 REVIEW
Miglustat as a therapeutic agent: prospects and caveats
Rosemarie E Venier,1 Suleiman A Igdoura1,2

ABSTRACT
A viable treatment for lysosomal storage disease has been very difficult to attain. One option is pharmacological inhibition of synthetic pathways to reduce substrate accumulations. Miglustat N-butyldeoxynojirimycin (NBDNJ), an inhibitor of glucosylceramide synthase, has shown much promise in clinical trials for the treatment of Type I Gaucher disease. The molecular events invoked by NBDNJ in cell culture and in animal models have not been so definitive. This review discusses the biochemical and molecular impact of NBDNJ as it relates to its potential as a therapeutic drug.

Glycosphingolipids (GSLs) are glycolipids found within the plasma membrane. They are important molecules that facilitate cell–cell interactions and effective signalling. GSLs are localised in microdomains called lipid rafts; these rafts are enriched in sphingolipids, cholesterol, and glycosylphosphatidylinositol (GPI)-anchored proteins positioned to facilitate the efficient localisation of signalling proteins. Gangliosides are a subclass of sialylated GSLs. As part of normal turnover, gangliosides are internalised and targeted to the lysosome for degradation. There, individual sugars are cleaved from the molecule in a stepwise fashion by specific lysosomal hydrolases.1 Genetic defects in lysosomal enzymes result in the lysosomal storage of substrates and manifest as lysosomal storage diseases (LSDs), such as Tay-Sachs disease (TSD) and Sandhoff disease (SD). In addition to substrate accumulation, there is evidence that inflammation,2–4 microglial activation,5–7 and apoptosis,2,8,9 lead to characteristic neurodegeneration in patients affected by these and related disorders.

LSDs pose many unique challenges to the development of effective long-term therapy. Although glycosphingolipidoses manifest in similar ways, the accumulation of distinct substrates for each disease requires specialised treatment. Therapies currently under investigation include enzyme replacement, bone marrow transplantation, progenitor cell transplantation, viral gene therapy and substrate deprivation therapy. Each of these treatment types addresses a few of the challenges associated with lysosomal storage disorders, but an optimal therapy that can be applied universally to these devastating diseases is not currently available. The greatest hope for such a drug lies with N-butyldeoxynojirimycin (NBDNJ, Miglustat), an iminosugar that reduces GSL substrate synthesis.

Generally, iminosugars act as structural mono-saccharide mimics with nitrogen in place of the ring oxygen (figure 1).10 NBDNJ inhibits glucosylceramide synthase, and as a result prevents the formation of glycosylceramide (figure 2). NBDNJ facilitates this process through competitive inhibition of ceramide.11 Its ability to block GSL synthesis at such an early step as well as across the blood–brain barrier,12 makes NBDNJ a potential therapeutic drug for the treatment of multiple LSDs. Presently, NBDNJ is marketed under the name Miglustat and has been approved by the US Food and Drug Administration, the European Union and over 30 other countries for the treatment of adults with type I Gaucher disease for whom enzyme replacement therapy (ERT) is unsuitable. In addition, it has been approved in the European Union for the treatment of progressive neurological manifestations in adult and paediatric patients with Niemann-Pick disease type C (NPC).13 Gaucher disease results from a deficiency in acid β-glucosidase characterised by lysosomal storage of glucosylceramide, the substrate of acid β-glucosidase.14 In contrast, GSL accumulation is secondary to a cholesterol transport defect in NPC. This defect leads to the accumulation of cholesterol, sphingomyelin, phospholipids and glycolipids in the liver and spleen. Increased brain glycolipid levels are responsible for many of the symptoms experienced by NPC patients.15

Multiple studies have been performed to determine the efficacy of NBDNJ for treatment of other glycosphingolipid storage diseases. Overall, NBDNJ appears to be a useful and beneficial drug for substrate reduction therapy, particularly for the treatment of Gaucher disease, in which the drug target is immediately upstream of the accumulating substrate, and NPC, in which GSL accumulation is secondary (figure 3); however, the exact degree to which GSLs can be safely decreased in mammals is unknown. Gangliosides play a pivotal role in development and in neuronal function. It is therefore imperative to determine the possible molecular consequences of this therapy before implementing it as a standard treatment for all glycosphingolipidoses. This review aims to present evidence that illustrates the importance of gangliosides at the molecular level and cautions the widespread use of NBDNJ without further study.

LESSONS FROM MOUSE MODELS
Mouse models of LSDs have helped to elucidate the molecular mechanisms that may be involved in neurodegeneration and cell death. Mice that lack...
Therapeutics

Figure 1  Chemical structure of N-butyldeoxynojirimycin (NBDNJ). Imino sugars act as monosaccharide mimics with nitrogen in the place of oxygen in the ring. NBDNJ inhibits glucosylceramide synthase. Cer, ceramide; GlcCer, galactosylceramide; LacCer, lactosylceramide; SGMS, sphingomyelin synthase; GCS, glucosylceramide synthase; B4GalT6, lactosylceramide synthase; Siat 9, sialyltransferase 9; GalNACT, (NAcetylneuraminyl)-galactosylglucosylceramide N-acetylgalactosaminyltransferase; B3GalT4, ganglioside galactosyltransferase.

Figure 2  A-series ganglioside biosynthesis. Ganglioside biosynthesis occurs in a step-wise fashion by the addition of sugars. N-butyldeoxynojirimycin inhibits glucosylceramide synthase preventing the synthesis of downstream molecules. Sphingomyelin is also synthesised from ceramide. This pathway is activated when ceramide levels rise.

double knockouts showed GSL profiles identical to GalNAcT−/− single knockouts. The double knockouts lived for the entire experimental period and did not initially experience the behavioural deficiencies that HexB−/− mice did. Eventually, the double knockouts developed a progressively worsening ataxic gait at 7 months and muscle wasting and hunched posture at 9 months. Severe ataxia in these mice corresponded with the widespread loss of cerebellar Purkinje cells, which was not observed in GalNAcT−/− single knockouts. Nevertheless, double knockout of HexB and GalNAcT prolonged the life of the animals compared to single knockout of HexB, providing proof of principle for substrate reduction therapy.17

Studies in independently engineered GalNAcT−/− mice reinforced the idea that complex gangliosides play an important role in neuronal signal transduction. Takamiya et al.20 observed a reduced neural conduction velocity in the primary somatosensory cortex of GalNAcT knockouts, which was later found to be due to disrupted nodes of Ranvier.21 Another group noted axonal degeneration in both the central and peripheral nervous systems (CNS and PNS, respectively), reduced myelination in the CNS, and dysmyelination in both the CNS and PNS in their mice.22 23 These findings may be due to the lost interaction between ganglioside ligands and myelin-associated glycoprotein, which has been shown to maintain myelin stability.24 Similar results were found in GalNAcT−/− GM3 synthase (Siat9)−/− double knockout mice.24 Furthermore, in the presence of depolarising K+ levels or glutamate, apoptosis occurred in cerebellar granule neurons of GalNAcT−/− mice due to persistent intracellular Ca2+ elevation.25 These studies suggest that complex gangliosides are important in Ca2+ homeostasis, axonal maintenance and neuronal transmission. Therefore, the administration of substrate reduction therapy may be associated with negative consequences, especially in children and adolescents, in whom myelination may still be occurring as treatment begins.

Neuroinflammation is common among LSDs,26 and intrinsic molecular differences between diseased and wild type genotypes can exacerbate disease progression. For instance, the levels of the proinflammatory cytokines tumour necrosis factor α (TNF-α) and interleukin (IL)-1β are significantly higher in HexB−/− versus wild type mice, leading to pronounced microglia.2 These data suggest that a reduction of substrate accumulation may reduce the disease pathology caused by deregulated cytokine secretion. Recent investigations concerned with CD4 and CD8 T cell activation indicate that gangliosides play different roles in the maturation of T cell populations.
Work by Nagafuku et al.\textsuperscript{27} showed that the proliferation of CD4 T cells depends on a-series gangliosides, whereas CD8 T cell proliferation depends on the o-series during T cell receptor-mediated activation. T cells isolated from mice deficient in \textit{GalNAcT}\textsuperscript{-/-} also exhibited reduced IL-2 and interferon \(\gamma\) production, indicating the importance of gangliosides in immunity and the regulation of inflammatory responses. In contrast, \textit{GalNAcT}\textsuperscript{-/-} GD3 synthase (GD3S\textsuperscript{-/-}) double knockouts show significantly higher proinflammatory cytokine expression (IL1-\(\alpha\), IL1-\(\beta\), TNF-\(\alpha\)) and expression of the complement molecule C1q. Additionally, due to the absence of gangliosides, the lipid rafts in the cerebellar cells of these animals were diffuse. GPI-anchored regulatory complement proteins are normally found in these rafts. Thus, it is likely that the reduction of ganglioside levels leads to abnormal activation of the complement system, which propagates inflammation through the aforementioned cytokines. This hypothesis is supported by the rescue of this phenotype observed in a \textit{GalNAcT}\textsuperscript{-/-}\, GD3S\textsuperscript{-/-}\, C3\textsuperscript{-/-} triple knockout.\textsuperscript{28}

Mouse models of LSDs and synthesis mutants indicate that while gangliosides are necessary for proper development and the cell–cell interactions that affect immune system function, a balance between synthesis and degradation is essential. As previously mentioned, the iminosugar NBDNJ is a small molecule inhibitor that prevents the synthesis of gangliosides downstream of glucosylceramide.\textsuperscript{29} Thus, in cases where there is residual enzyme activity, substrate degradation can occur in the presence of NBDNJ at an effective, albeit slower rate. Conversely, in instances where enzyme activity is null, NBDNJ alone may not be sufficient to reduce ganglioside accumulation to an appreciable level unless administered very early. Nevertheless, early treatment of diseased individuals may hinder development, as shown by the effects of ganglioside depletion on neuronal signalling and cytokine secretion. The aforementioned mouse models, while extreme in nature, illustrate that the depletion of complex gangliosides can be detrimental at the molecular level (table 1).

In vitro studies in the promyelocytic cell line HL-60 showed that there were decreased levels of neutral glycolipids and gangliosides when cells were treated with NBDNJ. More specifically, GM1 was reduced by 90% in HL-60 cells, K-562 cells (another myeloid-derived cell line), and two lymphoid cell lines (MOLT-4 and H9) upon NBDNJ treatment, indicating inhibition of glycolipid biosynthesis. Further investigation by this group showed that lipid accumulation was prevented in the presence of NBDNJ in a murine macrophage cell line (WEHI-3B) treated with conduritol \(\beta\)-epoxide to mimic the lysosomal storage phenotype of Gaucher disease.\textsuperscript{30} These results led to the administration of NBDNJ in vivo.

The effects of NBDNJ were first studied in vivo in healthy C57Bl/6 mice. Treated mice presented with smaller spleens and thymuses and grew more slowly than untreated controls.\textsuperscript{31} These findings were encouraging because splenomegaly is a common symptom of LSDs\textsuperscript{32}; however, the concentration used to decrease lymphoid organ size was greater than the equivalent clinical dose in humans. Nevertheless, NBDNJ was found to alter the lymphocyte populations in these mice. Treated mice had more T cells, but fewer B cells in the spleen. Additionally, their thymuses showed increased levels of CD4 or CD8 cells at the expense of CD4/CD8 cells.\textsuperscript{31} Studies have shown the importance of lipids, specifically gangliosides, in lymphocyte development and maturation,\textsuperscript{27 33 34} indicating that NBDNJ has the ability to affect immune cell function, which must be considered when deciding on patient treatment. Overall, in C57Bl/6 mice, cell surface gangliosides decreased upon NBDNJ treatment, but sphingomyelin levels increased.\textsuperscript{31} These effects may be due to a compensatory mechanism that is in place to prevent ceramide accumulation, which is known to initiate apoptosis at high levels,\textsuperscript{35} by shunting ceramide towards other pathways. Nevertheless, the consequences of high sphingomyelin levels have not been investigated in this respect. This observation deserves attention to ensure that the increase in sphingomyelin does not manifest as symptoms such as those observed in Niemann Pick type A patients. Additionally, while lipid levels recovered 2 weeks after the drug was removed from the diet, the GM2 level was the slowest to return to normal.\textsuperscript{31} This difference may suggest a more specific, long-term effect of NBDNJ on GM2 synthesis, which may be useful for treatment of GM2 gangliosidoses. These findings led to the administration of NBDNJ to mouse models of LSDs, including TSD and SD.

TSD mice treated with NBDNJ showed improved neuropathology and significantly less GM2 ganglioside accumulation than age-matched untreated controls.\textsuperscript{12} Similar results were observed in SD mice.\textsuperscript{56} In addition to reducing ganglioside levels, NBDNJ significantly reduced the rate of decline of SD mice compared to untreated controls by increasing their life expectancy from 125 to 170 days. NBDNJ-treated animals performed well in behavioural testing when age-matched SD controls were at an advanced stage of the disease. These findings indicate that NBDNJ increases the quality of life, most likely through ganglioside reduction. Additionally, apoptosis assays performed in these mice showed that NBDNJ was able to prevent lymphocyte apoptosis, a common symptom of LSDs.\textsuperscript{32} These findings led to the administration of NBDNJ in vivo.
mice revealed apoptosis in untreated, but not NBDNJ-treated mice in most of the brain. However, the characteristic tremor observed at 12 weeks was unavoidable even with treatment. Other studies have observed apoptosis in the brains of SD mice and human TSD and SD patients, indicating a prevalent role in pathogenesis. Therefore, the ability of NBDNJ to reduce these impacts indicates its relevance for treating LSDs and the importance of early intervention.

Furthermore, NBDNJ can act in conjunction with other modes of treatment and has been found to show synergistic effects. SD mice administered NBDNJ after they had undergone bone marrow transplants (BMT) exhibited a significantly delayed onset of symptoms, performed better in behavioural tests, and presented the slowest rate of decline compared to untreated mice and those treated with either NBDNJ or BMT alone. Moreover, the GA2 levels in treated mice were comparable to those in untreated SD controls, although they lived longer, which suggests that GA2 is not affected by NBDNJ, and GA2 levels may not be a major life expectancy determinant. Additionally, while brain GM2 levels were similar in untreated, NBDNJ and BMT mice, the NBDNJ/BMT combination resulted in 50% higher GM2 levels. However, the combination therapy still enhanced the survival of SD mice. Based on these data, the authors suggest that pathology is not solely due to a GM2 storage level threshold. Further studies on the properties of NBDNJ indicate that it is effective in altering disease parameters. Nevertheless, the general improvements observed in mouse models of LSDs treated with NBDNJ were sufficient to result in clinical trials.

CLINICAL TRIALS
The findings of the aforementioned studies are largely responsible for the clinical trials of NBDNJ that took place in the early 2000s. The purpose of these studies was to evaluate the effectiveness of NBDNJ in patients with mild to moderate Gaucher disease, for whom enzyme replacement therapy was not appropriate. These clinical trials have generally been successful, with all reporting a significant reduction in liver and

**Table 1 Summary of mouse models described in text**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Enzyme affected</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>GalNAcT−/−</td>
<td>β-1,4-N-acetyl-galactosaminyl transferase</td>
<td>No complex gangliosides</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased GM3, GD3, lactosylceramide</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Seminiferous tubule degeneration causing male infertility</td>
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<tr>
<td></td>
<td></td>
<td>Reduced neural conduction velocity</td>
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<tr>
<td></td>
<td></td>
<td>Disrupted nodes of Ranvier</td>
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<tr>
<td></td>
<td></td>
<td>Axonal degeneration, reduced myelination, dysmyelination</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Apoptosis caused by depolarising K+ or glutamate</td>
</tr>
<tr>
<td>Siat9−/−</td>
<td>GM3 synthase</td>
<td>Heightened sensitivity to insulin</td>
</tr>
<tr>
<td>GD3−/−</td>
<td>GD3 synthase</td>
<td>b-series gangliosides deleted, a-series gangliosides accumulated</td>
</tr>
<tr>
<td>HexB−/−</td>
<td>Hexosaminidase A and B</td>
<td>GM2, GA2 accumulation throughout central nervous systems</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Severe neurodegeneration</td>
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<tr>
<td></td>
<td></td>
<td>High proinflammatory cytokine expression</td>
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<tr>
<td>GalNAcT−/− GD3−/−</td>
<td>β-1,4-N-acetyl-galactosaminyl transferase, GD3 synthase</td>
<td>Upregulated complement-related genes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High proinflammatory cytokine expression</td>
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<tr>
<td></td>
<td></td>
<td>Diffuse lipid rafts</td>
</tr>
<tr>
<td>GalNAcT−/− Siat9−/−</td>
<td>β-1,4-N-acetyl-galactosaminyl transferase, GM3 synthase</td>
<td>Complex gangliosides not present</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased lactosylceramide and lactosylceramide-3-sulfate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Smaller brain, vacuolisation in white matter and brainstem</td>
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<tr>
<td></td>
<td></td>
<td>Astrocyte activation, apoptosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Axon degeneration, impaired axonal-glial interactions</td>
</tr>
<tr>
<td>GalNAcT−/− HexB−/−</td>
<td>β-1,4-N-acetyl-galactosaminyl transferase, Hexosaminidase A and B</td>
<td>Missing complex gangliosides</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased GM3, GD3, lactosylceramide</td>
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<tr>
<td></td>
<td></td>
<td>No GM2 storage</td>
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<tr>
<td></td>
<td></td>
<td>Delayed onset of HexB−/− symptoms</td>
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<tr>
<td></td>
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<td>Extensive Purkinje cell loss</td>
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spleen volumes and a reduction of disease biomarkers. The major side effect noted by the majority of patients was diarrhoea, which responded well to loperamide or a lactose-free diet. This symptom is due to α-glucosidase inhibition. Two patients were withdrawn from the study performed by Cox et al due to paraesthesia and tremor was noted in a number of patients in two other studies. These findings indicate that NBDNJ treatment may have neurological side effects, although it is not certain that NBDNJ itself caused these problems. It is possible that these symptoms are related to myelin deterioration or GM3/GD3 ganglioside accumulation, as suggested by mouse models that are deficient in complex gangliosides. In addition, although type I Gaucher disease is traditionally defined as non-neuronopathic, 10.7% of patients experienced polyneuropathy, compared to 0.9–1.3% in the general population. The exact cause of the paraesthesia is unknown in these cases. Another study that followed patients who switched from ERT to NBDNJ found that most disease markers, including signalling levels, platelet counts, and liver and spleen sizes, were maintained.

Soon after the initial clinical trials for Gaucher patients, NBDNJ began being tested for the treatment of other LSDs. Although the mouse model of TSD is asymptomatic, the animals still accumulate GM2 ganglioside. Treatment with NBDNJ reduced this accumulation and should theoretically do the same in humans. Jacobs et al administered NBDNJ to a child with TSD after allogeneic bone marrow transplantation, and Bembi et al studied two young girls with TSD who were also given the drug. Neither study found that NBDNJ was able to arrest neurological deterioration, though Bembi et al found that it prevented macrocephaly. It is difficult to draw any concrete conclusions from these studies. However, it is likely that due to the severe nature of infantile TSD, NBDNJ must be administered at a very young age, before substantial accumulation has taken place, for it to have an observable affect. Maegawa et al found that the pharmacokinetics of NBDNJ in children are similar to those in adults, although most side effects are more severe in older patients. Peripheral neuropathy was observed in one patient, but because this is also a symptom of the disease itself, it may not have been caused by NBDNJ. Another patient experienced hypersensitivity of his hands and feet, which was improved upon dosage reduction. It is possible that these symptoms were due to disease progression and not treatment with NBDNJ, although it is difficult to be certain.

More recently, NBDNJ was approved for the treatment of NPC. Clinical studies of NPC indicate that NBDNJ is capable of stabilising disease progression in the majority of patients. However, it is apparent that NBDNJ is best suited for the treatment of late infantile or juvenile onset forms of the disease, in which there is likely to be greater enzyme function. Most adverse effects were gastrointestinal in nature, although some studies noted that patients developed tremor, which may be due to disease progression. Two patients in the study performed by Weath et al experienced tremor that was determined to be caused by NBDNJ. Of further interest, B lymphocytes from an NPC patient treated with NBDNJ showed increased fluid-phase endocytosis compared to normal controls. This observation may suggest that NBDNJ stimulates endosomal uptake, which would be expected to exacerbate lysosomal storage.

Studies performed in areas other than the brain support the broad therapeutic potential of NBDNJ. Misago et al found that NBDNJ stimulated the proliferation of human granuloid progenitors from bone marrow blood but suppressed the maturation of myelocytes to segmented neutrophils. In addition, NBDNJ inhibited apoptosis in these cells as measured by flow cytometry. Because neutrophils are producers of large amounts of reactive oxygen species, this evidence may indicate a role for neutrophils in neurodegeneration. Some evidence also suggests that NBDNJ can be atheroprotective by altering plasma lipid, lipoprotein and C reactive protein levels in Gaucher patients. These data further suggest a role for NBDNJ in the regulation of inflammation as well as GSL reduction, providing an additional contribution to neuroprotection. Overall, the results of the clinical studies investigating the use of NBDNJ for the treatment of glycosphingolipidoses have been favourable. Most studies indicate that NBDNJ is tolerable and capable of preventing or stabilising disease progression.

CONCLUSIONS

NBDNJ appears to have pleotropic effects in humans, as demonstrated by its ability to reduce GSL accumulation and inflammation. Nevertheless, it is vital to keep in mind the potential adverse molecular effects of NBDNJ on cellular processes, including signalling protein localisation. As illustrated by GalNAcT mice, systemic ganglioside depletion is detrimental to neuronal signalling. It causes reduced conduction velocity and myelination, axonal degeneration, and increases susceptibility to excitotoxicity. Gangliosides are important to immune cell development and function, which in turn affects cytokine responses. While some of these problems are due to complete loss of ganglioside synthesis, it is important to note that these modifications alter key cellular processes involved in disease pathogenesis of glycosphingolipidoses. That being said, ganglioside reduction and substrate deprivation by NBDNJ treatment is a more viable treatment in humans because the drug does not result in permanent depletion of the ganglioside reserves. Significantly, NBDNJ sets the stage for the development of more effective drugs with potentially less adverse physiological side effects.

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