MA, USA) was much longer, from IND filing in 2003, to initiation of clinical trials in 2007 and final approval for use in adults with type 1 Gaucher disease in 2014 [121]. The original prototype molecule was toxic and eventually, eliglustat tartrate was developed as one of a series of ceramide-analog glucocerebroside synthase inhibitors, derived from 1-phenyl-2-decanoylamino-3-morpholino-1-propanol entering clinical trials in 2007. Compared to miglustat, eliglustat was a very potent inhibitor of ceramide glucosyltransferase (IC_{50} 0.024 μ M). It was also highly specific with few off-target effects.

The clinical trial program was a massive multinational effort involving 12 countries, 24 centers and almost 400 patients in a phase 2 and three phase 3 clinical trials including a placebo-controlled randomized trial in untreated patients over age 16 with type 1 Gaucher disease [122–124]. Together, these studies set a new benchmark for the conduct of clinical trials of novel therapies in a rare disease, a far-cry from the pivotal trials of ERTs. The drug was well tolerated and the most common adverse reactions affecting ~10% of patients were fatigue, headache, nausea, diarrhea, back pain, pain in extremities, and upper abdominal pain. The randomized placebo-controlled clinical trial met the primary end-point of reduction in spleen volume [124]. Key secondary endpoints were also met (increase of blood hemoglobin concentration, reduction of liver volume and increase of platelet count) and these were analyzed with statistical significance with the use of a pre-specified fixed-

sequence hypothesis-testing approach to ensure strong control of the type I error rate. Additional clinical trials compared maintenance of therapeutic goals in patients stable on ERT who switched to eliglustat using a non-inferiority design [123]. The primary end-point of stability in composite therapeutic goals were met as well as in the individual domains of stability of hemoglobin concentration, platelets counts, liver volume and spleen volume. These studies and phase 2-open label clinical trials showed reduction of biomarkers of Gaucher disease including chitotriosidase, CCL18, plasma glucocerebroside, glucosylsphingosine, and MIP1- β . Additionally, bone marrow burden score and bone density studies also showed improvement.

Eliglustat was approved in 2014 for use in adults with type 1 Gaucher disease who have cytochrome P450 2d6 genotypes indicating that they are extensive or intermediate metabolizers [125]. Eliglustat is contraindicated in patients who are ultra-rapid metabolizers because clinically active blood levels may not be reliably attained. The recommended eliglustat dose is lower in patients who are slow metabolizers as well as in patients who are concurrent users of interacting medications. For patients on medication regimens that include strong or moderate CYP2D6 inhibitors concomitantly with strong or moderate CYP3A inhibitors, eliglustat is contraindicated. These drug combinations can potentially increase eliglustat plasma concentrations with risk of cardiac arrhythmias via prolongation of PR, QTc or QRS cardiac intervals. It is also recommended that eliglustat not be prescribed for pregnant women or those who are breast feeding.

Other studies have shown that responses in treatment-naive patients are comparable to those seen with ERT [126]. A most intriguing feature of eliglustat SRT is impressive reversal of disease in patients with massive disease burden reminiscent of that observed with ERT [122]. This observation seems to be at variance with the simple concept of substrate balance therapy as initially enunciated. A clue to this unexpected finding may lie in 1960 paper by Trams and Brady [5] where they measured incorporation of carbon-14 glucose into glucocerebroside and found it was actually increased. Indeed, expression of *UGCG*, which encodes ceramide glucosyltransferase is increased 2–3-fold in spleens of Gaucher mice [127]. Moreover, inflammation has been associated with increased activity of ceramide glucosyltransferase [128] and glucocerebroside synthesis and Gaucher disease is now recognized as an example of metabolic inflammation mediated by bioactive lipids [102]. Thus, these observations suggest that increased f glucocerebroside synthesis may function to accentuate basic catabolic defect due to *GBA1* mutations.

4.3 Prospects for Gene Therapy

Concurrent with successful development of ERT with macrophage-targeted placental glucocerebroside, Dr. Brady together with Dr. Karlsson at the NIH, turned their attention to assess feasibility of gene therapy as a more permanent cure. In 1990, they established the proof of principle using a high-titer, amphotropic retroviral vector in which human *GBA1* was driven by the mutant polyoma virus enhancer/herpesvirus thymidine kinase gene (tk) promoter (Py+/Htk) [129]. This vector normalized *GBA1* activity in transduced Gaucher fibroblasts and hemopoietic progenitor cells derived from patients with Gaucher disease. These studies were followed by a clinical protocol to explore the safety and feasibility of

retroviral transduction of peripheral blood or bone marrow CD34+ cells in three adults with Gaucher disease followed for 6–15 months. G-CSF was administered to mobilize target cells and CD34+ peripheral blood cells or CD34+ bone marrow cells were transduced ex vivo achieving transduction efficiency of 1–10% [130]. Gene-marked cells were found to engraft and persist for three months after cell infusion, without prior myeloablation. Nevertheless, the level of corrected cells (<0.02%) was too low and transient to confer any therapeutic benefit. Since these early efforts, the field of gene therapy has advanced greatly with demonstration of clinical benefits of gammaretroviral vectors in the treatment of primary immune deficiencies. Moreover, safety profiles have been further improved by transitioning to lentiviral vectors, as reflected in recent clinical success in the treatment of X-linked adrenoleukodystrophy, Wiskott-Aldrich syndrome and other disorders. Recently, Karlsson and his group have reported proof of principle studies using self-inactivating lentiviral vectors with *GBA1* under the control of a human phosphoglycerate kinase and CD68 promoter [131]. In their conditional KO mouse model of Gaucher disease, they demonstrated prevention, as well as reversal of, disease manifestations after lentiviral gene transfer. Therefore, these studies lay the foundation for the use of self-inactivating lentiviral vectors with cellular promoters which may play a role in future clinical gene therapy protocols for type 1 Gaucher disease.

4.5 Pharmacological Chaperones as Therapeutic Approach for Gaucher Disease

Small molecule chemical chaperones have been considered as a treatment approach for diseases caused by improperly folded proteins [132]. These small molecules are designed to selectively bind to a specific target protein and can increase enzyme stability, catalytic activity, and increase lysosomal translocation [133]. Lysosomal enzymes are folded in the endoplasmic reticulum (ER) with the aid of cellular chaperones, and are then translocated to the lysosome. When mutated, the enzyme may be misfolded, and undergo premature endoplasmic reticulum-associated degradation (ERAD) and never reach the lysosome [134]. Treatment with small molecule chaperones aid in protein folding and stabilization, as well as lysosomal translocation. Since many *GBA1* mutations are missense mutations, this strategy merits consideration.

Initially, pharmacologic chaperones developed for Gaucher disease were competitive inhibitors of the target enzyme that bind to the active site of the misfolded enzyme and facilitate proper folding and translocation. Early work in patient derived fibroblasts suggested that the iminosugar N-nonyl-1-deoxynojirimycin (NN-DNJ) increased mutant glucocerebrosidase activity up to 2-fold [135]. Subsequently, other iminosugar-based glucocerebrosidase inhibitors have been developed, but since they have a high affinity for glycosidases, they can have poor selectivity [136, 137] and also function as inhibitors at higher dosages. While preclinical studies appeared to be promising [138–142] a phase 2 clinical trial of isofagomine failed to improve clinical symptoms in patients with GD.

Recent efforts to develop chaperones for the treatment of GD have utilized high throughput screening (HTS) of small molecules. The screen of an FDA-approved drug library led to the identification of ambroxol, an agent widely used as cough medicine and a pH-dependent mixed inhibitor of glucocerebrosidase [143]. The efficacy of ambroxol as a potent chaperone

and translocator of mutant glucocerebrosidase to lysosomes was demonstrated in cell-based and mouse models [143–146] and in a very limited study in patients with neuronopathic Gaucher disease [147].

Another treatment approach is the use of non-inhibitory chaperones. These small molecules assist in the folding of mutant enzyme in the ER and in its translocation to lysosomes by binding to a site that is distinct from the active site and ideally they can restore lysosomal enzymatic function through chaperone and enzyme stimulatory effects [148]. While such chaperones are attractive candidates for therapeutic development, their identification is more challenging.

To improve the yield of non-inhibitory chaperones, a novel screening assay was designed using patient's tissue extracts as the source of mutant glucocerebrosidase. Such extracts also contain Saposin C, its native activator, and other potential cofactors. A patient spleen-based enzyme assay was used to screen a library of 250,000 compounds and identified several lead non-inhibitory compounds. The ability of these compounds to chaperone was confirmed in subsequent cell-based assays using patient-derived fibroblasts [149]. Two of the lead compounds were tested on primary macrophages differentiated from monocytes of patients with Gaucher disease, as well as from induced pluripotent stem cells (iPSCs) from dermal fibroblasts. The non-inhibitory chaperone increased glucocerebrosidase activity and lysosomal translocation and significantly reduced substrate storage in macrophages from patients with different genotypes and restored defective chemotaxis [150]. The lead chaperones were then tested in iPSC-derived dopaminergic neurons, where in addition to restoring enzymatic activity and reducing substrate storage, they were found to lower levels of alpha-synuclein [151]. Thus, such chaperones may ultimately also prove efficacious for the treatment of patients with Parkinson disease.

4.6 Histone Deacetylase Inhibitors

After the successful development of ERT for type 1 Gaucher disease, a major goal of Dr. Brady's research efforts was to identify tractable therapeutic targets for neuronopathic Gaucher disease. Using skin fibroblast cell lines derived from patient with neuronopathic Gaucher disease (L444P/L444P) and type 1 Gaucher disease (N370S/370S), in a series of studies, the last of which appeared soon after his 90th birthday, he proposed that histone deacetylase (HDAC) inhibitors might increase the quantity and catalytic activity of mutant glucocerebrosidases by affecting proteostasis [53, 55]. With NIH collaborators, he showed that both N370S and L444P mutant glucocerebrosidase result in a reduction of protein stability, rather than disruption of intrinsic enzymatic activity. Both mutations led to aberrant protein conformation that resulted in altered chaperone binding, hence making the nascent glucocerebrosidase protein vulnerable to recognition by E3 ligases (parkin and c-cbl) and proteasome-associated degradation. Further studies revealed that HDAC inhibitors increased the quantity and catalytic activity of mutant *GBA1* in fibroblast via impaired deacetylation of heat shock protein 90 that hindered the recognition of the mutant *GBA1* peptide and degradation, thereof. Hsp90 β was identified as the key chaperone that recognized misfolded mutant *GBA1* to guide the nascent protein through a valosin-containing protein (VCP)-associated degradation pathway. In these studies, HDAC inhibitors caused hyperacetylation

of the middle domain of Hsp90 β which impaired recognition of mutant *GBA1* by Hsp90 β and hence, resulted in increased levels of the active enzyme in the lysosomes. These proof-of-principle studies were performed using HDAC inhibitors, suberoylanilide hydroxamic acid (SAHA, vorinostat), a clinically available HDAC inhibitor which is CNS-penetrant, and a unique investigational HDAC inhibitor called LB-205. These studies are promising enough to advance to pre-clinical animal models and eventually to the clinic. Incidentally, vorinostat is clinically approved for cutaneous T cell lymphoma and of the various cancers in which it has been investigated, it has shown promising results in multiple myeloma. Thus, it is tempting to speculate a potential role of HDAC inhibitors as dual therapy for not only neuropathic Gaucher disease but also type 1 Gaucher disease complicated by multiple myeloma or another cancers [152].

The Development of New Research Tools

5.1 Mouse Models of Gaucher disease

While it has been long appreciated that mouse models of Gaucher disease are an extremely important tool for studying disease pathogenesis and drug development, the generation of an appropriate model has proved quite challenging. Genetic alteration of exons 9–10 of *Gba* has been the target of most animal model strategies since this region is most frequently mutated in Gaucher disease. The first mouse model of Gaucher disease was created by insertion of a Neo cassette in exons 9 and 10 by the group of Dr. Ginns at the NIH in 1992 [153], effectively knocking out all glucocerebrosidase activity. This model replicated severe type 2 Gaucher disease with glucocerebroside accumulation in liver, brain and lungs, and in the lysosomes of spleen and liver macrophages. However, the mice died in neonatal period due to dehydration related to permeability barrier defects in the skin [154]. These studies provided great insight into the collodion skin phenotype associated with the most severe type 2 Gaucher disease. It led to the recognition that alterations in epidermal biochemistry and ultrastructure in patients could be used to discriminate between type 2 and type 3 disease [155]. This has direct clinical utility as it is not uncommon to come across affected infants in whom it is not possible to distinguish clinically between type 2 and type 3. However, the early lethality of this model limited its utility for therapeutic development. Next, the Proia group, also at the NIH, utilized a single insertion mutagenesis procedure to develop mice harboring the ‘RecNciI’ mutation (L444P/A456P) and mice homozygous for the L444P mutation [156]. Both strains initially died after birth. In an attempt to develop a longer lived model homozygous for L444P mutation, Dr. Proia crossed heterozygote mice with heterozygous *Ugcg* knockout mice [97]. After serial intercrosses of *Gba*^{+/L444P};*Ugcg*^{+/-} mice, they obtained L444P/L444P homozygous (*Gba*^{L444P/L444P};*Ugcg*^{+/+} mice). It is very intriguing that despite wild type *Ugcg*, L444P homozygous mice were not lethal and although they lacked Gaucher features, the model recapitulated an inflammatory phenotype with B cell lymphoproliferation. More recently, mice have been generated harboring other missense mutations (V394L, D409H and D409V mice) [157]. These mice from the Grabowski laboratory are long lived and express a biochemical phenotype, but have mild to no accumulation of Gaucher cells. Surprisingly, N370S homozygous mice died within 24 hours of birth owing to a skin defect, similar to *Gba*^{-/-} mice, despite significant residual glucocerebrosidase activity. A conditional GD mouse was generated in 2006 by the

Karlsson's laboratory using *Cre*-mediated deletion of floxed *Gba* gene in hematopoietic cells, achieved upon treatment with polyinosinic-polycytidylic acid to activate the *Mx1* promoter after birth [158]. These mice had a normal life-span and recapitulated GD phenotype more fully, with massive Gaucher infiltration, splenomegaly and cytopenia. A conditional mouse model of type 2 GD was generated by the same group through insertion of a floxed Neo cassette into *Gba* intron 8, and homozygous mice were then bred with keratin-14-*Cre* transgenic mice to rescue *Gba* in the skin [159]. These mice developed classical phenotype of type 2 Gaucher disease and lived ~10 days. This conditional KO strategy was also used to generate another conditional deletion in hematopoietic cells by the Mistry group, which successfully recapitulated aspects the human phenotype and was instrumental in describing immune dysregulation and metabolic inflammation as well as the role of *Gba2* in Gaucher disease [101, 127, 160]. An important aspect of Gaucher disease that is being explored in this murine model is biliary excretion of glucocerebroside and its bioactive lipids. In fact, the first and only such study was by Dr. Brady's group 35 years ago showing impressive biliary secretion of the stored lipids in Gaucher disease [161]. Dr. Brady revisited this topic recently, working to characterize the nature of biliary transport of Gaucher lipids in mouse models with Dr. Mistry's group [101].

Recently, there has been a resurgence of interest in a chemically induced mouse model using conduritol β -epoxide (CBE), an inhibitor of glucocerebrosidase first developed in the 1970s [162]. The Futerman lab improved on this model though optimizing dose and timing of CBE administration to generate longer-lived models replicating neuronopathic Gaucher disease [30]. This system was recently employed to describe impressive variability of Gaucher disease phenotypes in different strains of mice and a genome-wide association study has led to a list of compelling candidate modifier genes.

5.2 Cell Based Models of Gaucher Disease

Research in the field of Gaucher disease has long sought effective cell models to pursue a better understanding of disease pathophysiology and to develop novel therapeutics. Fibroblasts, while helpful for some studies, do not store the lipid substrate. Recently, attention has turned to developing induced pluripotent stem cell (iPSC) lines from patients with Gaucher disease. Several groups have successfully generated such lines and differentiated them into different lineages. In one study, macrophages differentiated from these Gaucher iPSC lines were evaluated and found to have enzyme deficiency, lipid storage, impaired chemotaxis, and impaired respiratory burst [150]. Other investigators used Gaucher iPSCs to explore defects in hematopoiesis, offering insight into elements of Gaucher pathogenesis outside of macrophages [163].

One field where the use of Gaucher iPSCs is especially exciting is in exploring the link between *GBA1* and Parkinson disease. Dopaminergic neurons have been generated both from iPSCs from Gaucher carriers and patients. In fact, one study investigated the differences between iPSC-derived neurons from monozygotic twins with *GBA1* mutations who were discordant for Parkinson disease [164]. Aflaki *et.al.* recently studied iPSC dopaminergic neurons and found that those made from patients with Gaucher disease showed low glucocerebrosidase activity, and elevated levels of glucocerebroside [151]. iPS-

derived dopaminergic neurons from patients with Gaucher disease with parkinsonism and type 2 Gaucher disease had elevated levels of α -synuclein. The cells were treated with a small molecule chaperone which increased glucocerebrosidase activity multifold in all Gaucher neurons and reduced α -synuclein levels in dopaminergic neurons derived from patients with Gaucher disease who also had parkinsonism. A second study by Mazzulli *et al* [165] also used iPSC-derived dopaminergic neurons from patients with mutations in PARK9, an A53T mutation in α -synuclein, an α -synuclein triplication, one patient with Gaucher disease, and one with idiopathic Parkinson disease to evaluate one of these previously described non-inhibitory chaperones of glucocerebrosidase. Treatment with the small molecule reduced substrate levels and pathological α -synuclein, also indicating that such chaperones may prove beneficial for the treatment of synucleinopathies.

6. Major Ongoing Challenges in the Field of Gaucher Disease

This is truly an exciting time for research in the field of Gaucher disease. When Dr. Brady began his work in the field the resources, tools and information available to him were quite limited. Currently, the number of publications, companies, organizations, websites and conferences focused on Gaucher disease are truly remarkable. The past decades have provided new insights, tools and treatments (Figure 1) and Gaucher disease has become a beacon, illuminating research directions for other lysosomal storage disorders.. In addition, the link with parkinsonism has opened new avenues of research and has infused additional energy into the field, and, as a result, the numbers of publications in this field are rising exponentially. It is especially gratifying to see that research directed toward a rare disease may have important implications for a common complex disorder.

Below are many of the questions and topics that the coauthors feel will be important to study in the coming years:

1. **Explaining phenotypic heterogeneity in a single gene disorder:** Determining (rather than speculating on) whether and to what extent environmental (both prenatal and post-natal) exposures (such as viral or other infections) influence the phenotypes of both type 1 and type 3 Gaucher disease remains elusive. This will be even more important if newborn screening becomes more prevalent. Studies of patients with identical genotypes and discordant phenotypes, especially discordant sibling pairs should help in this endeavor. Next-generation whole exome or whole genome strategies should facilitate these studies. Because of the relative rarity of Gaucher disease, large multicenter collaborative studies may be required in order to achieve the power necessary for the identification of genetic modifiers.
2. **Understanding the different organ involvement in Gaucher disease:** It will be important to determine why bone is such a susceptible target for infarction and necrosis and why these stochastic bone complications continue to occur despite ERT. What is the cause of the gibbus deformity in some patients with Gaucher disease? Furthermore, why do some patients develop pulmonary involvement and why is it relatively resistant to treatment? Defining the basic mechanisms by which splenectomy, especially when performed in young patients, exerts a

deleterious effect especially on bone and lung, might contribute to a better understanding of the pathophysiology in non-splenectomized patients.

3. **Establishing the full impact of Gaucher disease on macrophage function:** How do the cellular impairments seen in Gaucher macrophages influence disease manifestations and progression? To what extent do platelets, endothelial cells and/or circulating microparticles contribute to bone and other pathology either intrinsically or under the influence of inflammatory cytokines and chemokines? In addition to documenting abnormal increases in these inflammatory mediators, understanding why and which are abnormally increased in certain patients and not in others, and defining the actual pathways by which they contribute to clinical events will be important. From an epidemiologic perspective, what is the actual prevalence of the immunological (T cell, Tregs, NKT cells) abnormalities described in Gaucher disease models and patients, and do they consistently correlate with glucosylsphingosine levels in untreated and treated patients? Is there a way to prevent malignancies or provide targeted therapeutics?
4. **Determining the relative efficacy of different treatment modalities:** Are the longer term outcomes, including bone events and propensity for parkinsonism, myeloma and other malignancies, different in patients treated with SRT rather than ERT and how does this compare to untreated patients? Are there advantages in combining these different therapeutic strategies? How can we best compare therapeutic outcomes when new treatments like pharmacologic chaperones or HDAC inhibitors are developed? How can data be accessed by all when much is held in specific industry-sponsored registries? Why do autopsy studies indicate variable responses to therapy and what other factors contribute to the differences in effectiveness seen?
5. **Identifying improved biomarkers to inform treatment decisions and to predict prognosis:** How can we discern which subjects are likely to need therapeutic intervention and when? This will be particularly critical as more patients are identified by screening.
6. **Reducing the cost of treatment for Gaucher disease:** How can we make therapy accessible to all patients around the globe?
7. **Clearly defining the mechanism underlying the association between Gaucher disease and parkinsonism:** Why are carriers of a single *GBA1* mutation at increased risk of developing parkinsonism? What are the implications for treatment of Parkinson disease and for understanding pathways contributing to disease pathogenesis? Why is *GBA1*-associated parkinsonism associated with an earlier disease onset and often with cognitive impairment? What determines the anatomical distribution of protein aggregation responsible for the different clinical parkinsonian presentations? Identification of novel ligands and or chemical changes that could act as surrogate biomarkers of disease progression and can be followed over time to assess treatment efficacy are crucial to accurate determination of response in future clinical trials.

8. **Identifying the mechanisms underlying neuronopathic Gaucher disease:** How can we explain the vast diversity of phenotypes encountered? What is the reason for involvement of the saccadic eye movements? Why is mutation D409H associated with such a unique phenotype (Gaucher disease type 3C)? What determines who develops myoclonic epilepsy?
9. **Elucidating the best therapeutic strategy for neuronopathic GD:** Assuming that new treatments (SRTs, chaperones) are active in the CNS, how early does treatment have to be started to prevent or arrest clinical CNS pathology. Can existent disease be reversed and when does it become irreversible?
10. **Understanding the natural history and prevalence of GD in other continents:** Much of the data collected on Gaucher disease has primarily come from specific regions such as North and South America, Europe and Israel. How does Gaucher disease present and develop in other continents and what do these differences teach us about the disorder?
11. **Identifying and training the next generation of Gaucher investigators:** Many of the current investigators were trained in the previous century. How do we ensure leadership and continuity in the field? In which specialty programs will they find the best home?

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Abbreviations

ERT	enzyme replacement therapy
SRT	substrate reduction therapy
iPSC	induced pluripotent stem cells
GBAI	the glucocerebrosidase gene
CBE	conduritol β -epoxide
HSP	Heat Shock Protein
ER	endoplasmic reticulum

HDAC	histone deacetylase
NIH	National Institutes of Health
FDA	Food and Drug Administration

References

1. Gaucher P. De L'epithelioma primitif de la rate, hypertrophie idiopathique de la rate sans leucemie. 1882
2. Brill NE. Primary splenomegaly with a report of three cases occurring in one family. American Journal of Medical Science. 1901; 121:377–392.
3. Oberling CWP. Rev Franc de Ped III. 1927:475.
4. AE. La maladie de Gaucher dans l'enfance. Faculté de Médecine; Paris: 1934.
5. Trams EG, Brady RO. Cerebroside synthesis in Gaucher's disease. The Journal of clinical investigation. 1960; 39:1546–1550. [PubMed: 13777865]
6. Brady RO, Kanfer J, Shapiro D. THE METABOLISM OF GLUCOCEREBROSIDES. I. PURIFICATION AND PROPERTIES OF A GLUCOCEREBROSIDE-CLEAVING ENZYME FROM SPLEEN TISSUE. The Journal of biological chemistry. 1965; 240:39–43. [PubMed: 14253443]
7. Brady RO, Kanfer JN, Shapiro D. METABOLISM OF GLUCOCEREBROSIDES. II. EVIDENCE OF AN ENZYMATIC DEFICIENCY IN GAUCHER'S DISEASE. Biochemical and biophysical research communications. 1965; 18:221–225. [PubMed: 14282020]
8. Patrick AD. A Deficiency of Glucocerebrosidase in Gaucher Disease. Biochemical Journal. 1965; 97:17C–24C. [PubMed: 16749099]
9. Brady RO. The sphingolipidoses. The New England journal of medicine. 1966; 275:312–318. [PubMed: 5940695]
10. Barton, NW., Brady, RO. Macrophage-targeted glucocerebrosidase : a therapeutically effective enzyme replacement product for Gaucher disease. In: LAaSS, editor. Pharmaceutical Enzymes. Marcel Dekker; New York: 1997. p. 261-283.
11. Kraus EJ. Zur Kenntnis der Splenomegalie Gaucher, insbesondere der Histogenese der Grobzellen Wucherung Z. Angew Anat. 1920; 7:186.
12. Hillborg PO. Gaucher's disease in Norrbotten. Nordisk medicin. 1959; 61:303–306. [PubMed: 13633040]
13. Kampine JP, Brady RO, Kanfer JN, Feld M, Shapiro D. Diagnosis of gaucher's disease and niemann-pick disease with small samples of venous blood. Science (New York, NY). 1967; 155:86–88.
14. Schneider EL, Ellis WG, Brady RO, McCulloch JR, Epstein CJ. Infantile (type II) Gaucher's disease: in utero diagnosis and fetal pathology. The Journal of pediatrics. 1972; 81:1134–1139. [PubMed: 4674572]
15. Weinreb NJ, Brady RO, Tappel AL. The lysosomal localization of sphingolipid hydrolases. Biochimica et biophysica acta. 1968; 159:141–146. [PubMed: 5650428]
16. Pentchev PG, Brady RO, Hibbert SR, Gal AE, Shapiro D. Isolation and characterization of glucocerebrosidase from human placental tissue. The Journal of biological chemistry. 1973; 248:5256–5261. [PubMed: 4768898]
17. Furbish FS, Blair HE, Shiloach J, Pentchev PG, Brady RO. Enzyme replacement therapy in Gaucher's disease: large-scale purification of glucocerebrosidase suitable for human administration. Proceedings of the National Academy of Sciences of the United States of America. 1977; 74:3560–3563. [PubMed: 269414]
18. Ginns EI, Brady RO, Stowens DW, Furbish FS, Barranger JA. A new group of glucocerebrosidase isozymes found in human white blood cells. Biochemical and biophysical research communications. 1980; 97:1103–1107. [PubMed: 7470138]

19. Ginns EI, Brady RO, Pirruccello S, Moore C, Sorrell S, Furbish FS, Murray GJ, Tager J, Barranger JA. Mutations of glucocerebrosidase: discrimination of neurologic and non-neurologic phenotypes of Gaucher disease. *Proceedings of the National Academy of Sciences of the United States of America*. 1982; 79:5607–5610. [PubMed: 6957882]
20. Rapoport JM, Ginns EI. Bone-marrow transplantation in severe Gaucher's disease. *The New England journal of medicine*. 1984; 311:84–88. [PubMed: 6377066]
21. Takasaki S, Murray GJ, Furbish FS, Brady RO, Barranger JA, Kobata A. Structure of the Nasparagine-linked oligosaccharide units of human placental beta-glucocerebrosidase. *The Journal of biological chemistry*. 1984; 259:10112–10117. [PubMed: 6469957]
22. Martin BM, Sidransky E, Ginns EI. Gaucher's disease: advances and challenges. *Advances in pediatrics*. 1989; 36:277–306. [PubMed: 2675571]
23. Miao S, McCarter JD, Grace ME, Grabowski GA, Aebersold R, Withers SG. Identification of Glu340 as the active-site nucleophile in human glucocerebrosidase by use of electrospray tandem mass spectrometry. *The Journal of biological chemistry*. 1994; 269:10975–10978. [PubMed: 7908905]
24. Ginns EI, Choudary PV, Tsuji S, Martin B, Stubblefield B, Sawyer J, Hozier J, Barranger JA. Gene mapping and leader polypeptide sequence of human glucocerebrosidase: implications for Gaucher disease. *Proceedings of the National Academy of Sciences of the United States of America*. 1985; 82:7101–7105. [PubMed: 3863141]
25. Barneveld RA, Keijzer W, Tegelaers FP, Ginns EI, Geurts van Kessel A, Brady RO, Barranger JA, Tager JM, Galjaard H, Westerveld A, Reuser AJ. Assignment of the gene coding for human beta-glucocerebrosidase to the region q21–q31 of chromosome 1 using monoclonal antibodies. *Human genetics*. 1983; 64:227–231. [PubMed: 6885065]
26. Reiner O, Wigderson M, Horowitz M. Structural analysis of the human glucocerebrosidase genes. *DNA (Mary Ann Liebert, Inc)*. 1988; 7:107–116.
27. Tsuji S, Choudary PV, Martin BM, Stubblefield BK, Mayor JA, Barranger JA, Ginns EI. A mutation in the human glucocerebrosidase gene in neuronopathic Gaucher's disease. *The New England journal of medicine*. 1987; 316:570–575. [PubMed: 2880291]
28. Tsuji S, Martin BM, Barranger JA, Stubblefield BK, LaMarca ME, Ginns EI. Genetic heterogeneity in type 1 Gaucher disease: multiple genotypes in Ashkenazic and non-Ashkenazic individuals. *Proceedings of the National Academy of Sciences of the United States of America*. 1988; 85:2349–2352. [PubMed: 3353383]
29. Hruska KS, LaMarca ME, Scott CR, Sidransky E. Gaucher disease: mutation and polymorphism spectrum in the glucocerebrosidase gene (GBA). *Human mutation*. 2008; 29:567–583. [PubMed: 18338393]
30. Klein AD, Ferreira NS, Ben-Dor S, Duan J, Hardy J, Cox TM, Merrill AH Jr, Futerman AH. Identification of Modifier Genes in a Mouse Model of Gaucher Disease. *Cell reports*. 2016; 16:2546–2553. [PubMed: 27568557]
31. Tayebi N, Stubblefield BK, Park JK, Orvisky E, Walker JM, LaMarca ME, Sidransky E. Reciprocal and nonreciprocal recombination at the glucocerebrosidase gene region: implications for complexity in Gaucher disease. *American journal of human genetics*. 2003; 72:519–534. [PubMed: 12587096]
32. Grabowski GA. Phenotype, diagnosis, and treatment of Gaucher's disease. *Lancet (London, England)*. 2008; 372:1263–1271.
33. Goker-Alpan O, Hruska KS, Orvisky E, Kishnani PS, Stubblefield BK, Schiffmann R, Sidransky E. Divergent phenotypes in Gaucher disease implicate the role of modifiers. *Journal of medical genetics*. 2005; 42:e37. [PubMed: 15937077]
34. Brady RO, Pentchev PG, Gal AE, Hibbert SR, Dekaban AS. Replacement therapy for inherited enzyme deficiency. Use of purified glucocerebrosidase in Gaucher's disease. *The New England journal of medicine*. 1974; 291:989–993. [PubMed: 4415565]
35. Furbish FS, Steer CJ, Krett NL, Barranger JA. Uptake and distribution of placental glucocerebrosidase in rat hepatic cells and effects of sequential deglycosylation. *Biochimica et biophysica acta*. 1981; 673:425–434. [PubMed: 6784774]

36. Barton NW, Brady RO, Dambrosia JM, Doppelt SH, Hill SC, Holder CA, Mankin HJ, Murray GJ, Zirzow GC, Parker RI. Dose-dependent responses to macrophage-targeted glucocerebrosidase in a child with Gaucher disease. *The Journal of pediatrics*. 1992; 120:277–280. [PubMed: 1735829]
37. Barton NW, Brady RO, Dambrosia JM, Di Bisceglie AM, Doppelt SH, Hill SC, Mankin HJ, Murray GJ, Parker RI, Argoff CE, et al. Replacement therapy for inherited enzyme deficiency--macrophage-targeted glucocerebrosidase for Gaucher's disease. *The New England journal of medicine*. 1991; 324:1464–1470. [PubMed: 2023606]
38. Brady RO, Barton NW, Grabowski GA. The role of neurogenetics in Gaucher disease. *Archives of neurology*. 1993; 50:1212–1224. [PubMed: 8215980]
39. Tytki-Szymanska AVA, El-Beshlawy A, Cole JA, Kolodny E. Neuronopathic Gaucher disease: demographic and clinical features of 131 patients enrolled in the International Collaborative Gaucher Group Neurological Outcomes Subregistry. *Journal of inherited metabolic disease*. 2010; 33:339–346. [PubMed: 20084461]
40. Patterson MC, Horowitz M, Abel RB, Currie JN, Yu KT, Kaneski C, Higgins JJ, O'Neill RR, Fedio P, Pikus A, et al. Isolated horizontal supranuclear gaze palsy as a marker of severe systemic involvement in Gaucher's disease. *Neurology*. 1993; 43:1993–1997. [PubMed: 8413956]
41. Sidransky E, Tsuji S, Stubblefield BK, Currie J, FitzGibbon EJ, Ginns EI. Gaucher patients with oculomotor abnormalities do not have a unique genotype. *Clinical genetics*. 1992; 41:1–5. [PubMed: 1633639]
42. Park JK, Orvisky E, Tayebi N, Kaneski C, Lamarca ME, Stubblefield BK, Martin BM, Schiffmann R, Sidransky E. Myoclonic epilepsy in Gaucher disease: genotype-phenotype insights from a rare patient subgroup. *Pediatric research*. 2003; 53:387–395. [PubMed: 12595585]
43. Abrahamov A, Elstein D, Gross-Tsur V, Farber B, Glaser Y, Hadas-Halpern I, Ronen S, Tafakjdi M, Horowitz M, Zimran A. Gaucher's disease variant characterised by progressive calcification of heart valves and unique genotype. *Lancet (London, England)*. 1995; 346:1000–1003.
44. Desnick SJ, Desnick RJ, Brady RO, Pentchev PG, Simmons RL, Najarian JS, Swaiman K, Sharp HL, Krivit W. Renal transplantation in Type II Gaucher disease. *Birth defects original article series*. 1973; 9:109–119. [PubMed: 4611522]
45. Schiffmann R, Heyes MP, Aerts JM, Dambrosia JM, Patterson MC, DeGraba T, Parker CC, Zirzow GC, Oliver K, Tedeschi G, Brady RO, Barton NW. Prospective study of neurological responses to treatment with macrophage-targeted glucocerebrosidase in patients with type 3 Gaucher's disease. *Annals of neurology*. 1997; 42:613–621. [PubMed: 9382473]
46. Altarescu G, Hill S, Wiggs E, Jeffries N, Kreps C, Parker CC, Brady RO, Barton NW, Schiffmann R. The efficacy of enzyme replacement therapy in patients with chronic neuronopathic Gaucher's disease. *The Journal of pediatrics*. 2001; 138:539–547. [PubMed: 11295718]
47. Benko W, Ries M, Wiggs EA, Brady RO, Schiffmann R, Fitzgibbon EJ. The saccadic and neurological deficits in type 3 Gaucher disease. *PloS one*. 2011; 6:e22410. [PubMed: 21799847]
48. Zirzow GC, Sanchez OA, Murray GJ, Brady RO, Oldfield EH. Delivery, distribution, and neuronal uptake of exogenous mannose-terminal glucocerebrosidase in the intact rat brain. *Neurochemical research*. 1999; 24:301–305. [PubMed: 9972879]
49. Bembi BCG, Zanatta M, Bottega M, Pelos G, Gornati R, Berra B. Cerebrospinal-fluid infusion of Alglucerase in the Treatment of Acuteneuronopathic Gauchers-Disease. *Pediatric research*. 1995; 38:425.
50. Lonser RR, Schiffman R, Robison RA, Butman JA, Quezado Z, Walker ML, Morrison PF, Walbridge S, Murray GJ, Park DM, Brady RO, Oldfield EH. Image-guided, direct convective delivery of glucocerebrosidase for neuronopathic Gaucher disease. *Neurology*. 2007; 68:254–261. [PubMed: 17065591]
51. Weiss K, Gonzalez AN, Lopez G, Pedoeim L, Groden C, Sidransky E. The clinical management of Type 2 Gaucher disease. *Molecular genetics and metabolism*. 2015; 114:110–122. [PubMed: 25435509]
52. Schiffmann R, Fitzgibbon EJ, Harris C, DeVile C, Davies EH, Abel L, van Schaik IN, Benko W, Timmons M, Ries M, Vellodi A. Randomized, controlled trial of miglustat in Gaucher's disease type 3. *Annals of neurology*. 2008; 64:514–522. [PubMed: 19067373]

53. Lu J, Yang C, Chen M, Ye DY, Lonser RR, Brady RO, Zhuang Z. Histone deacetylase inhibitors prevent the degradation and restore the activity of glucocerebrosidase in Gaucher disease. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108:21200–21205. [PubMed: 22160715]
54. Brady RO, Yang C, Zhuang Z. An innovative approach to the treatment of Gaucher disease and possibly other metabolic disorders of the brain. *Journal of inherited metabolic disease*. 2013; 36:451–454. [PubMed: 22814681]
55. Yang C, Rahimpour S, Lu J, Pacak K, Ikejiri B, Brady RO, Zhuang Z. Histone deacetylase inhibitors increase glucocerebrosidase activity in Gaucher disease by modulation of molecular chaperones. *Proceedings of the National Academy of Sciences of the United States of America*. 2013; 110:966–971. [PubMed: 23277556]
56. Sidransky E. Gaucher disease: complexity in a “simple” disorder. *Molecular genetics and metabolism*. 2004; 83:6–15. [PubMed: 15464415]
57. Savica R, Boeve BF, Logroscino G. Epidemiology of alpha-synucleinopathies: from Parkinson disease to dementia with Lewy bodies. *Handbook of clinical neurology*. 2016; 138:153–158. [PubMed: 27637957]
58. Reichmann H, Brandt MD, Klingelhofer L. The nonmotor features of Parkinson’s disease: pathophysiology and management advances. *Current opinion in neurology*. 2016; 29:467–473. [PubMed: 27262147]
59. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL. Mutation in the alpha-synuclein gene identified in families with Parkinson’s disease. *Science (New York, NY)*. 1997; 276:2045–2047.
60. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. *Nature*. 1997; 388:839–840. [PubMed: 9278044]
61. Hernandez DG, Reed X, Singleton AB. Genetics in Parkinson disease: Mendelian versus non-Mendelian inheritance. *Journal of neurochemistry*. 2016
62. Neudorfer O, Giladi N, Elstein D, Abrahamov A, Turezkite T, Aghai E, Reches A, Bembi B, Zimran A. Occurrence of Parkinson’s syndrome in type I Gaucher disease. *QJM : monthly journal of the Association of Physicians*. 1996; 89:691–694. [PubMed: 8917744]
63. Tayebi NCM, Madike V, Stubblefield BK, Orvisky E, Krasnewich D, Fillano JJ, Sidransky E. Gaucher disease and parkinsonism: a phenotypic and genotypic characterization. *Molecular genetics and metabolism*. 2001; 73:313–321. [PubMed: 11509013]
64. Wong KTH, Tresser N, Schiffmann R. Dementia with Lewy bodies and parkinsonism in a type 1 Gaucher disease patient. *J Neuropathol Exp Neurol*. 2000; 59:454.
65. Wong K, Sidransky E, Verma A, Mixon T, Sandberg GD, Wakefield LK, Morrison A, Lwin A, Colegial C, Allman JM, Schiffmann R. Neuropathology provides clues to the pathophysiology of Gaucher disease. *Molecular genetics and metabolism*. 2004; 82:192–207. [PubMed: 15234332]
66. Tayebi N, Walker J, Stubblefield B, Orvisky E, LaMarca ME, Wong K, Rosenbaum H, Schiffmann R, Bembi B, Sidransky E. Gaucher disease with parkinsonian manifestations: does glucocerebrosidase deficiency contribute to a vulnerability to parkinsonism? *Molecular genetics and metabolism*. 2003; 79:104–109. [PubMed: 12809640]
67. Goker-Alpan O, Schiffmann R, LaMarca ME, Nussbaum RL, McInerney-Leo A, Sidransky E. Parkinsonism among Gaucher disease carriers. *Journal of medical genetics*. 2004; 41:937–940. [PubMed: 15591280]
68. Lwin A, Orvisky E, Goker-Alpan O, LaMarca ME, Sidransky E. Glucocerebrosidase mutations in subjects with parkinsonism. *Molecular genetics and metabolism*. 2004; 81:70–73. [PubMed: 14728994]
69. Aharon-Peretz JRH, Gershoni-Baruch R. Mutations in the glucocerebrosidase gene and Parkinson’s disease in Ashkenazi Jews. *The New England journal of medicine*. 2004; 351:1972. [PubMed: 15525722]
70. Sidransky E, Nalls MA, Aasly JO, Aharon-Peretz J, Annesi G, Barbosa ER, Bar-Shira A, Berg D, Bras J, Brice A, Chen CM, Clark LN, Condroyer C, De Marco EV, Durr A, Eblan MJ, Fahn S,

Farrer MJ, Fung HC, Gan-Or Z, Gasser T, Gershoni-Baruch R, Giladi N, Griffith A, Gurevich T, Januario C, Kropp P, Lang AE, Lee-Chen GJ, Lesage S, Marder K, Mata IF, Mirelman A, Mitsui J, Mizuta I, Nicoletti G, Oliveira C, Ottman R, Orr-Urtreger A, Pereira LV, Quattrone A, Rogaeva E, Rolfs A, Rosenbaum H, Rozenberg R, Samii A, Samaddar T, Schulte C, Sharma M, Singleton A, Spitz M, Tan EK, Tayebi N, Toda T, Troiano AR, Tsuji S, Wittstock M, Wolfsberg TG, Wu YR, Zabetian CP, Zhao Y, Ziegler SG. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. *The New England journal of medicine*. 2009; 361:1651–1661. [PubMed: 19846850]

71. Sardi SP, Cheng SH, Shihabuddin LS. Gaucher-related synucleinopathies: the examination of sporadic neurodegeneration from a rare (disease) angle. *Progress in neurobiology*. 2015; 125:47–62. [PubMed: 25573151]
72. Nalls MA, Duran R, Lopez G, Kurzawa-Akanbi M, McKeith IG, Chinnery PF, Morris CM, Theuns J, Crosiers D, Cras P, Engelborghs S, De Deyn PP, Van Broeckhoven C, Mann DM, Snowden J, Pickering-Brown S, Halliwell N, Davidson Y, Gibbons L, Harris J, Sheerin UM, Bras J, Hardy J, Clark L, Marder K, Honig LS, Berg D, Maetzler W, Brockmann K, Gasser T, Novellino F, Quattrone A, Annesi G, De Marco EV, Rogaeva E, Masellis M, Black SE, Bilbao JM, Foroud T, Ghetti B, Nichols WC, Pankratz N, Halliday G, Lesage S, Klebe S, Durr A, Duyckaerts C, Brice A, Giasson BI, Trojanowski JQ, Hurtig HI, Tayebi N, Landazabal C, Knight MA, Keller M, Singleton AB, Wolfsberg TG, Sidransky E. A multicenter study of glucocerebrosidase mutations in dementia with Lewy bodies. *JAMA neurology*. 2013; 70:727–735. [PubMed: 23588557]
73. Mitsui J, Matsukawa T, Sasaki H, Yabe I, Matsushima M, Durr A, Brice A, Takashima H, Kikuchi A, Aoki M, Ishiura H, Yasuda T, Date H, Ahsan B, Iwata A, Goto J, Ichikawa Y, Nakahara Y, Momose Y, Takahashi Y, Hara K, Kakita A, Yamada M, Takahashi H, Onodera O, Nishizawa M, Watanabe H, Ito M, Sobue G, Ishikawa K, Mizusawa H, Kanai K, Hattori T, Kuwabara S, Arai K, Koyano S, Kuroiwa Y, Hasegawa K, Yuasa T, Yasui K, Nakashima K, Ito H, Izumi Y, Kaji R, Kato T, Kusunoki S, Osaki Y, Horiuchi M, Kondo T, Murayama S, Hattori N, Yamamoto M, Murata M, Satake W, Toda T, Filla A, Klockgether T, Wullner U, Nicholson G, Gilman S, Tanner CM, Kukull WA, Stern MB, Lee VM, Trojanowski JQ, Masliah E, Low PA, Sandroni P, Ozelius LJ, Foroud T, Tsuji S. Variants associated with Gaucher disease in multiple system atrophy. *Annals of clinical and translational neurology*. 2015; 2:417–426. [PubMed: 25909086]
74. Gan-Or ZOL, Bar-Shira A, Saunders-Pullman R, Mirelman A, Kornerich R, Gana-Weisz M, Raymond D, Rozenkrantz L, Deik A, Gurevich T, Gross SJ, Schreiber-Agus N, Giladi N, Bressman SB, Orr-Urtreger A. The p.L302P mutation in the lysosomal enzyme gene SMPD1 is a risk factor for Parkinson disease. *Neurology*. 2013; 80:1606–1610. [PubMed: 23535491]
75. Lopez G, Kim J, Wiggs E, Cintron D, Groden C, Tayebi N, Mistry PK, Pastores GM, Zimran A, Goker-Alpan O, Sidransky E. Clinical course and prognosis in patients with Gaucher disease and parkinsonism *Neurology. Genetics*. 2016; 2:e57.
76. Goker-Alpan O, Lopez G, Vithayathil J, Davis J, Hallett M, Sidransky E. The spectrum of parkinsonian manifestations associated with glucocerebrosidase mutations. *Archives of neurology*. 2008; 65:1353–1357. [PubMed: 18852351]
77. McNeill A, Duran R, Proukakis C, Bras J, Hughes D, Mehta A, Hardy J, Wood NW, Schapira AH. Hyposmia and cognitive impairment in Gaucher disease patients and carriers. *Movement disorders : official journal of the Movement Disorder Society*. 2012; 27:526–532. [PubMed: 22344629]
78. Gan-Or Z, Amshalom I, Kilarski LL, Bar-Shira A, Gana-Weisz M, Mirelman A, Marder K, Bressman S, Giladi N, Orr-Urtreger A. Differential effects of severe vs mild GBA mutations on Parkinson disease. *Neurology*. 2015; 84:880–887. [PubMed: 25653295]
79. Cilia RTS, Marotta G, Cereda E, Siri C, Tesi S, Zecchinelli AL, Canesi M, Mariani CB, Meucci N, Sacilotto G, Zini M, Barichella M, Magnani C, Duga S, Asselta R, Soldà G, Seresini A, Seia M, Pezzoli G, Goldwurm S. Survival and dementia in GBA-associated Parkinson's disease: The mutation matters. *Annals of neurology*. 2016
80. Liu GBB, Locascio JJ, Jansen IE, Winder-Rhodes S, Eberly S, Elbaz A, Brice A, Ravina B, van Hilten JJ, Cormier-Dequaire F, Corvol JC, Barker RA, Heutink P, Marinus J, Williams-Gray CH, Scherzer CR. for theInternational Genetics of Parkinson Disease Progression (IGPP) Consortium. Neuropathic Gaucher's mutations accelerate cognitive decline in Parkinson's. *Annals of neurology*. 2016

81. Davis MYJC, Leverenz JB, Weintraub D, Trojanowski JQ, Chen-Plotkin A, Van Deerlin VM, Quinn JF, Chung KA, Peterson-Hiller AL, Rosenthal LS, Dawson TM, Albert MS, Goldman JG, Stebbins GT, Bernard B, Wszolek ZK, Ross OA, Dickson, Eidelberg D, Mattis PJ, Niethammer M, Yearout D, Hu SC, Cholerton BA, Smith M, Mata IF, Montine TJ, Edwards KL, Zabetian CP. Association of GBA Mutations and the E326K Polymorphism With Motor and Cognitive Progression in Parkinson Disease. *JAMA neurology*. 2016; 73:1217–1224. [PubMed: 27571329]
82. Mallett V, Ross JP, Alcalay RN, Ambalavanan A, Sidransky E, Dion PA, Rouleau GA, Gan-Or Z. GBA p.T369M substitution in Parkinson disease: Polymorphism or association? A meta-analysis *Neurology. Genetics*. 2016; 2:e104.
83. Goker-Alpan O, Masdeu JC, Kohn PD, Ianni A, Lopez G, Groden C, Chapman MC, Cropp B, Eisenberg DP, Maniwang ED, Davis J, Wiggs E, Sidransky E, Berman KF. The neurobiology of glucocerebrosidase-associated parkinsonism: a positron emission tomography study of dopamine synthesis and regional cerebral blood flow. *Brain : a journal of neurology*. 2012; 135:2440–2448. [PubMed: 22843412]
84. Siebert M, Sidransky E, Westbroek W. Glucocerebrosidase is shaking up the synucleinopathies. *Brain : a journal of neurology*. 2014; 137:1304–1322. [PubMed: 24531622]
85. Beavan MS, Schapira AH. Glucocerebrosidase mutations and the pathogenesis of Parkinson disease. *Annals of medicine*. 2013; 45:511–521. [PubMed: 24219755]
86. Sidransky E, Lopez G. The link between the GBA gene and parkinsonism *The Lancet. Neurology*. 2012; 11:986–998. [PubMed: 23079555]
87. Mazzulli JR, Xu YH, Sun Y, Knight AL, McLean PJ, Caldwell GA, Sidransky E, Grabowski GA, Krainc D. Gaucher disease glucocerebrosidase and alpha-synuclein form a bidirectional pathogenic loop in synucleinopathies. *Cell*. 2011; 146:37–52. [PubMed: 21700325]
88. Gegg ME, Burke D, Heales SJ, Cooper JM, Hardy J, Wood NW, Schapira AH. Glucocerebrosidase deficiency in substantia nigra of parkinson disease brains. *Annals of neurology*. 2012; 72:455–463. [PubMed: 23034917]
89. Murphy KE, Gysbers AM, Abbott SK, Tayebi N, Kim WS, Sidransky E, Cooper A, Garner B, Halliday GM. Reduced glucocerebrosidase is associated with increased alpha-synuclein in sporadic Parkinson's disease. *Brain : a journal of neurology*. 2014; 137:834–848. [PubMed: 24477431]
90. Mistry PKSS, Yang R, Yee J, Yang M. Consequences of diagnostic delays in type 1 Gaucher disease: the need for greater awareness among hematologists-oncologists and an opportunity for early diagnosis and intervention. *Am J Hematol*. 2007; 82:697–701. [PubMed: 17492645]
91. Weinreb NJLR. Causes of death due to hematological and non-hematological cancers in 57 US patients with type 1 Gaucher Disease who were never treated with enzyme replacement therapy. *Critical reviews in oncogenesis*. 2013; 18:177–195. [PubMed: 23510063]
92. Arends M, van Dussen L, Biegstraaten M, Hollak CE. Malignancies and monoclonal gammopathy in Gaucher disease; a systematic review of the literature. *British journal of haematology*. 2013; 161:832–842. [PubMed: 23594419]
93. Ogretmen BHY. Biologically active sphingolipids in cancer pathogenesis and treatment. *Nat Rev Cancer*. 2004; 4:604–616. [PubMed: 15286740]
94. Rosenbloom BEWN, Zimran A, Kacena KA, Charrow J, Ward E. Gaucher disease and cancer incidence: a study from the Gaucher Registry. *Blood*. 2005; 105:4560–4572.
95. de Fost MVDS, Weverling GJ, Brill N, Brett S, Häussinger D, Hollak CE. Increased incidence of cancer in adult Gaucher disease in Western Europe. *Blood cells, molecules & diseases*. 2006; 36:53–58.
96. Taddei THKK, Yang M, Yang R, Malhotra A, Boxer M, Aleck KA, Rennert G, Pastores GM, Mistry PK. The underrecognized progressive nature of N370S Gaucher disease and assessment of cancer risk in 403 patients. *Am J Hematol*. 2009; 84:208–214. [PubMed: 19260119]
97. Mizukami H, Mi Y, Wada R, Kono M, Yamashita T, Liu Y, Werth N, Sandhoff R, Sandhoff K, Proia RL. Systemic inflammation in glucocerebrosidase-deficient mice with minimal glucosylceramide storage. *The Journal of clinical investigation*. 2002; 109:1215–1221. [PubMed: 11994410]

98. Allen MJMB, Khokher AM, Rushton N, Cox TM. Pro-inflammatory cytokines and the pathogenesis of Gaucher's disease: increased release of interleukin-6 and interleukin-10. *QJM : monthly journal of the Association of Physicians*. 1997; 90:19–25. [PubMed: 9093585]
99. Castaneda JALM, Cooper JD, Pearce DA. Immune system irregularities in lysosomal storage disorders. *Acta Neuropathol*. 2008; 115:159–174. [PubMed: 17924126]
100. vDL, Dekker N, Hollak CE, Overkleeft H, Scheij S, Ghauharali K, van Breemen MJ, Ferraz MJ, Groener JE, Maas M, Wijburg FA, Speijer D, Tylki-Szymanska A, Mistry PK, Boot RG, Aerts JM. Elevated plasma glucosylsphingosine in Gaucher disease: relation to phenotype, storage cell markers, and therapeutic response. *Blood*. 2011; 118:e118–127. [PubMed: 21868580]
101. Mistry PK, Liu J, Sun L, Chuang WL, Yuen T, Yang R, Lu P, Zhang K, Li J, Keutzer J, Stachnik A, Mennone A, Boyer JL, Jain D, Brady RO, New MI, Zaidi M. Glucocerebrosidase 2 gene deletion rescues type 1 Gaucher disease. *Proceedings of the National Academy of Sciences of the United States of America*. 2014; 111:4934–4939. [PubMed: 24639522]
102. Nair S, Boddupalli CS, Verma R, Liu J, Yang R, Pastores GM, Mistry PK, Dhodapkar MV. Type II NKT-TFH cells against Gaucher lipids regulate B-cell immunity and inflammation. *Blood*. 2015; 125:1256–1271. [PubMed: 25499455]
103. Nair SBA, Liu J, Boddupalli CS, Mistry PK, Dhodapkar MV. Clonal Immunoglobulin against Lysolipids in the Origin of Myeloma. *The New England journal of medicine*. 2016; 374:555–561. [PubMed: 26863356]
104. Pavlova EV, Archer J, Wang S, Dekker N, Aerts JM, Karlsson S, Cox TM. Inhibition of UDP-glucosylceramide synthase in mice prevents Gaucher disease-associated B-cell malignancy. *The Journal of pathology*. 2015; 235:113–124. [PubMed: 25256118]
105. Ilyas AAQR, Dalakas MC, Brady RO. Polyneuropathy with monoclonal gammopathy: glycolipids are frequently antigens for IgM paraproteins. *Proceedings of the National Academy of Sciences of the United States of America*. 1985; 82:6697–6700. [PubMed: 2413445]
106. Serratrice CCS, Serratrice J, Stirnemann J. Imiglucerase in the management of Gaucher disease type I: an evidence-based review of its place in therapy. *Core Evid*. 2016; 11:37–47. [PubMed: 27790078]
107. Mistry PKDP, Vellodi A, Cole JA, Yeh M, Weinreb NJ. Timing of initiation of enzyme replacement therapy after diagnosis of type 1 Gaucher disease: effect on incidence of avascular necrosis. *British journal of haematology*. 2009; 147:561–570. [PubMed: 19732054]
108. Rogaeva E, Hardy J. Gaucher and Parkinson diseases: unexpectedly related. *Neurology*. 2008; 70:2272–2273. [PubMed: 18541881]
109. Lee NCCY, Wong SL, Sheen JM, Tsai FJ, Peng SF, Leung JH, Chao MC, Shun CT, Hwu WL. Outcome of early-treated type III Gaucher disease patients. *Blood cells, molecules & diseases*. 2014; 53:105–109.
110. Monestime G, Borger DK, Kim J, Lopez G, Allgaeuer M, Jain D, Vortmeyer A, Wang HW, Sidransky E. Varied autopsy findings in five treated patients with Gaucher disease and parkinsonism include the absence of Gaucher cells. *Molecular genetics and metabolism*. 2016; 118:55–59. [PubMed: 26992326]
111. Burrow TA, Sun Y, Prada CE, Bailey L, Zhang W, Brewer A, Wu SW, Setchell KD, Witte D, Cohen MB, Grabowski GA. CNS, lung, and lymph node involvement in Gaucher disease type 3 after 11 years of therapy: clinical, histopathologic, and biochemical findings. *Molecular genetics and metabolism*. 2015; 114:233–241. [PubMed: 25219293]
112. Starzyk KRS, Yee J, Smith SE, Kingma W. The long-term international safety experience of imiglucerase therapy for Gaucher disease. *Molecular genetics and metabolism*. 2007; 90:157–163. [PubMed: 17079176]
113. Zimran APG, Tylki-Szymanska A, Hughes DA, Elstein D, Mardach R, Eng C, Smith L, Heisel-Kurth M, Charrow J, Harmatz P, Fernhoff P, Rhead W, Longo N, Giraldo P, Ruiz JA, Zahrieh D, Crombez E, Grabowski GA. Safety and efficacy of velaglucerase alfa in Gaucher disease type 1 patients previously treated with imiglucerase. *Am J Hematol*. 2013;3.
114. Pastores GMRB, Weinreb N, Goker-Alpan O, Grabowski G, Cohn GM, Zahrieh D. A multicenter open-label treatment protocol (HGT-GCB-058) of velaglucerase alfa enzyme replacement

- therapy in patients with Gaucher disease type 1: safety and tolerability. *Genet Med.* 2014; 16:359–366. [PubMed: 24263462]
115. Pastores GMSS, Petakov M, Giraldo P, Rosenbaum H, Amato DJ, Szer J, Chertkoff R, Brill-Almon E, Zimran A. Enzyme replacement therapy with taliglucerase alfa: 36-month safety and efficacy results in adult patients with Gaucher disease previously treated with imiglucerase. *Am J Hematol.* 2016; 91:661–665. [PubMed: 27102949]
116. Platt FM, Neises GR, Karlsson GB, Dwek RA, Butters TD. N-butyldeoxygalactonojirimycin inhibits glycolipid biosynthesis but does not affect N-linked oligosaccharide processing. *The Journal of biological chemistry.* 1994; 269:27108–27114. [PubMed: 7929454]
117. Radin NS. Treatment of Gaucher disease with an enzyme inhibitor. *Glycoconjugate journal.* 1996; 13:153–157. [PubMed: 8737239]
118. Platt FM, Jeyakumar M. Substrate reduction therapy. *Acta paediatrica (Oslo, Norway : 1992).* 2008; 97:88–93.
119. Cox T, Lachmann R, Hollak C, Aerts J, van Weely S, Hrebicek M, Platt F, Butters T, Dwek R, Moyses C, Gow I, Elstein D, Zimran A. Novel oral treatment of Gaucher's disease with N-butyldeoxynojirimycin (OGT 918) to decrease substrate biosynthesis. *Lancet (London, England).* 2000; 355:1481–1485.
120. Cox TM, Amato D, Hollak CE, Luzy C, Silkey M, Giorgino R, Steiner RD. Evaluation of miglustat as maintenance therapy after enzyme therapy in adults with stable type 1 Gaucher disease: a prospective, open-label non-inferiority study. *Orphanet journal of rare diseases.* 2012; 7:102. [PubMed: 23270487]
121. Cox TM. Eliglustat tartrate, an orally active glucocerebroside synthase inhibitor for the potential treatment of Gaucher disease and other lysosomal storage diseases. *Current opinion in investigational drugs (London, England : 2000).* 2010; 11:1169–1181.
122. Lukina E, Watman N, Arreguin EA, Banikazemi M, Dragosky M, Iastrebner M, Rosenbaum H, Phillips M, Pastores GM, Rosenthal DI, Kaper M, Singh T, Puga AC, Bonate PL, Peterschmitt MJ. A phase 2 study of eliglustat tartrate (Genz-112638), an oral substrate reduction therapy for Gaucher disease type 1. *Blood.* 2010; 116:893–899. [PubMed: 20439622]
123. Cox TM, Drelichman G, Cravo R, Balwani M, Burrow TA, Martins AM, Lukina E, Rosenbloom B, Ross L, Angell J, Puga AC. Eliglustat compared with imiglucerase in patients with Gaucher's disease type 1 stabilised on enzyme replacement therapy: a phase 3, randomised, open-label, non-inferiority trial. *Lancet (London, England).* 2015; 385:2355–2362.
124. Mistry PK, Lukina E, Ben Turkia H, Amato D, Baris H, Dasouki M, Ghosn M, Mehta A, Packman S, Pastores G, Petakov M, Assouline S, Balwani M, Danda S, Hadjiev E, Ortega A, Shankar S, Solano MH, Ross L, Angell J, Peterschmitt MJ. Effect of oral eliglustat on splenomegaly in patients with Gaucher disease type 1: the ENGAGE randomized clinical trial. *Jama.* 2015; 313:695–706. [PubMed: 25688781]
125. Balwani M, Burrow TA, Charrow J, Goker-Alpan O, Kaplan P, Kishnani PS, Mistry P, Ruskin J, Weinreb N. Recommendations for the use of eliglustat in the treatment of adults with Gaucher disease type 1 in the United States. *Molecular genetics and metabolism.* 2016; 117:95–103. [PubMed: 26387627]
126. Ibrahim J, Underhill LH, Taylor JS, Angell J, Peterschmitt MJ. Clinical response to eliglustat in treatment-naïve patients with Gaucher disease type 1: Post-hoc comparison to imiglucerase-treated patients enrolled in the International Collaborative Gaucher Group Gaucher Registry. *Molecular genetics and metabolism reports.* 2016; 8:17–19. [PubMed: 27408819]
127. Mistry PK, Liu J, Yang M, Nottoli T, McGrath J, Jain D, Zhang K, Keutzer J, Chuang WL, Mehal WZ, Zhao H, Lin A, Mane S, Liu X, Peng YZ, Li JH, Agrawal M, Zhu LL, Blair HC, Robinson LJ, Iqbal J, Sun L, Zaidi M. Glucocerebroside gene-deficient mouse recapitulates Gaucher disease displaying cellular and molecular dysregulation beyond the macrophage. *Proceedings of the National Academy of Sciences of the United States of America.* 2010; 107:19473–19478. [PubMed: 20962279]
128. Brennan PJ, Tatituri RV, Brigl M, Kim EY, Tuli A, Sanderson JP, Gadola SD, Hsu FF, Besra GS, Brenner MB. Invariant natural killer T cells recognize lipid self antigen induced by microbial danger signals. *Nature immunology.* 2011; 12:1202–1211. [PubMed: 22037601]

129. Fink JK, Correll PH, Perry LK, Brady RO, Karlsson S. Correction of glucocerebrosidase deficiency after retroviral-mediated gene transfer into hematopoietic progenitor cells from patients with Gaucher disease. *Proceedings of the National Academy of Sciences of the United States of America*. 1990; 87:2334–2338. [PubMed: 2315324]
130. Dunbar CEK, Schiffmann R, Barton NW, Nolte JA, Esplin JA, Pensiero M, Long Z, Lockey C, Emmons RV, Csik S, Leitman S, Krebs CB, Carter C, Brady RO, Karlsson S. Retroviral transfer of the glucocerebrosidase gene into CD34+ cells from patients with Gaucher disease: in vivo detection of transduced cells without myeloablation. *Hum Gene Ther*. 1998; 9:2629–2640. [PubMed: 9853529]
131. Dahl MDA, Olsson K, Mansson JE, Marques AR, Mirzaian M, Aerts JM, Ehinger M, Rothe M, Modlich U, Schambach A, Karlsson S. Lentiviral gene therapy using cellular promoters cures type 1 Gaucher disease in mice. *Mol Ther*. 2015; 23:835–844. [PubMed: 25655314]
132. Compain P, Martin OR, Boucheron C, Godin G, Yu L, Ikeda K, Asano N. Design and synthesis of highly potent and selective pharmacological chaperones for the treatment of Gaucher's disease. *Chembiochem : a European journal of chemical biology*. 2006; 7:1356–1359. [PubMed: 16871601]
133. Lieberman RL, Wustman BA, Huertas P, Powe AC Jr, Pine CW, Khanna R, Schlossmacher MG, Ringe D, Petsko GA. Structure of acid beta-glucosidase with pharmacological chaperone provides insight into Gaucher disease. *Nature chemical biology*. 2007; 3:101–107. [PubMed: 17187079]
134. Meusser B, Hirsch C, Jarosch E, Sommer T. ERAD: the long road to destruction. *Nature cell biology*. 2005; 7:766–772. [PubMed: 16056268]
135. Sawkar AR, Cheng WC, Beutler E, Wong CH, Balch WE, Kelly JW. Chemical chaperones increase the cellular activity of N370S beta-glucosidase: a therapeutic strategy for Gaucher disease. *Proceedings of the National Academy of Sciences of the United States of America*. 2002; 99:15428–15433. [PubMed: 12434014]
136. Gloster TM, Davies GJ. Glycosidase inhibition: assessing mimicry of the transition state. *Organic & biomolecular chemistry*. 2010; 8:305–320. [PubMed: 20066263]
137. Butters TD, Dwek RA, Platt FM. Imino sugar inhibitors for treating the lysosomal glycosphingolipidoses. *Glycobiology*. 2005; 15:43r–52r. [PubMed: 15329358]
138. Khanna RBE, Pellegrino L, Schilling A, Rigat BA, Soska R, Nafar H, Raney BE, Feng J, Lun Y, Powe AC, Palling DJ, Wustman BA, Schiffmann R, Mahuran DJ, Lockhart DJ, Valenzano KJ. The pharmacological chaperone isofagomine increases the activity of the Gaucher disease L444P mutant form of beta-glucosidase. *The FEBS journal*. 2010; 277:1618–1638. [PubMed: 20148966]
139. Sun Y, Liou B, Xu YH, Quinn B, Zhang W, Hamler R, Setchell KD, Grabowski GA. Ex vivo and in vivo effects of isofagomine on acid beta-glucosidase variants and substrate levels in Gaucher disease. *The Journal of biological chemistry*. 2012; 287:4275–4287. [PubMed: 22167193]
140. Sun Y, Ran H, Liou B, Quinn B, Zamzow M, Zhang W, Bielawski J, Kitatani K, Setchell KD, Hannun YA, Grabowski GA. Isofagomine in vivo effects in a neuropathic Gaucher disease mouse. *PloS one*. 2011; 6:e19037. [PubMed: 21533102]
141. Panicker LM, Miller D, Park TS, Patel B, Azevedo JL, Awad O, Masood MA, Veenstra TD, Goldin E, Stubblefield BK, Tayebi N, Polumuri SK, Vogel SN, Sidransky E, Zambidis ET, Feldman RA. Induced pluripotent stem cell model recapitulates pathologic hallmarks of Gaucher disease. *Proceedings of the National Academy of Sciences of the United States of America*. 2012; 109:18054–18059. [PubMed: 23071332]
142. Zimran A. How I treat Gaucher disease. *Blood*. 2011; 118:1463–1471. [PubMed: 21670466]
143. Maegawa GH, Tropak MB, Buttner JD, Rigat BA, Fuller M, Pandit D, Tang L, Kornhaber GJ, Hamuro Y, Clarke JT, Mahuran DJ. Identification and characterization of ambroxol as an enzyme enhancement agent for Gaucher disease. *The Journal of biological chemistry*. 2009; 284:23502–23516. [PubMed: 19578116]
144. Luan Z, Li L, Higaki K, Nanba E, Suzuki Y, Ohno K. The chaperone activity and toxicity of ambroxol on Gaucher cells and normal mice. *Brain & development*. 2013; 35:317–322. [PubMed: 22682976]

145. Babajani G, Tropak MB, Mahuran DJ, Kermode AR. Pharmacological chaperones facilitate the post-ER transport of recombinant N370S mutant beta-glucocerebrosidase in plant cells: evidence that N370S is a folding mutant. *Molecular genetics and metabolism*. 2012; 106:323–329. [PubMed: 22592100]
146. Bendikov-Bar I, Maor G, Filocamo M, Horowitz M. Ambroxol as a pharmacological chaperone for mutant glucocerebrosidase. *Blood cells, molecules & diseases*. 2013; 50:141–145.
147. Narita A, Shirai K, Itamura S, Matsuda A, Ishihara A, Matsushita K, Fukuda C, Kubota N, Takayama R, Shigematsu H, Hayashi A, Kumada T, Yuge K, Watanabe Y, Kosugi S, Nishida H, Kimura Y, Endo Y, Higaki K, Nanba E, Nishimura Y, Tamasaki A, Togawa M, Saito Y, Maegaki Y, Ohno K, Suzuki Y. Ambroxol chaperone therapy for neuronopathic Gaucher disease: A pilot study. *Annals of clinical and translational neurology*. 2016; 3:200–215. [PubMed: 27042680]
148. Jung O, Patnaik S, Marugan J, Sidransky E, Westbroek W. Progress and potential of non-inhibitory small molecule chaperones for the treatment of Gaucher disease and its implications for Parkinson disease. *Expert review of proteomics*. 2016; 13:471–479. [PubMed: 27098312]
149. Goldin E, Zheng W, Motabar O, Southall N, Choi JH, Marugan J, Austin CP, Sidransky E. High throughput screening for small molecule therapy for Gaucher disease using patient tissue as the source of mutant glucocerebrosidase. *PLoS one*. 2012; 7:e29861. [PubMed: 22272254]
150. Aflaki E, Stubblefield BK, Maniwang E, Lopez G, Moaven N, Goldin E, Marugan J, Patnaik S, Dutra A, Southall N, Zheng W, Tayebi N, Sidransky E. Macrophage models of Gaucher disease for evaluating disease pathogenesis and candidate drugs. *Science translational medicine*. 2014; 6:240ra273.
151. Aflaki E, Borger DK, Moaven N, Stubblefield BK, Rogers SA, Patnaik S, Schoenen FJ, Westbroek W, Zheng W, Sullivan P, Fujiwara H, Sidhu R, Khaliq ZM, Lopez GJ, Goldstein DS, Ory DS, Marugan J, Sidransky E. A New Glucocerebrosidase Chaperone Reduces alpha-Synuclein and Glycolipid Levels in iPSC-Derived Dopaminergic Neurons from Patients with Gaucher Disease and Parkinsonism. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2016; 36:7441–7452. [PubMed: 27413154]
152. Mistry PK, Taddei T, vom Dahl S, Rosenbloom BE. Gaucher disease and malignancy: a model for cancer pathogenesis in an inborn error of metabolism. *Critical reviews in oncogenesis*. 2013; 18:235–246. [PubMed: 23510066]
153. Tybulewicz VL, Tremblay ML, LaMarca ME, Willemsen R, Stubblefield BK, Winfield S, Zablocka B, Sidransky E, Martin BM, Huang SP, et al. Animal model of Gaucher's disease from targeted disruption of the mouse glucocerebrosidase gene. *Nature*. 1992; 357:407–410. [PubMed: 1594045]
154. Holleran WM, Ginns EI, Menon GK, Grundmann JU, Fartasch M, McKinney CE, Elias PM, Sidransky E. Consequences of beta-glucocerebrosidase deficiency in epidermis. Ultrastructure and permeability barrier alterations in Gaucher disease. *The Journal of clinical investigation*. 1994; 93:1756–1764. [PubMed: 8163674]
155. Sidransky E, Fartasch M, Lee RE, Metlay LA, Abella S, Zimran A, Gao W, Elias PM, Ginns EI, Holleran WM. Epidermal abnormalities may distinguish type 2 from type 1 and type 3 of Gaucher disease. *Pediatric research*. 1996; 39:134–141. [PubMed: 8825398]
156. Liu Y, Suzuki K, Reed JD, Grinberg A, Westphal H, Hoffmann A, Doring T, Sandhoff K, Proia RL. Mice with type 2 and 3 Gaucher disease point mutations generated by a single insertion mutagenesis procedure. *Proceedings of the National Academy of Sciences of the United States of America*. 1998; 95:2503–2508. [PubMed: 9482915]
157. Xu YH, Quinn B, Witte D, Grabowski GA. Viable mouse models of acid beta-glucosidase deficiency: the defect in Gaucher disease. *The American journal of pathology*. 2003; 163:2093–2101. [PubMed: 14578207]
158. Enquist IB, Nilsson E, Ooka A, Mansson JE, Olsson K, Ehinger M, Brady RO, Richter J, Karlsson S. Effective cell and gene therapy in a murine model of Gaucher disease. *Proceedings of the National Academy of Sciences of the United States of America*. 2006; 103:13819–13824. [PubMed: 16954197]
159. Enquist IB, Lo Bianco C, Ooka A, Nilsson E, Mansson JE, Ehinger M, Richter J, Brady RO, Kirik D, Karlsson S. Murine models of acute neuronopathic Gaucher disease. *Proceedings of the*

- National Academy of Sciences of the United States of America. 2007; 104:17483–17488. [PubMed: 17954912]
160. Liu J, Halene S, Yang M, Iqbal J, Yang R, Mehal WZ, Chuang WL, Jain D, Yuen T, Sun L, Zaidi M, Mistry PK. Gaucher disease gene GBA functions in immune regulation. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109:10018–10023. [PubMed: 22665763]
161. Pentchev PG, Gal AE, Wong R, Morrone S, Neumeier B, Massey J, Kanter R, Sawitsky A, Brady RO. Biliary excretion of glycolipid in induced or inherited glucosylceramide lipidosis. Biochimica et biophysica acta. 1981; 665:615–618. [PubMed: 7295755]
162. Kanfer JN, Stephens MC, Singh H, Legler G. The Gaucher mouse. Progress in clinical and biological research. 1982; 95:627–644. [PubMed: 6812089]
163. Sgambato JAPT, Miller D, Panicker LM, Sidrnasky E, Lun Y, Awad O, Bentzen SM, Zambidis ET, Feldman RA. Gaucher Disease-Induced Pluripotent Stem Cells Display Decreased Erythroid Potential and Aberrant Myelopoiesis. Stem cells translational medicine. 2015; 4:878–886. [PubMed: 26062980]
164. Woodard CM, Campos BA, Kuo SH, Nirenberg MJ, Nestor MW, Zimmer M, Mosharov EV, Sulzer D, Zhou H, Paull D, Clark L, Schadt EE, Sardi SP, Rubin L, Eggan K, Brock M, Lipnick S, Rao M, Chang S, Li A, Noggle SA. iPSC-derived dopamine neurons reveal differences between monozygotic twins discordant for Parkinson’s disease. Cell reports. 2014; 9:1173–1182. [PubMed: 25456120]
165. Mazzulli JR, Zunke F, Tsunemi T, Toker NJ, Jeon S, Burbulla LF, Patnaik S, Sidransky E, Marugan JJ, Sue CM, Krainc D. Activation of beta-Glucocerebrosidase Reduces Pathological alpha-Synuclein and Restores Lysosomal Function in Parkinson’s Patient Midbrain Neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2016; 36:7693–7706. [PubMed: 27445146]

Highlights

- With the recent passing of Roscoe Brady, an era has ended in the field of lysosomal biology in health and disease.
- In the past decades considerable progress has been achieved in many aspects of Gaucher disease, including treatment.
- The association of glucocerebrosidase and parkinsonism has broadened the relevance of this research.

Gaucher Disease – Advances and Challenges

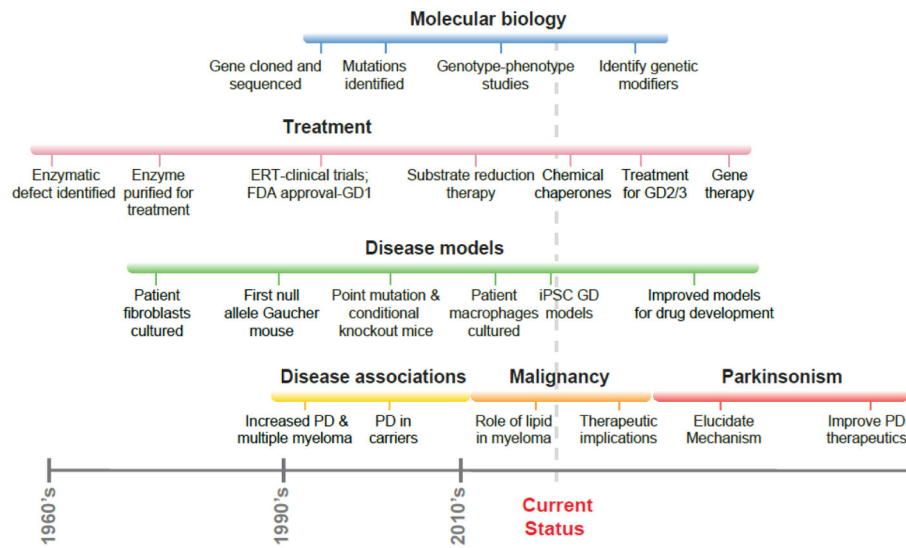


Figure 1. Timeline illustrating some of the progress made in different aspects of Gaucher disease and the ongoing challenges.

Table 1**Gaucher Disease: NIH Milestones**

1965 - Defect in glucocerebrosidase identified [7]
1967 - Diagnostic test for Gaucher disease [13]
1968 - Lysosomal localization of glucocerebrosidase [15]
1972 - Prenatal diagnosis of Gaucher disease [14]
1973 - Method for purifying the enzyme [16]
1980 - Different glucocerebrosidase isoforms found [18]
1984 - First bone marrow transplant [20]
1984 - Protein sequence identified [21]
1985 - Localization of gene to chromosome 1q21 [24]
1987 - First mutations identified [27, 28]
1991 - Successful ERT in type 1 Gaucher disease [37]
1992 - First mouse model of Gaucher disease generated [140]
1992 - Initial genotype-phenotype studies [40]
1994 - Identification of skin ultrastructural changes [141]
1996 - Neonatal Gaucher disease described [142]
1997 - ERT Trials in type 3 Gaucher disease [44]
2003 - Appreciation of link with Parkinson disease [63]
2004 - Parkinsonism in <i>GBA1</i> carriers [65]
2007 - High throughput screens for small molecules impacting GCase [153]
2007 - Intracerebral infusion of ERT attempted [49]
2008 - Description of randomized controlled trial of miglustat in patients with type 3 Gaucher disease
2009 - Multicenter study of <i>GBA1</i> mutations in Parkinson disease [67]
2011 - Histone deacetylation inhibitors proposed for Gaucher disease [52]
2012 Identification of non-inhibitory chaperones of glucocerebrosidase [136]
2012 PET imaging studies of <i>GBA1</i> -associated Parkinson disease [77]
2013 Multicenter study of <i>GBA1</i> mutations in Dementia with Lewy Bodies [69]
2014 Development of iPSC-derived models of Gaucher disease [137]
2016 Demonstration that a <i>GBA1</i> chaperone decreases a-synuclein in iPSC neurons [138]
