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Evaluation of the Landscape of Pharmacodynamic Biomarkers in GM1 and GM2 Gangliosidosis

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ABSTRACT

GM1 and GM2 gangliosidosis are inherited, progressive, neurodegenerative lysosomal disorders of variable onset and disease progression. GM1 gangliosidosis is a result of biallelic pathogenic variants in the *GLB1* gene, which confer absent or reduced β-galactosidase enzyme activity and lead to the accumulation of glycoconjugates such as glycosphingolipid GM1-gangliosides. GM2 is caused by biallelic pathogenic variants in one of the three genes (*HEXA*, *HEXB*, and *GM2A*) which confer deficiency of β-hexosaminidase or the GM2 ganglioside activator protein, responsible for the catabolism of GM2 gangliosides. In both gangliosidoses, glycosphingolipids accumulate primarily in neurons, with subsequent neuronal death, which translates to early mortality for patients. The clinical course is commonly differentiated by age of symptom onset. To date, no disease-modifying therapy has been approved globally, and treatment is typically supportive. The lack of mature biomarker development in these diseases contributes to challenges associated with quantifying treatment response. However, recent advancements in the detection of neurodegenerative biomarkers and treatment innovation have spurred interest in biomarker identification in plasma and cerebrospinal fluid in patients with GM1 and GM2 gangliosidosis as pharmacodynamic endpoints to support clinical trials and regulatory decision-making. In this review, we assess the landscape of lipid and protein biomarkers, the extent of evidence, and propose considerations for future biomarker development to measure treatment response and support drug development in GM1 and GM2 gangliosidosis.

JEL Classification: Biomarkers

1 | Introduction

GM1 and GM2 gangliosidosis (GM1 and GM2, respectively) are rare genetic sphingolipidoses that are clinically heterogeneous and progressive. GM1 has an estimated incidence of 1 in 100,000–200,000 live births, whereas GM2, known as Tay Sachs and Sandhoff disease, has an estimate of approximately 1 in 222,000 and 1 in 422,000 live births, respectively [1, 2]. Although rare in the general population, certain populations have higher incidences and increased carrier rates, such as Ashkenazi Jews, French Canadians, and Cajun populations [3]. GM1 and GM2 can be classified based on the age of symptom

Abbreviations: AAV, adeno-associated viral; AST, aspartate aminotransferase; CNS, central nervous system; CSF, cerebrospinal fluid; GAGs, glycosaminoglycans; LDH, lactate dehydrogenase; NfL, neurofilament light chain.

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onset into subtypes: infantile, late-infantile and juvenile, and adult [4, 5]. Disease severity is inversely correlated with enzyme activity. For example, the infantile subtype has an age of onset prior to 12 months of age, presents with absent or negligible enzyme activity, and has the most severe and rapid clinical course [1]. Individuals with gangliosidoses have progressive neurological impairment including motor deficits, progressive weakness, hypotonia, decreased responsiveness, seizures, and ultimately premature death [6–9].

GM1 is characterized by biallelic variants in *GLB1*, which encodes β-galactosidase, while GM2 is characterized by biallelic variants in *HEXA*, *HEXB*, or *GM2A*, which encode α or β subunits of isozymes that dimerize to form β-hexosaminidase A or the GM2 ganglioside activator protein [10]. GM2 is classified into Tay-Sachs, caused by variants in *HEXA* (α subunit), and Sandhoff disease, caused by variants in *HEXB* (β subunit); these conditions are clinically indistinguishable. In GM1 and GM2, variants in its respective genes lead to an absence or reduction of enzyme activity and a build-up of gangliosides, including accumulation in neuronal tissue, which results in morbidity and premature mortality [6].

Gangliosides are glycolipids formed from a ceramide on the cytoplasmic side of the rough endoplasmic reticulum, processed in the Golgi network to add carbohydrates, and, lastly, processed by LacCer-α-2-3 sialyltransferase to add sialic acids to form GM3 gangliosides and precursor gangliosides for the synthesis of GM2 ganglioside [11–13]. GM1 and GM2 gangliosides are predominantly located in the brain and are distributed in caveolae-rich microdomains in the plasma membrane where they are critical to neuronal differentiation, cell adhesion, signal transduction, and inflammation [14–19]. GM1 and GM2 gangliosides differ by the number of sialic acids and the type of monosaccharides present (Figure 1).

While the pathophysiology of these sphingolipidoses is relatively well characterized, mechanisms of neurodegeneration are not yet fully elucidated. Animal models have enabled understanding of the human condition, including disease pathophysiology, and assisted in the testing of numerous investigational products. For instance, feline models of naturally occurring GM1 and GM2 have paved the way for advancements in the understanding of the pathophysiology, the potential to test therapeutics, and are widely used in research [20, 21]. While mouse models have historically lacked the neurological similarity to infantile and juvenile presentations of GM1 and GM2, mouse models have been capable of measuring the accumulation of glycosphingolipids in the brain and more closely model a less severe phenotype of GM1 [5, 22]. Other rodent models appear nonexistent, presumably due to the well-established genetically engineered strains of mice recapitulating key features of GM1 or GM2, including the accumulation of GM1 or GM2 gangliosides, and ease of access to mice. To understand the impact of severe presentations, feline models recapitulate infantile and juvenile phenotypes, including neurological manifestations, and allow measurement of treatment response [21, 23, 24].

Despite advancements in the understanding of pathophysiology and well-characterized animal models, no GM1 or GM2 disease-modifying therapies have been approved globally; treatment

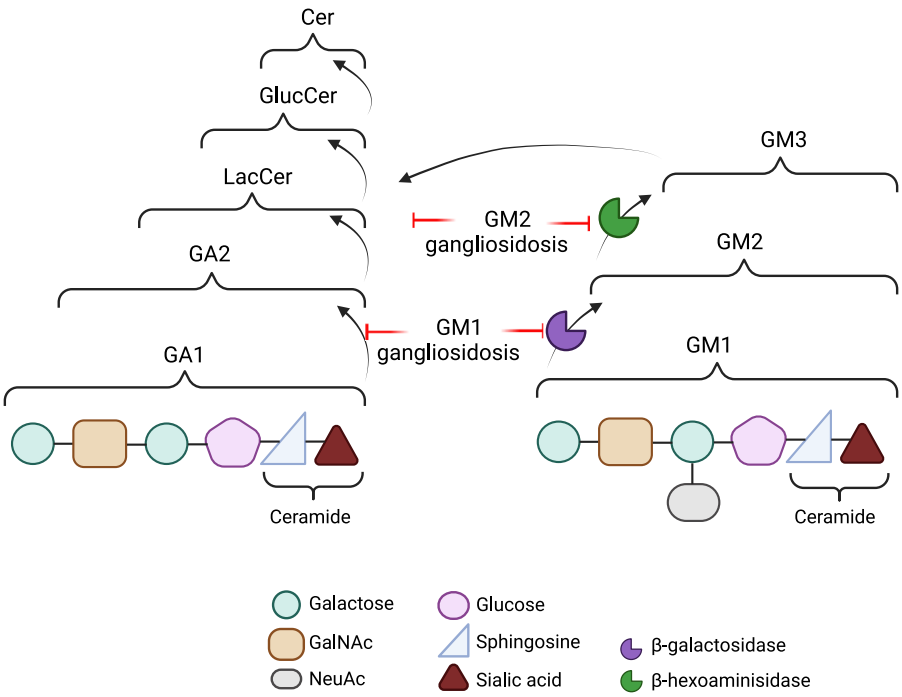

FIGURE 1 | Catabolism of GM1 and GM2 gangliosides and other sphingolipids. GM1 ganglioside is metabolized to GM2 ganglioside by β-galactosidase and, subsequently, GM2 is converted to GM3 by β-hexosaminidase and/or GM2 activator protein (not pictured). Deficiencies in these enzymes lead to accumulation in these sphingolipids leading to GM1 or GM2 based on enzyme defect. Abbreviations: GalNAc, N-acetylgalactosamine; NeuAc, N-acetylneuraminic acid; GM1, GM1 ganglioside; GM2, GM2 ganglioside; GM3, GM3 ganglioside; GA1, GA1 ganglioside; GA2, GA2 ganglioside; LacCer, lactosylceramide; GlucCer, glucosylceramide; Cer, ceramide.

focuses on supportive and palliative care. Miglustat, an iminosugar that inhibits glucosylceramide synthase, has been used to potentially stabilize the disease, despite no improvement in neurological impairment [25, 26]. The majority of treatments being developed aim to reduce substrate accumulation or restore functional enzyme activity in the brain (e.g., https://clinicaltrials.gov/ NCT #04221451, NCT #03759665, NCT #01102686, NCT #00672022, NCT #03822013, NCT #05758922 or NCT #04273269, NCT #04713475, NCT #03952637, NCT #01869270, NCT #04669535, and NCT #04798235). Small-molecule investigational products, such as pyrimethamine and venglustat, intend to enhance the deficient protein expression and activity within the lysosome or inhibit the biosynthesis of glycosphingolipids upstream of the deficient protein [27, 28].

2 | Biomarkers

Biomarkers may support and expedite clinical trial development in sphingolipidoses. Biomarkers may be used to overcome intrinsic challenges associated with clinical trials in these small populations, such as phenotypic heterogeneity and the long duration of observation needed to evaluate clinical benefits related to motor activity, coordination, swallowing, behavior, and development. Monitoring biomarkers may be useful indicators of treatment response at an individual level in clinic (i.e., response biomarker); they may also be useful to guide dose selection in clinical trials (i.e., pharmacodynamic biomarkers) or establish evidence of effectiveness (i.e., surrogate endpoint biomarkers). However, for the value of biomarkers to truly be realized, understanding the biomarkers' relation to the disease mechanism and clinical outcomes is critical to the interpretation of a treatment effect.

Sphingolipidoses such as GM1 and GM2 are monogenic with a well-characterized downstream pathophysiology. Thus, these diseases may be suitable candidates for the use of cellular biomarkers to serve as proxies for clinical outcomes. This review examines the landscape of biomarkers for GM1 and GM2, focusing on biomarkers associated with neurodegeneration that have been reported in published literature. Specific biomarkers of interest are those with clear biological plausibility and relationship to the disease pathology and those with evidence to suggest they have potential to inform treatment response (Table 1). Biomarkers are classified into lipid and protein biomarkers. Imaging biomarkers were out of scope for this review. For each biomarker, an introduction is provided followed by a summary of relevant results from studies demonstrating baseline alteration in disease compared to healthy states, pharmacodynamic studies derived from animal models, and human studies when available. Each biomarker section concludes with a synthesis of findings and potential considerations for future biomarker development.

2.1 | Lipid Biomarkers

2.1.1 | GM1 and GM2 Gangliosides

GM1 and GM2 are a direct result of genetic variation, which leads to the accumulation of gangliosides in many tissues and organs, most notably in the brain. Thus, it may seem obvious to measure the accumulating substrate as a potential treatment response biomarker. GM1 and GM2 gangliosides are major glycosphingolipids in the central nervous system (CNS), and it is believed that GM1 and GM2 gangliosides are neuroprotective and neurotrophic in nature [40–43]. However, the role of GM1 and GM2 ganglioside accumulation, along with lyso-GM1 and GM2 derivatives, in the development of neurodegeneration is not fully understood [44, 45]. Substrate reduction therapy relies on residual enzymatic activity to remove the gangliosides that have accumulated. In theory, substrate reduction therapy may not be beneficial in patients with little or no residual enzyme activity, such as patients with infantile GM1 or infantile GM2 [46].

Plasma lyso-GM1 and GM2 concentrations can be measured using liquid chromatography–tandem mass spectrometry, and most patients with GM1 or GM2 have high basal plasma concentrations relative to healthy individuals. Welford and colleagues found that 31 of 33 patients with GM2 had detectable plasma lyso-GM1 and GM2 concentrations, and the concentration range was similar in patients with Tay-Sachs and Sandhoff disease [39]. Similarly, 12 of 13 patients with GM1 and 5 patients with GM2 with the highest lyso-GM2 concentrations had detectable lyso-GM1 concentrations. Plasma lyso-GM1 and GM2 had a strong negative correlation with age; the few patients with GM2 who had undetectable concentrations were adults, and lyso-GM1 concentrations could not be quantified in the oldest patient with GM1 [39]. In GM1 and GM2 dog models, CSF GM1 ganglioside concentration elevations have been observed whereby concentrations increased in an age-proportional manner in GM1 dogs (9-fold at 5 months to 51-fold at 12 months) and were 7-fold higher in GM2 dogs compared to normal dogs [37, 47].

Kodama and colleagues demonstrated that lyso-GM2 was elevated in a Sandhoff mouse model compared to wild-type mice [32]. Mice treated with intracerebroventricular administration of modified Hex B enzyme had reduced plasma and CSF lyso-GM2 concentrations 1 week posttreatment, depicting the potential pharmacodynamic and therapeutic effects of enzyme replacement therapy [32]. Plasma lyso-GM2 concentrations were similarly elevated in patients with Sandhoff and Tay-Sachs diseases as compared to healthy controls.

Using an adult murine GM2 model, oral administration of N-butyldeoxynojirimycin, a glucose analog of a competitive inhibitor of ceramide glucosyltransferases, led to reduced GM2 ganglioside accumulation in the brain [29, 30]. This reduction corresponded with improved survival. Additional studies by Kasperzyk and colleagues demonstrated a similar reduction of total brain gangliosides and GM1 ganglioside content at early ages following the intraperitoneal administration of N-butyldeoxynojirimycin to neonatal C57BL/6J and β-gal knockout mice [31]. N-butyldeoxynojirimycin had a dose-dependent reduction in brain ganglioside content by 25% and 28% in C57BL/6J mice and 19% and 32% in knockout mice following either 600 mg or 1200 mg/kg/day, respectively. No behavioral or clinical outcomes were assessed in these studies.

In a naturally occurring GM1 feline model, cats were treated with an intracranial injection of an adeno-associated viral (AAV) vector-based gene therapy, which led to a significant reduction in 16 CSF sphingolipids after 8 months compared to untreated cats and healthy controls [34]. The authors found that CSF GM1

TABLE 1 | Summary of GM1 and GM2 biomarker changes in studies that evaluate therapeutic interventions.

Biomarker	Studies and trials	Findings[a]	Reference
GM1 and GM2 gangliosides and derivatives	Platt et al. (1997): (GM2 mouse model) N-butyldeoxynojirimycin ~4800 mg/kg/day administered orally	Reduction in GM2 ganglioside accumulation in the brain up to 12 weeks of age (No statistics)	[29]
	Jeyakumar et al. (1999): (GM2 mouse model) N-butyldeoxynojirimycin 2400 or 4800 mg/kg/day administered orally	Reduction in GM2 ganglioside accumulation in the brain at 16 weeks of age (Statistically significant compared to untreated)	[30]
	Kasperzyk et al. (2004): (GM1 mouse model) N-butyldeoxynojirimycin 600 or 1200 mg/kg/day for 4 days administered by intraperitoneal injection	Reduction in GM1 ganglioside content at neonatal ages (Statistically significant compared to controls)	[31]
	Kodama et al. (2011): (GM2 mouse model) Modified hexosaminidase B 20 μmol/h/25 μL administered by intracerebroventricular injection	Reduction in plasma and CSF lyso-GM2 concentrations at 1-week posttreatment (Statistically significant compared to untreated)	[32]
	Maeda et al. (2015): *(Fibroblasts from patients with GM1) Cyclodextrins 0.01, 0.1, 1 mM for 24 h, or 1, 10 mM for 1 h*	*Reduction in GM1 ganglioside concentration in endolysosomes at 0.01 mM and 1 mM for 24 h (Statistically significant compared to controls)*	[33]
	Gray-Edwards et al. (2017): (GM1 feline model) AAVrh8 vectors encoding for feline β-galactosidase at doses of 1.2×10^{13}, 3×10^{12} to 4×10^{12}, or 3×10^{11} vector genomes administered once intracranially (presymptomatic)	Reduction in CSF GM1 ganglioside peak area at ~5 years posttreatment (Statistically significant compared to untreated)	[34]
	Gross et al. (2022): (GM1 feline model) AAV9 vector encoding for feline β-galactosidase at a dose of 1.5×10^{13} vector genomes/kg body weight administered once intravenously (presymptomatic)	Reduction of GM1 ganglioside concentrations in brain regions at 16 weeks posttreatment (Statistically significant compared to untreated for cerebellum and cervical intumescence of spinal cord) Reduction of GM1 ganglioside concentrations in long-term cohort (No statistics)	[35]
GAGs	Gray-Edwards et al. (2017): (GM1 feline model) AAV1 or AAVrh8 vectors encoding for feline β-galactosidase at doses of 1.2×10^{13}, 3×10^{12} to 4×10^{12}, or 3×10^{11} vector genomes administered once intracranially (presymptomatic)	Reduction in urinary concentrations at 3–5 years of age (Statistically significant compared to untreated, but significantly higher than controls)	[36]
	Gross et al. (2022): (GM1 feline model) AAV9 vector encoding for feline β-galactosidase at a dose of 1.5×10^{13} vector genomes/kg body weight administered once intravenously (pre-symptomatic)	Increases in urinary concentrations at 16 weeks posttreatment (Statistically significant compared to untreated and healthy controls) Reduction in urinary concentrations in long-term cohort (~40 months of age) (No statistics)	[35]

(Continues)

TABLE 1 | (Continued)

Biomarker	Studies and trials	Findings[a]	Reference
AST	Satoh et al. (2007): (GM1 dog model) Prednisolone 0.5 mg/kg body weight every other day administered orally for 6 months	No changes in CSF concentrations at 2 to 13 months of age (Not statistically significant compared to untreated)	[37]
	Gray-Edwards et al. (2017): (GM1 feline model) AAV1 or AAVrh8 vectors encoding for feline β-galactosidase at doses of 1.2×10^{13}, 3×10^{12} to 4×10^{12}, or 3×10^{11} vector genomes administered once intracranially (presymptomatic)	Reduction in CSF concentrations from 16 weeks posttreatment to 2–3 years posttreatment (long term) for cats with minimal clinical disease (Statistically significant compared to untreated; not statistically significant compared to normal controls) Reduction in serum concentrations from 50–80 weeks to > 100 weeks (Not statistically significant compared to normal controls)	[36]
	Gross et al. (2022): (GM1 feline model) AAV9 vector encoding for feline β-galactosidase at a dose of 1.5×10^{13} vector genomes/kg body weight administered once intravenously (presymptomatic)	Reduction in CSF concentrations at 17.8–30.4 months of age (long term) (No statistics)	[35]
LDH	Satoh et al. (2007): (GM1 dog model) Prednisolone 0.5 mg/kg body weight every other day administered orally for 6 months	No changes in CSF concentrations at 2 to 13 months of age (Not statistically significant compared to untreated)	[37]
	Gray-Edwards et al. (2017): (GM1 feline model) AAV1 or AAVrh8 vectors encoding for feline β-galactosidase at doses of 1.2×10^{13}, 3×10^{12} to 4×10^{12}, or 3×10^{11} vector genomes administered once intracranially (pre-symptomatic)	Reduction in CSF concentrations from 16 weeks posttreatment to 2–3 years posttreatment (long term) for cats with minimal clinical disease (Statistically significant compared to untreated; not statistically significant compared to normal controls)	[36]
	Gross et al. (2022): (GM1 feline model) AAV9 vector encoding for feline β-galactosidase at a dose of 1.5×10^{13} vector genomes/kg body weight administered once intravenously (pre-symptomatic)	Reduction in CSF concentrations at 17.8 to 30.4 months of age (long term) (No statistics)	[35]
	Kaya et al. (2022): (GM2 mouse model) Acetyl-DL-leucine 0.1 g/kg/day administered orally	Increased expression in the cerebellum at 12 weeks of age (Statistically significant compared to untreated)	[38]
NfL	Welford et al. (2022): (GM2 mouse model) Sinbaglustat 30 or 300 mg/kg/day administered orally	Reduction in plasma concentrations at 105 days of age (Statistically significant compared to untreated)	[39]
Chitotriosidase	None		

Note: Studies that used human materials are italicized.
Abbreviations: AAV, adeno-associated viral; AST, aspartate aminotransferase; CSF, cerebrospinal fluid; GAGs, Glycosaminoglycans; LDH, lactate dehydrogenase; NfL, neurofilament light chain.
[a]Findings as reported in the literature.

ganglioside concentrations were strongly correlated with disease progression (e.g., score based on clinical signs/symptoms) and, following AAV treatment, GM1 ganglioside concentrations were reduced by 97% compared to untreated cats. AAV treatment resulted in a 6-fold longer life expectancy compared to untreated cats [34]. After intravenous injection of an AAV vector-based gene therapy in a GM1 feline model, GM1 ganglioside concentrations were reduced in different brain regions, including the cerebellum, at 16 weeks posttreatment and long term [35].

GM1 ganglioside concentrations were investigated in fibroblasts from patients with GM1 who were exposed to cyclodextrins, which are cyclic oligosaccharides that form complexes with hydrophobic molecules [33]. Exposure to 10 mM cyclodextrin for 1 h led to improved cholesterol efflux, though the mechanism by which cyclodextrins exert potential benefit is unclear. Dimethyl-α-cyclodextrin led to improvements in phospholipid efflux, while other cyclodextrins did not induce efflux (e.g., methyl-β-cyclodextrin, glucuronylglucosyl-β-cyclodextrin, and 2-hydroxypropyl-cyclodextrin). Using fluorescence microscopy, treatment with 1 mM cyclodextrins for 24 h led to decreased GM1 ganglioside concentration in the endolysosomes [33].

GM1 and GM2 gangliosides present sensitive and specific biochemical diagnostic biomarkers for GM1 and GM2, respectively. Although premature as a surrogate biomarkers in clinical trials, findings from animal models are promising for the predictability of clinical benefit and may support the use of gangliosides as pharmacodynamic biomarkers. However, it is unknown if blood-based GM1 and GM2 gangliosides are reflective of dynamics in target tissue, such as the CNS. In combination with the well-characterized pathophysiology and proximity to the enzymatic defect, GM1 and GM2 gangliosides are appealing biomarkers; however, future investigations to demonstrate an association with treatment response and clinical outcomes in humans are warranted.

2.1.2 | Glycosaminoglycan

β-Galactosidase and β-hexosaminidase are involved in the degradation of glycosaminoglycans (GAGs) [48]. GAGs are negatively charged polysaccharides that play a role in cell hydration, scaffolding, and biochemical processes and are excreted in urine [49].

It has been observed that GM1 cats had elevated baseline urinary GAGs and, following intracranial AAV treatment, urinary GAGs were reduced despite remaining elevated compared to healthy cats [36]. Urinary GAGs remained significantly elevated at 16 weeks following intravenous AAV treatment in GM1 cats compared to normal cats despite observed functional improvements and reductions were only observed in the long-term cohort, demonstrating a potential delay in the biomarker response [35].

Biological plausibility, findings from early animal models, and potential ease of sample collection support further investigation into the use of GAGs as a treatment response biomarker. Urinary GAGs may be of particular interest as a treatment response biomarker for therapeutics that replace or enhance enzyme activity given its downstream relation to the enzymatic defect.

2.2 | Protein Biomarkers

2.2.1 | Aspartate Aminotransferase

In GM1 and GM2, neuronal death is responsible for the progressive deterioration of CNS function. Analysis of cerebrospinal fluid (CSF) in other neurological conditions (e.g., Alzheimer disease) demonstrates aspartate aminotransferase (AST) elevations during CNS damage, which may indicate a potential nonspecific biomarker for neurological damage [50–52]. Cell damage leads to AST release in the extracellular space, potentially serving as a direct measure of CNS damage [53]. AST can act as a scavenger for excess glutamate in the brain and is involved in redox reactions to regulate hydrogen sulfide production [54]. Building upon the historical evidence of increased AST catalytic activity following CNS damage, studies have shown that AST may be a reliable and feasible biomarker to determine the prognosis of patients after CNS injury and with neurodegenerative diseases [54–56].

Elevations in CSF AST concentrations have been shown in patients with late-infantile GM1 compared to those with juvenile GM1 or healthy normal subjects [36]. The baseline elevation in these patients is consistent with other studies [57, 58]. Serum AST concentrations correlated with disease severity and were significantly elevated in those with a younger age of onset, reflective of the age-based phenotypes. Serum AST concentrations collected over time for each patient with infantile GM1 demonstrated the capability of AST to track disease progression. However, with increased age of onset, there was more variability in AST concentrations such that the trend became less clear in late-infantile GM1 compared to infantile GM1 [36].

CSF AST concentrations were studied in GM1 dogs as an indicator of treatment response and clinical course. Satoh and colleagues administered glucocorticoid steroids to GM1 dogs with neurological symptoms of progressive motor dysfunction and assessed CSF AST concentrations compared to normal dogs [37]. Baseline CSF AST concentrations were significantly higher in GM1 dogs compared to normal dogs. CSF AST concentrations increased with disease progression and correlated with age until reaching a plateau after 7 months of age. Administration of oral prednisolone did not improve clinical features, and there were no changes in AST concentration compared to untreated GM1 dogs [37].

Gray-Edwards and colleagues observed that in a naturally occurring GM1 feline model, baseline CSF and serum AST concentrations were significantly elevated compared to normal cats, supporting cellular damage [36]. Intracranial injection of an AAV vector encoding feline β-galactosidase prior to symptom onset demonstrated improved mean survival of >6.7 times longer than untreated cats. After 16 weeks posttreatment, improvement in survival correlated with a normalization in CSF AST concentrations similar to concentrations observed in normal cats. The authors state that the response was durable and, upon follow-up, cats had attenuation of disease progression by 1–3 years following gene therapy and exhibited normalized

serum AST concentrations beyond 100 weeks [36]. At all timepoints evaluated, untreated GM1 cats had elevated serum AST concentrations compared to treated cats. Additionally, serum AST concentrations were reflective of CSF AST concentrations and were found to be robust indicators of treatment response regardless of seizure status.

Gross and colleagues studied CSF AST concentrations in a GM1 feline model and found significant elevation compared to normal cats [35]. After intravenous administration of an AAV vector encoding feline β-galactosidase, CSF AST concentrations were variable at 16 weeks posttreatment but normalized at ~2 years posttreatment. CSF AST concentrations were partially reflective of the clinical course for untreated cats and the long-term cohort, which survived 5.3 times longer than untreated cats. However, at 16 weeks posttreatment, CSF AST concentrations were not normalized despite cats showing either little or no clinical symptoms [35].

Studies that assessed AST as a treatment response biomarker in GM1 and GM2 are nonclinical in nature and have demonstrated that AST has the potential to monitor treatment efficacy as a general marker for CNS damage [44, 48, 56]. In the limited studies conducted in humans, elevation in baseline CSF AST concentrations in patients with GM1 and GM2 was observed and differed by symptoms and age compared to healthy subjects [36]. Specifically, evidence from the study by Gray-Edwards and colleagues suggests an association between CSF and blood-based AST concentrations and that increases in AST concentrations align with disease progression, reflecting its potential as a blood-based prognostic biomarker [36]. Despite elevation observed in GM1 and GM2, AST elevation has not been seen in other lysosomal storage disorders with CNS involvement, which may be due to the rate of disease progression and neurotoxicity of gangliosides [57]. AST elevation is seen in many unrelated pathologies such as hepatitis, requiring exclusion of comorbidities that may result in elevation. AST may provide a biomarker for CNS damage relevant to gangliosidoses that may correspond with other nonlysosomal storage neurological disorders. Further research is warranted to understand the relationship of AST concentrations with progression and what threshold of change is associated with a clinical benefit in humans.

2.2.2 | Lactate Dehydrogenase

Lactate dehydrogenase (LDH) is a housekeeping protein expressed in all organs, notably the heart, liver, muscles, lungs, blood, and kidneys. LDH is a soluble cytoplasmic enzyme responsible for the catalysis of lactate to pyruvate in an essential reaction for glycolysis and gluconeogenesis [59]. Although LDH expression may differ in each cell type, the total enzymatic activity and LDH released are proportional to the concentration of cells in the area and can provide a sensitive and active measurement of cell death [60]. Upon membrane damage, LDH is released into the extracellular space, allowing it to be measured and serve as a potential biomarker for neurodegeneration in GM1 and GM2.

CSF LDH concentrations in pediatric patients with late-infantile GM1 were significantly higher compared to those with juvenile GM1, although the sample size was small for pediatric patients with late-infantile GM1 ($n = 2$). Higher CSF LDH concentrations were noted in the setting of rapid neurological decline in a patient with juvenile GM1 [36]. In a 10-year prospective study of patients with late-infantile and juvenile GM1, blood LDH concentrations were in the normal pediatric range at the first visit, and no additional sampling was reported [61]. CSF LDH concentrations were also not reported.

CSF LDH concentrations were evaluated as a biomarker for neurodegeneration in a GM1 dog model, and the study found average CSF LDH concentrations were significantly elevated in affected dogs compared to controls [37]. CSF LDH concentrations remained higher in affected dogs compared to controls from 2 to 13 months of age, with the highest concentrations at 7 months of age. Treatment of affected dogs with oral prednisolone did not improve clinical features, and CSF LDH concentrations were similar to those of untreated affected dogs [37].

CSF LDH concentrations were evaluated in two studies using a GM1 feline model. Gray-Edwards and colleagues compared concentrations among GM1 cats treated with intracranial gene therapy, untreated GM1 cats at a humane endpoint, and normal control cats [36, 62]. CSF LDH concentrations in untreated GM1 cats at the humane endpoint were significantly higher compared to controls. Treated GM1 cats had significantly lower CSF LDH concentrations compared to untreated GM1 cats at 16 weeks and long term (1–3 years of age). Treated GM1 cats that reached the humane endpoint had high variability in concentrations. CSF LDH concentrations were higher in GM1 cats with seizures and had a stronger correlation with clinical assessments (i.e., slight tremors, overt tremors, spastic front legs, spastic hind legs, and inability to ambulate) in cats grouped as without compared to with/without breakthrough seizures ($R^2 = 0.64$ vs. 0.08). Findings showed the potential of CSF LDH concentrations to correlate with disease progression and treatment response. Unlike CSF LDH concentrations, serum LDH concentrations were not elevated in GM1 cats [36].

Gross and colleagues studied CSF LDH concentrations in a GM1 feline model treated with intravenous gene therapy [35]. Untreated GM1 cats had significantly higher CSF LDH concentrations compared to controls. High variability in CSF LDH concentrations was observed at 16 weeks posttreatment, but the authors noted that the two cats with the highest levels of β-gal activity had lower CSF LDH concentrations. Treated cats in the long-term cohort had normalized CSF LDH concentrations [35]. CSF LDH concentrations may have reflected the clinical course in part, although variable and lacking normalization of LDH concentrations at 16 weeks posttreatment in the presence of little or no clinical symptoms [35].

LDH expression was evaluated in a GM2 mouse model ($Hexb^{-/-}$) treated with acetyl-DL-leucine to explore the drug's potential mechanism of action [38]. LDH expression levels at baseline were not captured and were only evaluated in 12-week-old mice. LDH expression in the cerebellum of treated $Hexb^{-/-}$ mice was significantly higher compared to untreated $Hexb^{-/-}$ mice. In contrast to dog and cat models, no difference in LDH expression was found between untreated $Hexb^{-/-}$ and wild-type mice, potentially reflecting a model-specific limitation [38].

Studies investigating LDH concentrations as a treatment response biomarker in GM1 and GM2 are limited to animal models. To summarize, both GM1 cat and dog models showed higher CSF LDH concentrations compared to controls. CSF LDH concentrations generally trended with the clinical course and therapeutic response, including lower concentrations in the setting of improvements in clinical assessments and no change in the setting of a lack of improvement. Observations in a GM2 mouse model were inconsistent with GM1 cat and dog models, depicting potential differences between animal models or in naturally occurring models compared to genetic knockout models. Similar to felines and children, CSF LDH concentrations were elevated with neurological events (e.g., seizures). However, since CSF LDH concentrations were often evaluated at singular timepoints in studies, concentration changes during disease progression and timing of changes in response to treatment remains unclear. Blood LDH concentrations were within the normal range in a cohort of pediatric patients with GM1, while CSF LDH concentrations were not reported in that study [61]. Thus, CSF LDH concentrations may not be reflected by the blood-based biomarker. Additionally, various diseases result in elevated LDH concentrations, such as infection, liver pathologies, and cancers; thus, blood-based LDH concentrations may be indicative of other comorbidities.

2.2.3 | Neurofilament Light Chain

Neurofilaments are cytoskeletal proteins involved in axonal radial growth and neuroaxonal scaffolding that are released upon axonal damage. These proteins are released in an age-dependent manner into the CSF, and elevations have been associated with neurodegenerative diseases such as amyotrophic lateral sclerosis and Parkinson disease [63–68]. Neurofilament light chain (NfL) is predominantly expressed in large-caliber myelinated axons. NfL is a soluble unit that can be measured in CSF and blood, with the potential to be an indicator of disease progression and pharmacodynamics [69–71]. Plasma NfL concentrations have been used as a biomarker in many diseases with neurodegeneration, such as Alzheimer disease, amyotrophic lateral sclerosis, and multiple sclerosis [72–76].

Plasma NfL concentrations were elevated in patients with GM1 and GM2 [39]. The significant elevations in plasma NfL concentrations were similar to those observed in patients with other severe neurodegenerative diseases [72]. NfL concentrations may also increase as a natural response to aging. Interestingly, plasma NfL concentrations were not correlated with age at sampling in healthy subjects but were correlated with age in patients with GM1 and GM2. Samples collected from patients with GM1 and GM2, with an age of onset of 0–2, 3–4, and 5–10 years, had median plasma NfL concentrations 42.5-, 13.0-, and 4.3-fold higher than that in healthy subjects, respectively, reflective of disease severity associated with younger age of onset [39].

Plasma NfL concentrations have been investigated in relation to treatment in a *Hexb*−/− mouse model [39]. Welford and colleagues investigated the use of sinbaglustat, an iminosugar that acts as a dual inhibitor of glucocerebrosidase-2 and glucosylceramide synthase, on plasma NfL concentrations in *Hexb*−/− mice manifesting a neurological and pathological phenotype consistent with Sandhoff disease [39]. As expected, untreated mice at 30 days of age until euthanized at 105 days demonstrated significant elevations in plasma NfL concentrations over time. Plasma NfL concentrations were reduced in a dose-dependent manner in mice treated with sinbaglustat.

Few studies have investigated the role of NfL as a biomarker for treatment response in GM1 and GM2 specifically, and clinical data are lacking. Given the lack of natural history studies and clinical trials showing clinical benefits of treatment, it is difficult to discern if NfL concentration changes are sensitive to predict beneficial responses to therapy. However, accumulating data seem to support its use as a pharmacodynamic biomarker in other neurodegenerative diseases and may potentially shed light on the utility of this biomarker in GM1 and GM2.

2.2.4 | Chitotriosidase

Chitotriosidase, also referred to as chitinase 1, is expressed by leukocytes including macrophages [77]. The 50-kDa protein isoform is predominantly secreted into blood and is the precursor to the 39-kDa protein, which is predominantly localized in the lysosome and, when elevated, may indicate increased tissue macrophage infiltration [78, 79]. Reduction in serum chitotriosidase activity has been observed after treatment with glucocerebrosides in Gaucher disease, and a rebound effect in chitotriosidase concentrations was observed following cessation of enzyme replacement therapy [80]. Genetic variability in chitotriosidase activity has been described across ancestral groups, including chitotriosidase activity deficiency [81]. Chitotriosidase has been investigated as a biomarker for macrophage activation in GM1 and GM2, despite the unclear association between the molecular mechanism of macrophage infiltration and chitotriosidase activity in GM1 or GM2 disease development.

Wajner and colleagues evaluated the plasma activity and kinetic parameters of chitotriosidase in healthy individuals, patients with GM1, and other diseases, including Gaucher disease [82]. In patients with GM1, plasma chitotriosidase activity was significantly higher than that in healthy individuals. Patients with GM1 had significantly higher chitotriosidase K_m and V_{max} values compared to healthy individuals. Heat stability studies showed that chitotriosidase was more stable in healthy individuals than in patients with GM1. The authors observed differences in activity and kinetic parameters between groups of patients and speculated that the differential expression of chitotriosidase isoforms may contribute to these findings [82]. Plasma chitotriosidase activity was also evaluated in a retrospective analysis of patients with GM1 and was higher in patients with infantile GM1 compared to late-infantile GM1 [83]. Association with the clinical course is unknown since the authors did not report the time of chitotriosidase activity assessment in relation to disease onset or progression [83].

Kim and colleagues investigated the relationship between CSF and serum chitotriosidase activity with disease burden in patients with various lysosomal storage disorders, including GM1 and GM2 [84]. Three patients with infantile GM1 had increasing CSF and serum chitotriosidase activity over time. One patient with late-infantile GM1 had decreasing CSF chitotriosidase

activity (except for the final sampling point) but increasing serum chitotriosidase activity. Two patients with juvenile GM1 had stable serum chitotriosidase activity over time and CSF samples over time were not available. CSF chitotriosidase activity generally did not significantly differ within GM1 and GM2 subtypes. Serum chitotriosidase activity was significantly higher in infantile compared to juvenile GM1 but was similar across GM2 phenotypes [84]. Using samples collected within 1 week of neuropsychological testing, CSF chitotriosidase activity was a significant predictor of raw scores of the cognitive domain of the Bayley Scales of Infant and Toddler Development—III test after adjusting for disease [84]. Increases in CSF chitotriosidase activity were associated with decreases in raw scores. Serum chitotriosidase activity correlated with raw scores for GM1 but not for GM2. Most chitotriosidase activity measurements were collected after patients had seizures and, therefore, it is unclear whether the findings are similar in stable patients. However, the authors noted that CSF and serum chitotriosidase activity increased after initial seizure onset in two patients [84].

Most studies evaluated chitotriosidase activity as a potential measure to inform diagnosis among various lysosomal storage disorders. There was a paucity of nonclinical studies that evaluate chitotriosidase as a potential biomarker in GM1 and GM2. Future research may include chitotriosidase activity assessment in GM1 and GM2 nonclinical models to provide additional insights into its mechanism in relation to disease development, its relationship with disease progression, and its potential as a pharmacodynamic biomarker. Similar to other biomarkers described in this manuscript, chitotriosidase is not a specific biomarker for GM1 or GM2. Chitotriosidase activity levels in patients with GM1 were higher than in healthy individuals in one study, but the relationship within clinical subtypes of disease (e.g., infantile vs. juvenile GM1) is less clear and appears to have high variability. The relationship between CSF and plasma chitotriosidase activity warrants further investigation as disparate findings have been observed. Additionally, biomarker characterization using prospective longitudinal data, measurement of activity before and after therapies, measurement in relation to neurological events, and correlation with disease severity measures would strengthen support for chitotriosidase as a potential biomarker to inform treatment response. Future work for this biomarker may also consider genetic variability and chitotriosidase isoforms.

3 | Conclusion

GM1 and GM2 are characterized by a neurodegenerative disease process resulting from the loss of enzyme activity and accumulation of gangliosides and ceramide substrates in both the CNS and peripheral organs. Due to the progressive and fatal nature of GM1 and GM2, the development of therapeutics aims to prevent or slow the worsening of clinical symptoms. Drug development in these diseases is challenging due to rarity, heterogeneity in presentation and progression, ethical considerations for diseases predominant in children, and an unclear relationship between the pathophysiology of the disease and neurodegenerative mechanisms. Several products in development for gangliosidoses are underway. However, unvalidated clinical measurement tools that may not be sensitive to detect or quantify a delay in worsening, nonspecific and unclear biomarkers, as well as imprecise patient-reported outcomes, significantly hinder advancement.

A variety of biomarkers have the potential to act as quantitative pharmacodynamic biomarkers to inform treatment response and assess neurodegeneration by correlating biomarker concentrations with clinical disease severity. These biomarkers can be characterized as protein biomarkers such as AST, NfL, LDH, chitotriosidase, and lipid biomarkers such as GM1 and GM2 gangliosides and GAGs. This review outlined the available nonclinical and clinical data supporting the potential use of various biomarkers for drug development in GM1 and GM2. Characterization of these biomarkers in humans has largely focused on their role in diagnosis and differences across clinical subtypes, with limited longitudinal data to track biomarker changes throughout disease progression. While some are promising in the evaluation of treatment response, only nonclinical data are available to support this context of use. Although studies to date provide valuable information, additional clinical data are needed to support biomarker use as a treatment response biomarker to support drug development. It is noteworthy that the biomarkers referenced throughout this review are often measured using unvalidated methods. Thus, it is important that future biomarker development prioritizes bioanalytical method validation fit for the intended purpose of the study to ensure the data are reliable [85, 86].

The pathophysiology of GM1 and GM2 is relatively well characterized. GM1 gangliosides accumulate in both CNS and systemic organs, while GM2 gangliosides are restricted to the CNS [3]. Ganglioside accumulation has been associated with the loss of motor function and lysosomal distention, although the role of these gangliosides and other sphingolipids in neurodegeneration is not fully understood [87, 88]. Additionally, it is currently unknown if pharmacological reduction of sphingolipid metabolites can reverse or halt the neurodegenerative effects of the disease. Given that the clinical course of these gangliosidoses is a result of the deficiencies in the catabolic enzymes and the accumulation of sphingolipids in the lysosome, lipid-associated biomarkers (i.e., GM1 and GM2 gangliosides) are proximal to the pathophysiology and provide biologically plausible pharmacodynamic biomarker candidates. GM1 and GM2 gangliosides are accessible biomarkers measurable in plasma, and in combination with other biomarkers, may shed light on a drug's clinical pharmacology and indicate a treatment response. However, the relationship between these gangliosides and disease severity and progression has yet to be illustrated. Natural history studies, such as the RETRIEVE study, could be leveraged to understand how these lipid biomarkers change over the clinical course of the disease and their relationship with clinical outcomes [89].

Protein biomarkers such as NfL have gained increasing support for their potential utility to provide information on the identification and progression of neurodegeneration in many diseases. It is important to note that the protein biomarkers described in this article are potentially reflective of neurodegeneration but are not specific to GM1 or GM2. Using gene therapy, evidence has been generated to support CSF AST and LDH concentrations as potential treatment response biomarkers in naturally occurring animal models [35, 36]. It remains unclear if plasma

concentrations of these protein biomarkers are reflective of CNS involvement and whether changes in plasma concentrations would reflect proportional changes in CSF concentrations. Longitudinal sampling in these studies was limited, such that the relationship between biomarker concentrations and disease progression and the timing of biomarker changes in response to treatment is not fully known.

Contradictory trends in biomarkers have been observed between the mouse and the other mammalian models, such as the naturally occurring cat and dog model. For instance, in the GM2 (*Hexb*$^{-/-}$) mouse model, no difference in cerebellar LDH expression was observed between untreated *Hexb*$^{-/-}$ mice and wild-type mice at 12 weeks of age [38], while CSF LDH concentrations were significantly elevated in affected cats and dogs compared to controls [36, 37]. It is known that GM1 and GM2 mouse models lack the neurological involvement seen in the other animal models. This is potentially due to compensatory mechanisms in mice whereby stored GM2 gangliosides do not exceed a threshold to elicit neurodegeneration [29]. While GM1 and GM2 share similarities in clinical presentation, differences in animal model origin (e.g., genetic knockout vs. naturally occurring) or genetic etiology (GM1 vs. GM2) may also contribute to discrepant findings.

Questions remain about the relationship of these biomarkers to disease severity and progression in humans, and natural history data may be used to support the role of these biomarkers in the disease pathway. Additionally, these biomarkers have yet to be anchored with clinical outcomes, making it difficult to interpret whether these biomarkers can distinguish between changes in dose or drug effect. This is further complicated by the lack of validated clinical measurement tools, which creates additional challenges for drug development in gangliosidoses. To build upon studies in this review, future research is warranted to bridge the knowledge gaps with regard to the ability of these biomarkers to discern a treatment effect using validated assays and mapping changes in these biomarkers to the clinical course of the disease.

Author Contributions

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The authors have nothing to report.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

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