Small Molecule Inhibitors of Ganglioside Biosynthesis as a Substrate Reduction Therapy for the Gangliosidoses

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Abstract

The primary goal of this research is to identify novel CNS penetrant inhibitors of ganglioside biosynthesis as a treatment for lysosomal storage disease.

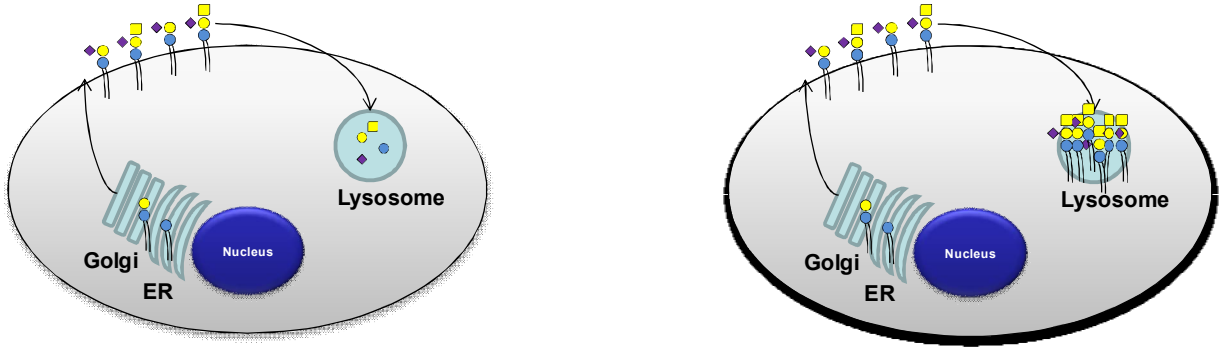
Tay-Sachs and Sandhoff diseases are lysosomal storage diseases caused by a deficiency in the lysosomal hexosaminidase required for the degradation of gangliosides. This deficiency leads to high levels of GM2 accumulation in the central nervous system which leads to progressive neurological deterioration and death. To date there is no effective therapy for these devastating neurological disorders.

To address this unmet medical need, we are developing a small molecule CNS penetrant substrate reduction therapy. The goal of substrate reduction is to reduce the synthesis of the substrate (gangliosides) to alleviate the burden on the compromised lysosomal system. The therapeutic approach has been validated with glucosylceramide synthase inhibitors. However, the efficacy of these compounds is limited due to low CNS penetration, limited ability to reduce ganglioside synthesis through the inhibition of upstream precursors (glucosylceramide), and dose-limiting toxicity.

Using a cell-based high throughput screen, we have identified a set of compounds that inhibit the synthesis of gangliosides without inhibiting the synthesis of early precursor glycolipids such as lactosylceramide. By focusing only on ganglioside inhibition, we believe this therapeutic strategy can reduce ganglioside accumulation in Tay Sachs and Sandhoff diseases while avoiding the potential toxicity associated with inhibiting a broad set of glycolipids. Three unique scaffolds have been identified that i) inhibit ganglioside synthesis downstream of the lactosylceramide synthase, ii) penetrate the blood-brain barrier and iii) reduce GM2 storage in human Tay-Sachs and Sandhoff fibroblasts. We are currently testing a series of analogs of these three scaffolds to identify the best compound series to further develop as a novel CNS penetrant substrate reduction therapy for gangliosidoses.

Clinical Application:
Lysosomal Storage Disease

Gangliosidoses: Lysosomal Storage of Gangliosides



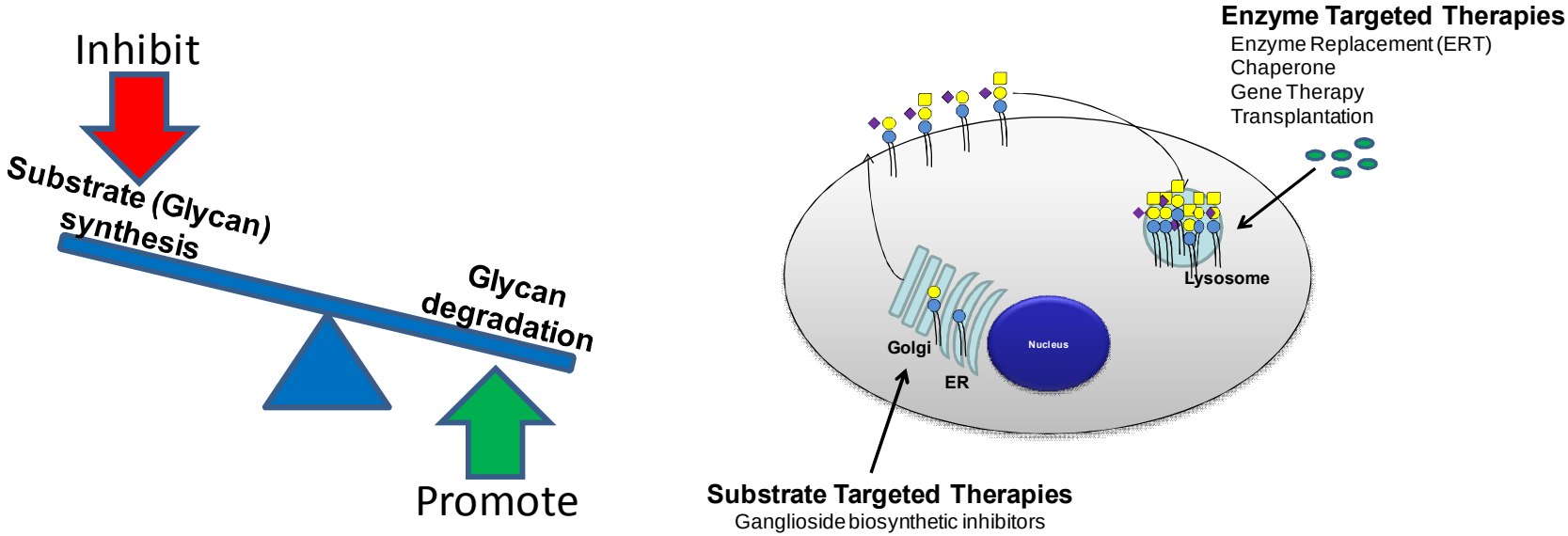
Panel 2. **Ganglioside Biosynthesis and Degradation are in Equilibrium.** Normally, ganglioside biosynthesis is in equilibrium with lysosomal degradation, so that all of the gangliosides that are internalized are quickly broken down and the components are recycled. In Gangliosidosis, this equilibrium is disrupted by a deficient lysosomal enzyme (or essential accessory) required for ganglioside degradation. This leads to a massive accumulation of gangliosides (and other glycans) in the lysosomes of affected cells.

Gangliosidoses: Clinical Overview

- **Lysosomal accumulation of gangliosides**
  - Fatal genetic disease typically presents in early childhood
  - Central nervous system is most significantly affected
    - Deterioration of mental and physical abilities occurs
    - Blindness, deafness, and an inability to swallow
    - Muscles atrophy and paralysis follows
    - Death usually occurs before the age of four
  - No current treatments
- **The Gangliosidoses (Combined prevalence 1:55,000)**
  - GM2 Gangliosidoses
    - Tay-Sachs (Hexosaminidase A deficiency)
      - Prevalence = 1:320,000
    - Sandhoff (Hexosaminidase A and B deficiency)
      - Prevalence = 1:309,000
    - A/B Variant (rare GM2 activator deficiency)
      - Prevalence = very rare
  - GM1 Gangliosidosis (β-galactosidase deficiency)
    - Prevalence = 1:200,000
  - Niemann-Pick C (NPC1/2 deficiency)
    - Prevalence = 1:150,000

Therapeutic Approaches to Lysosomal Storage Diseases

Therapeutic Approaches to the Gangliosidoses



Panel 3. **Therapeutic Approaches to Lysosomal Storage Disease.** There are two fundamental approaches to reducing lysosomal storage of gangliosides in these diseases:

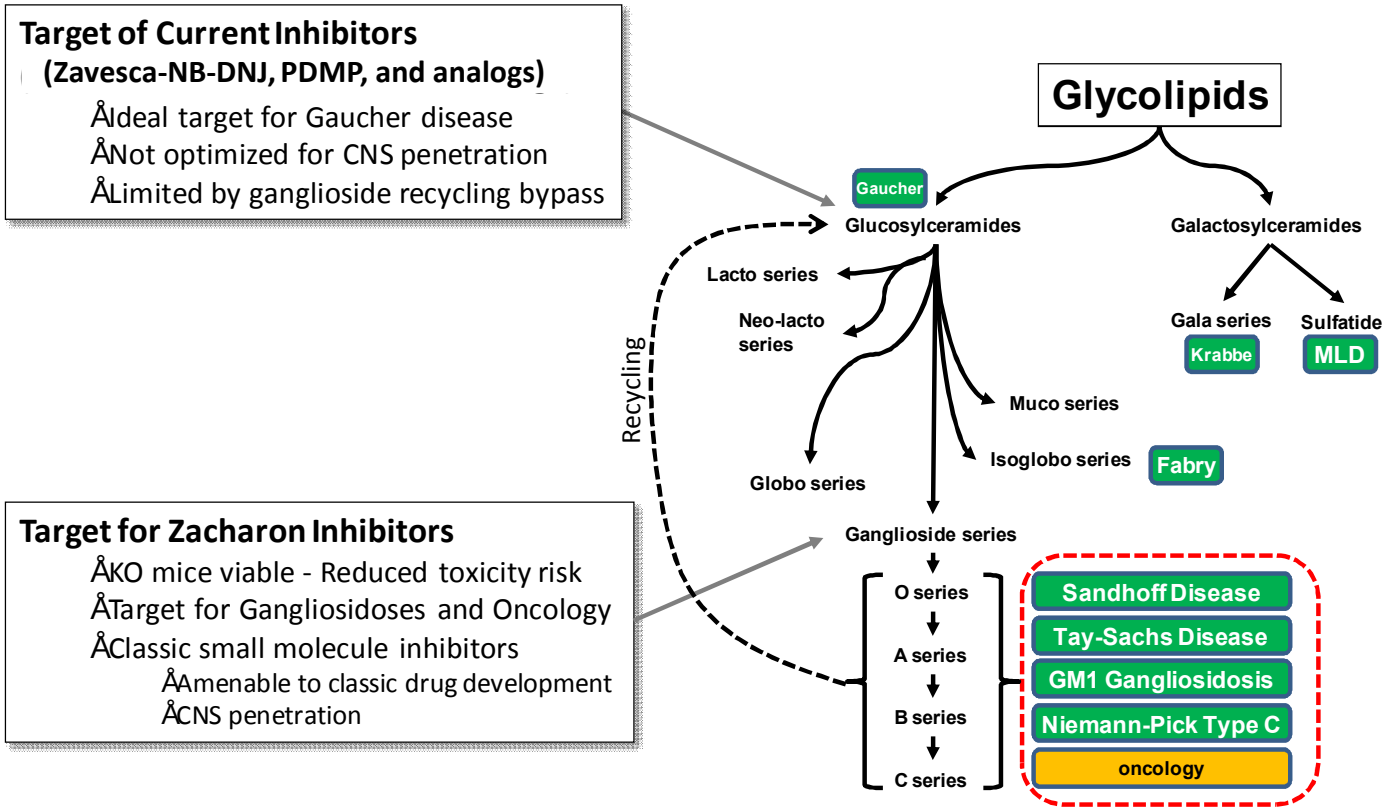
1. **Enzyme Targeted Therapies:** Increase the function of the lysosome by restoring the deficient enzyme activity.
2. **Substrate Targeted Therapies:** Reduce the substrate burden by inhibiting the synthesis of the glycans that cannot be degraded.

The most successful therapeutic approach for lysosomal storage diseases (LSDs) has been enzyme replacement therapy (ERT) where a recombinant form of the missing lysosomal enzyme is delivered to the patient. This approach has been very successful in treating the peripheral symptoms of other LSDs, but fails to treat the CNS.

Because Gangliosidoses are primarily neurological disorders, a CNS penetrant therapeutic is needed. **Our goal is to discover and develop a CNS penetrant inhibitor of ganglioside biosynthesis to reduce the burden of gangliosides accumulating in the CNS of Gangliosidoses patients.**

Therapeutic Approach: Selective Inhibition of Ganglioside Biosynthesis

Overview of Known Glycolipid Biosynthetic Inhibitors

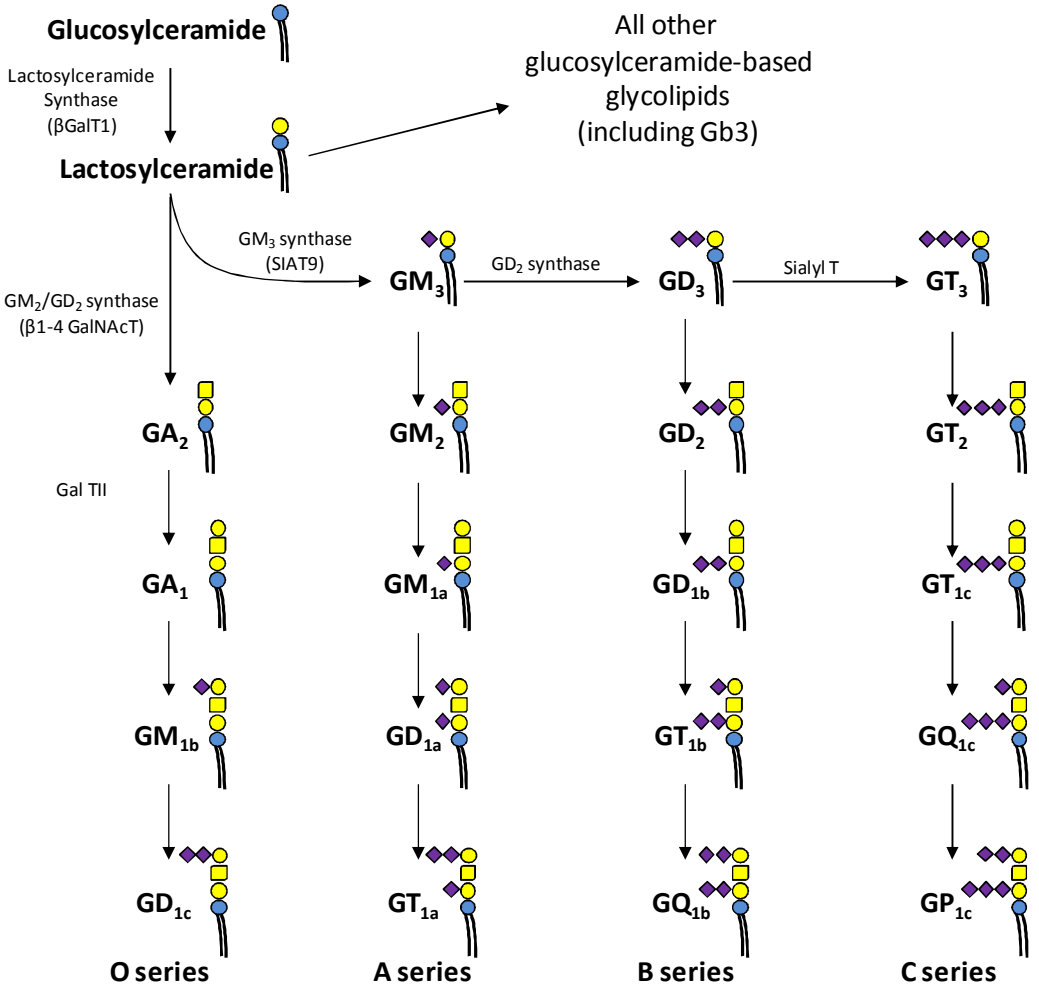


Panel 4. The Known Glycolipid Biosynthesis Inhibitors Target a Step Very Early in the Biosynthetic Pathway. The general flow of glycolipid biosynthesis is shown here. Most glycolipids are built from a glucosylceramide core that is extended through biosynthetic pathways indicated by the black lines. The green boxes indicate various lysosomal storage diseases and the primary glycolipid(s) that accumulate in the disease. The currently known small molecule inhibitors of glycolipid biosynthesis target the glucosylceramide synthase which is the first step in the synthesis of most glycolipids. Due to the point of inhibition, these compounds inhibit the synthesis of all glucosylceramide based glycolipids which has a high risk for toxicity in vivo. Additionally, most of the known inhibitors are not brain penetrant, limiting their use for lysosomal storage diseases that primary affect the CNS.

Our goal is to discover novel small molecule, CNS penetrant ganglioside selective inhibitors.

Therapeutic Target: Ganglioside Biosynthesis

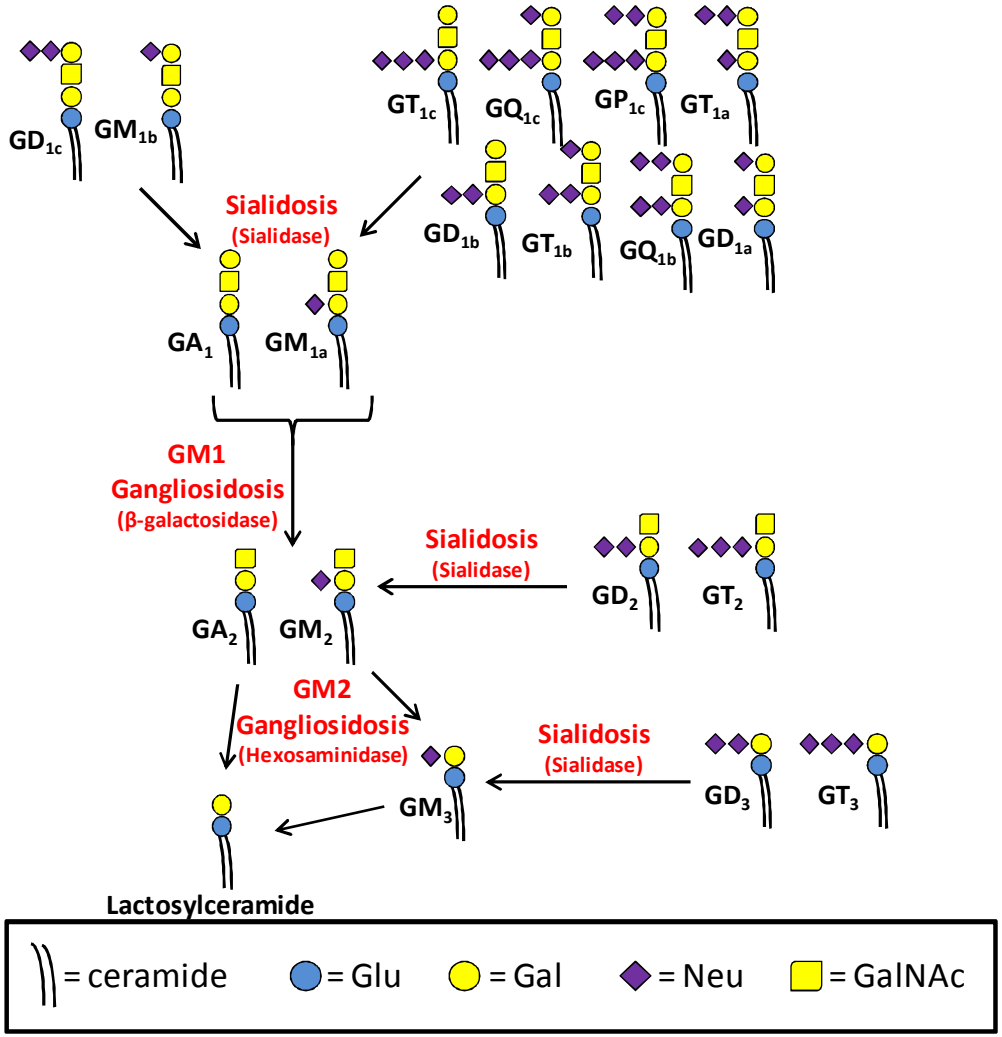
Ganglioside Biosynthesis is Catalyzed by a Set of Enzymes in the ER and Golgi



Panel 5. Ganglioside Biosynthesis. Gangliosides are synthesized by a series of glycosyltransferases from a glucosylceramide core. The biosynthesis proceeds down four distinct "series" of gangliosides. The composition of gangliosides produced by a cell is a reflection of the biosynthetic machinery and regulators expressed by the cell.

Ganglioside Degradation

The Gangliosidoses Define the Enzymes Required for The Lysosomal Degradation of Gangliosides



Panel 6. Ganglioside Degradation. Ganglioside degradation does not follow the reverse of ganglioside biosynthesis. Through the action of sialidases, the terminal sialic acid residues are removed converting the complex gangliosides to simpler derivatives (GA1, GM1a) which are then converted to GA2 and GM2 by the β-galactosidase (deficient in GM1 Gangliosidosis). GA2 and GM2 are converted to lactosylceramide and GM3 by the β-hexosaminidase that is deficient in Tay-Sachs and Sandhoff diseases. A disruption at any of these stages leads to ganglioside accumulation.

Genetic Validation of Substrate Targeted Therapy in GM2 Gangliosidoses

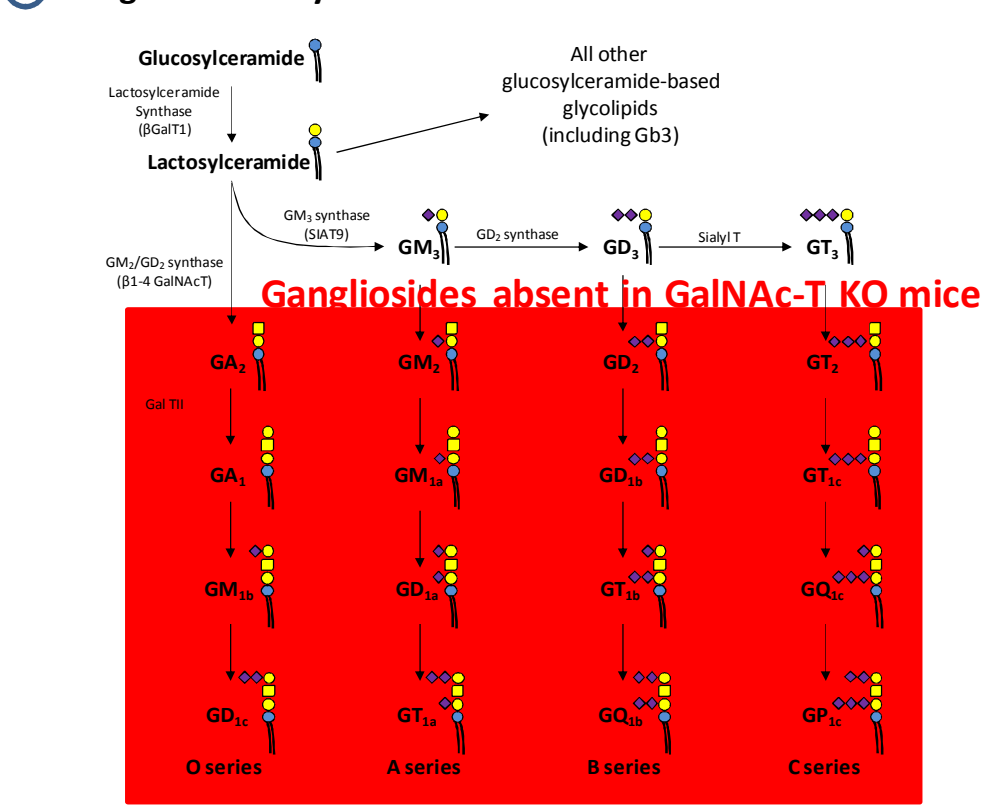
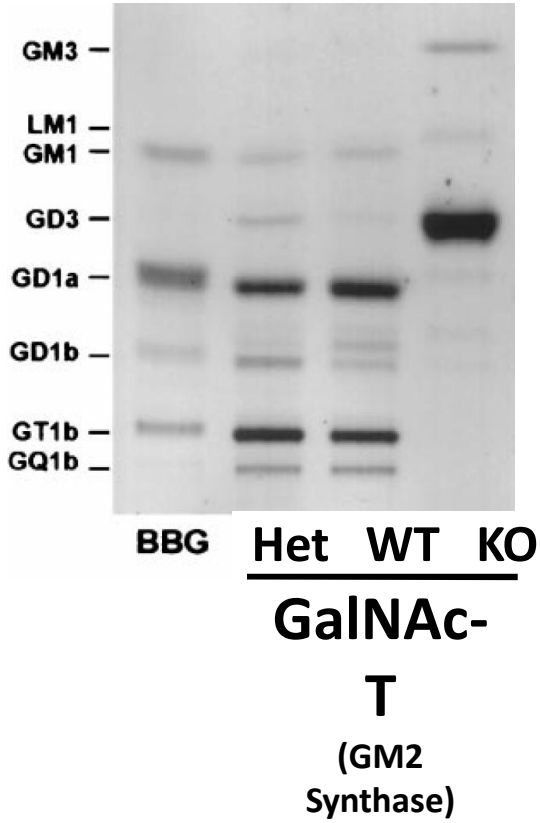


Figure from: Gusheng Wu, Xin Xie, Zi-Hua Lu, and Robert W. Ledeen. Cerebellar neurons lacking complex gangliosides degenerate in the presence of depolarizing levels of potassium. PNAS vol.98 no.1 2001.



Panel 7A. Genetic Disruption of Ganglioside Biosynthesis. GM2 Synthase deficient mice are viable and fertile and lack most complex gangliosides. TLC analysis of brain gangliosides in these mice reveal that the knockouts have a 30% reduction in total ganglioside content due to the loss of most complex gangliosides with a dramatic increase in GD3. In contrast, the heterozygote mice have very minor changes in ganglioside content (11.5% reduction in total gangliosides) with a subtle induction of GD3 content.

Genetic Validation of Substrate Targeted Therapy in GM2 Gangliosidoses, Cont.

B Genetic Target Validation of the Therapeutic Approach

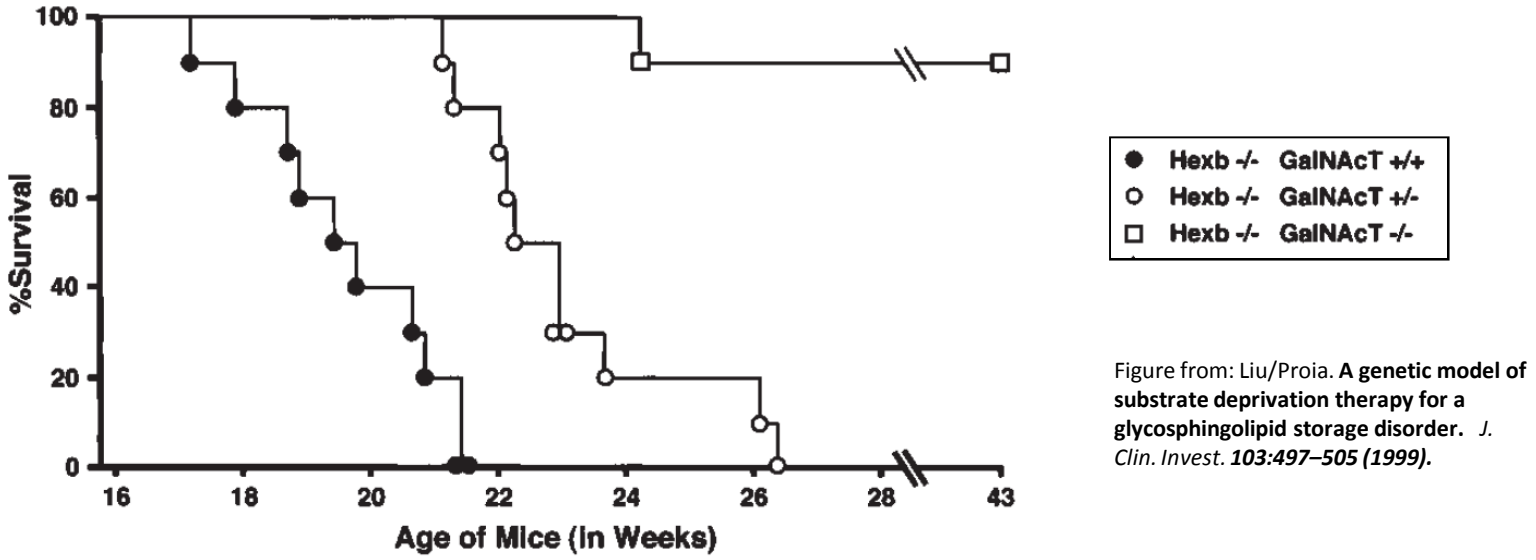
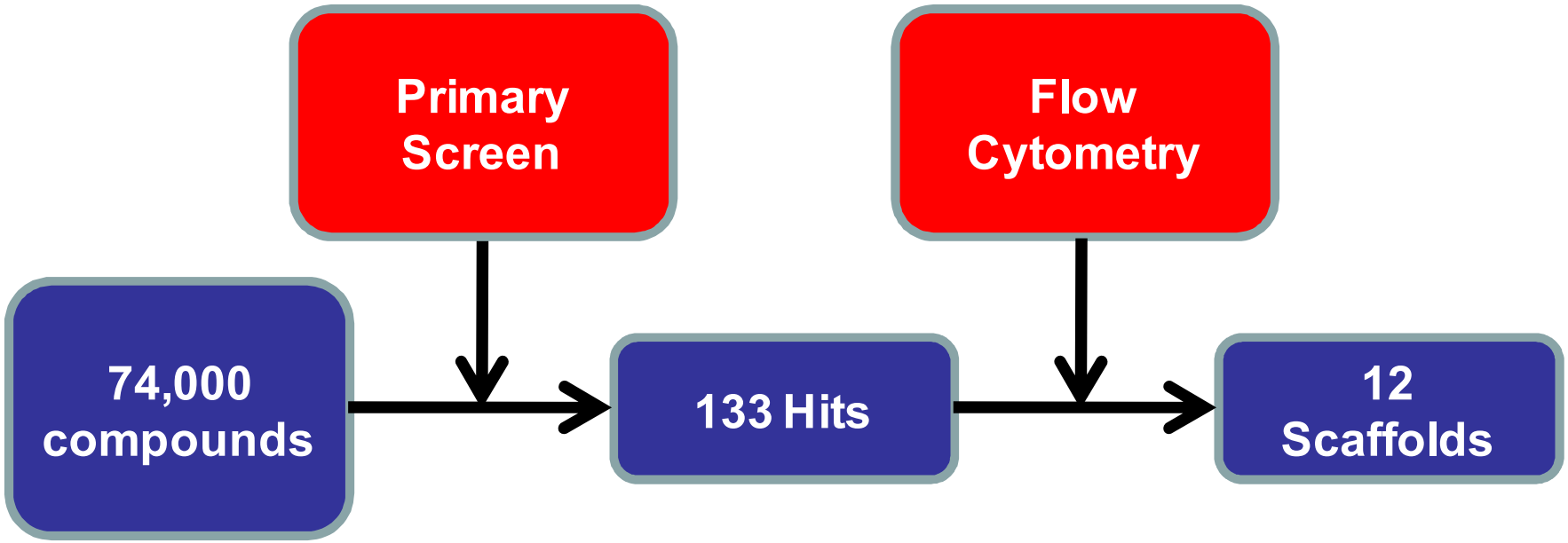


Figure from: Liu/Proia. **A genetic model of substrate deprivation therapy for a glycosphingolipid storage disorder.** *J. Clin. Invest.* ***103:497–505 (1999).***

Panel 7B. Genetic Validation of Substrate Targeted Therapy. The Sandhoff mice were crossed with GM2 Synthase knockout mice and the litter had significantly improved life expectancy. This study indicates that a the GM2 synthase knockout prevents the rapid progression to death that is normally observed in these mice. Even more significant, GM2 Synthase heterozygotes (which have a subtle 11.5% reduction in brain ganglioside content) have a significant increase in life span and delayed neurological progression.

This genetic data indicates that subtle inhibition of ganglioside synthesis via a CNS penetrant inhibitor would be expected to slow the progression of GM2 Gangliosidosis.

Ganglioside Inhibitor Discovery

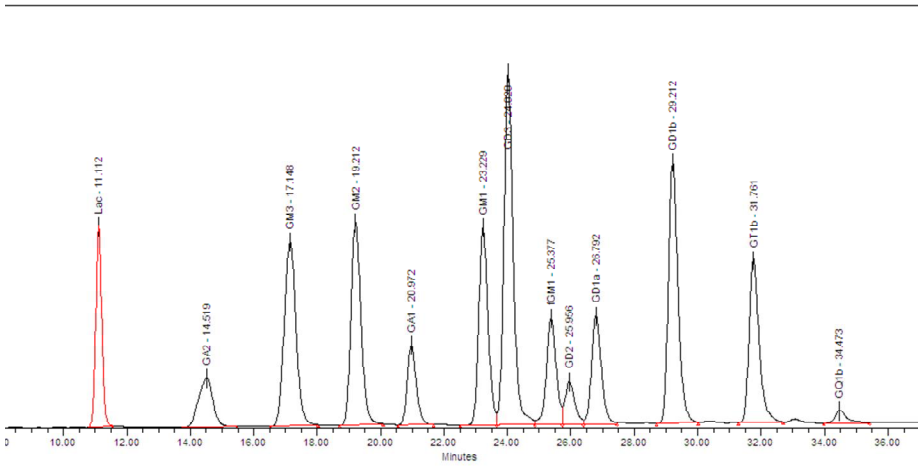


Panel 8. Discovery of Novel Small Molecule Inhibitors of Ganglioside Biosynthesis. We have developed a proprietary cell-based assay capable of screening for compounds that interfere with ganglioside biosynthesis using ganglioside binding lectins. Using this assay, we screened over 74,000 drug-like compounds and identified 133 hit compounds. These hit compounds reduce binding of ganglioside dependent lectins without affecting lectins that bind to unrelated glycans (such as heparan sulfate). 12 compounds were identified that dose-dependently reduced CTB binding by flow cytometry.

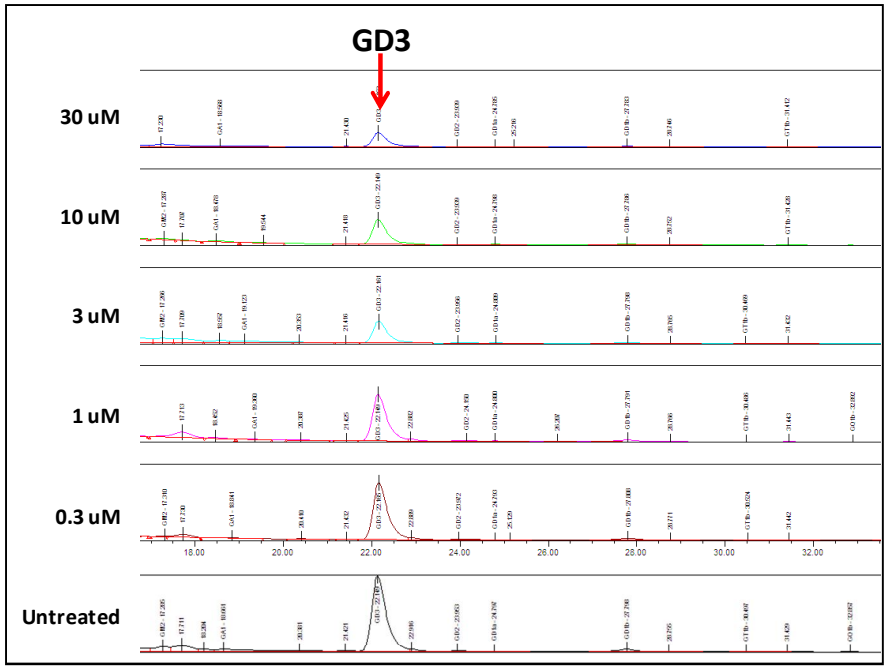
In Vitro Ganglioside Inhibition

Ⓐ Quantitation of Ganglioside Synthesis in Vitro

Bovine Brain Gangliosides – HPLC analysis



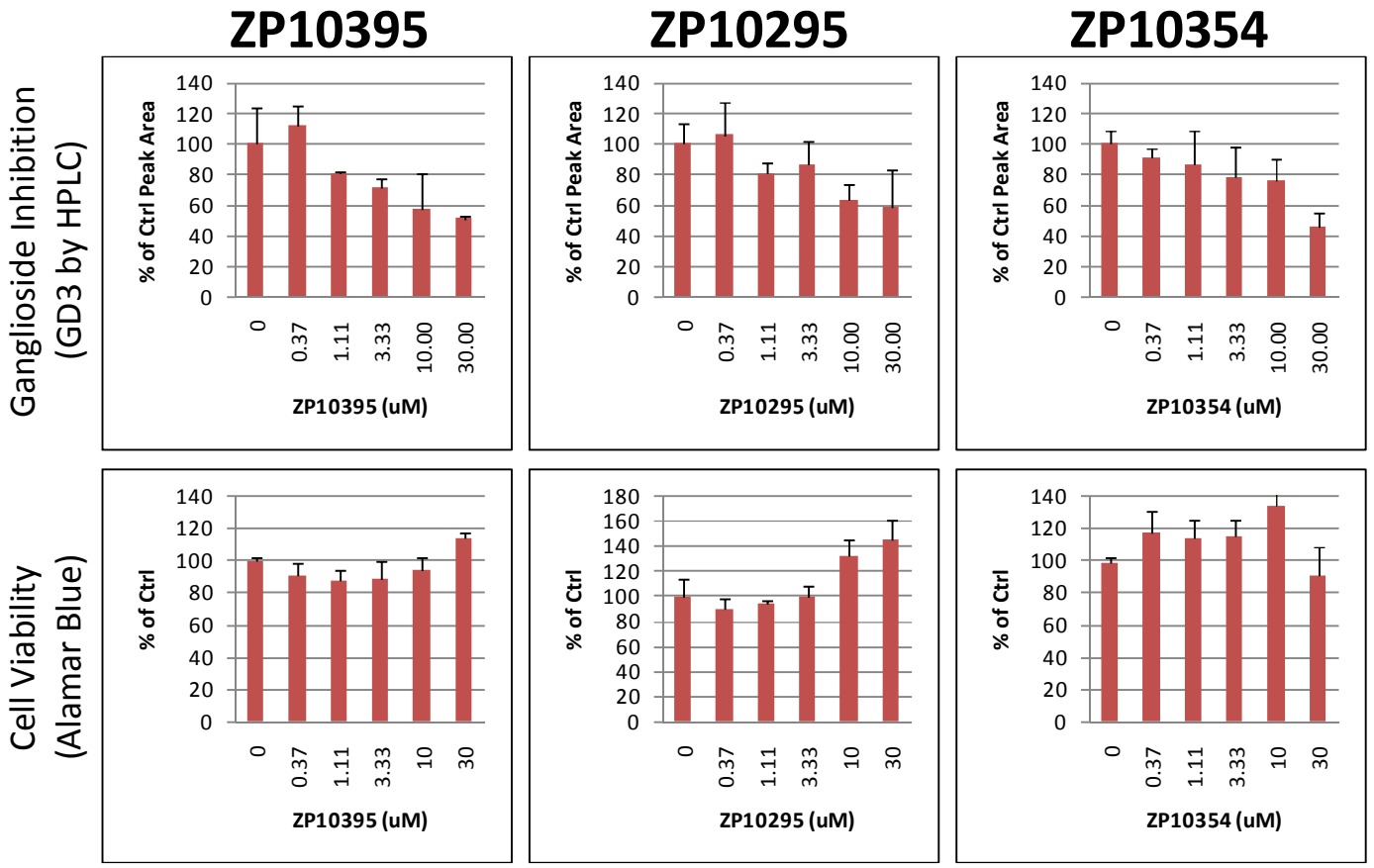
SKMEL28 - PDMP Dose Response



Panel 9A. Quantitation of Ganglioside Synthesis in Cultured Human Cells. In order to identify the subset of hit compounds that actually inhibit the biosynthesis of gangliosides, we used an HPLC-based ganglioside analysis method to quantify ganglioside content in cultured cells. The detection of GD3 inhibition induced by a dose range of PDMP is shown in the panel on the right.

In Vitro Ganglioside Inhibition, Cont.

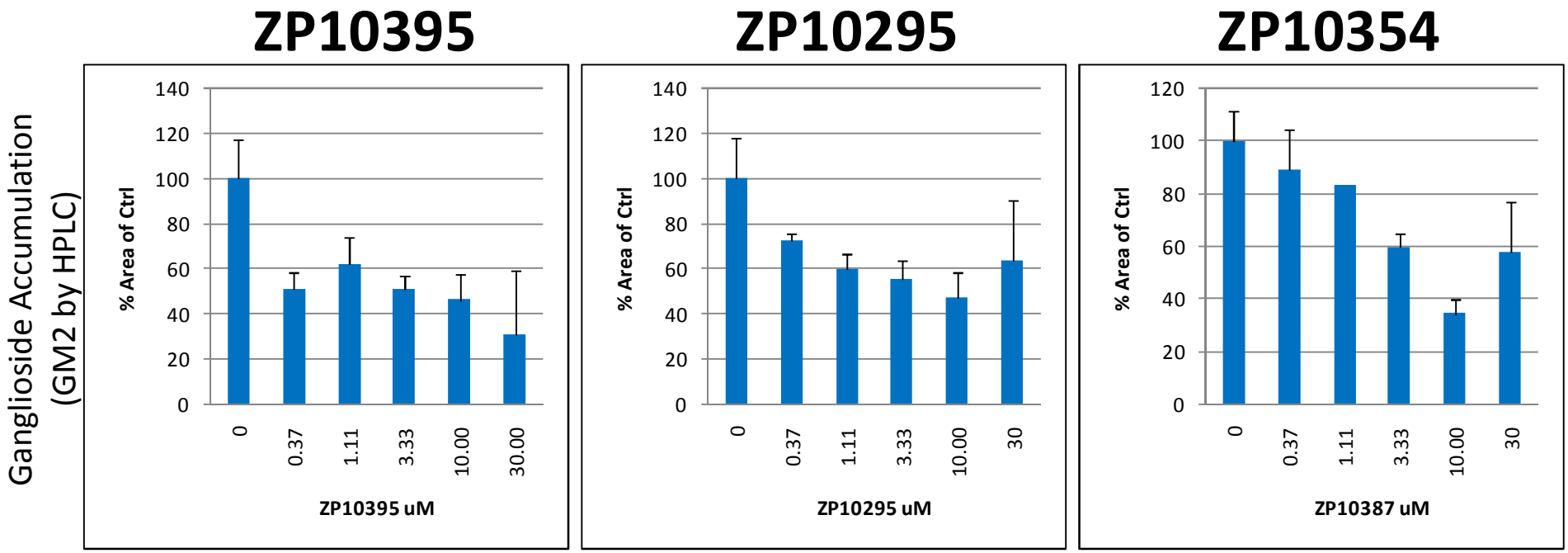
B Inhibition of Ganglioside Biosynthesis in Cultured Cells



Panel 9B. The Novel Small Molecules Inhibit Ganglioside Biosynthesis in Cultured Cells. In order to determine if the compounds can alter the composition of gangliosides in cultured human cells, we used the HPLC ganglioside compositional analysis method described above. This analysis revealed that 6 of the novel inhibitor scaffolds were able to alter ganglioside composition in cultured human cells. The data for three of these scaffolds in SKMEL28 (a human tumor line that over-expresses GD3) is shown above (top panels). The inhibition of ganglioside synthesis was accomplished without general toxicity as determined by Alamar blue oxidation (bottom panels).

Ganglioside Inhibitor Efficacy:
In Vitro Model of GM2 Gangliosidosis

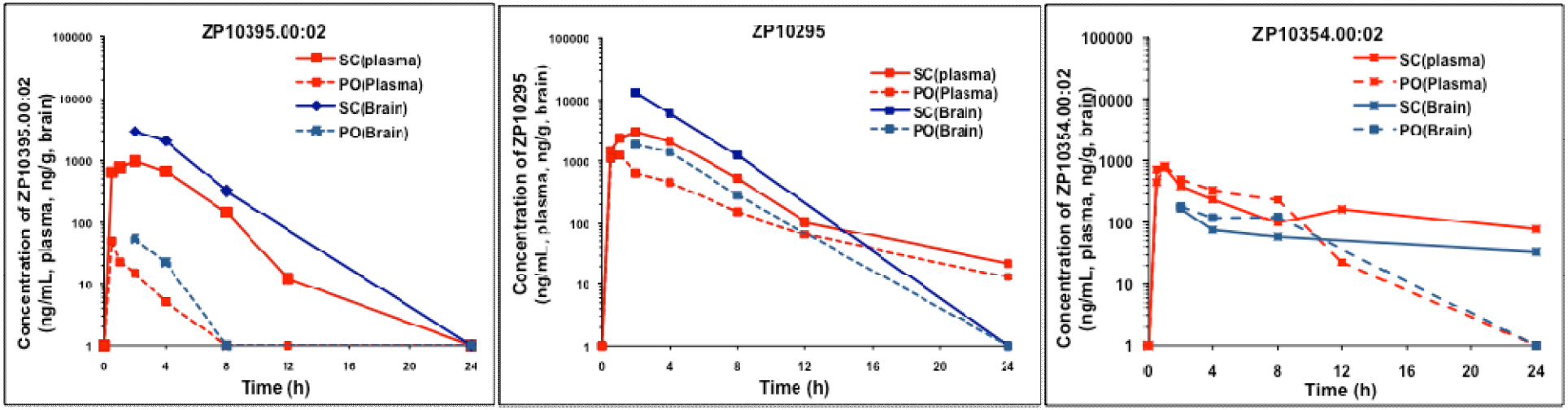
The Ganglioside Inhibitors Reduce GM2 Accumulation in Cells



Panel 10. Ganglioside Biosynthesis Inhibitors Reduce GM2 Accumulation in Primary Human Fibroblasts From GM2 Gangliosidoses Patients. The active ganglioside inhibitors were tested for their ability to reduce the lysosomal accumulation of GM2 in primary fibroblasts from Tay-Sachs and Sandhoff patients. Briefly, primary fibroblasts were grown in the presence of the test compounds at the indicated concentration period. Gangliosides were purified and quantified using HPLC. Each of these scaffolds (ZP10395, ZP10295, and ZP10354) reduced GM2 accumulation in a dose dependent manner without general toxicity.

Blood-Brain Barrier Penetration
Mouse PK Analysis

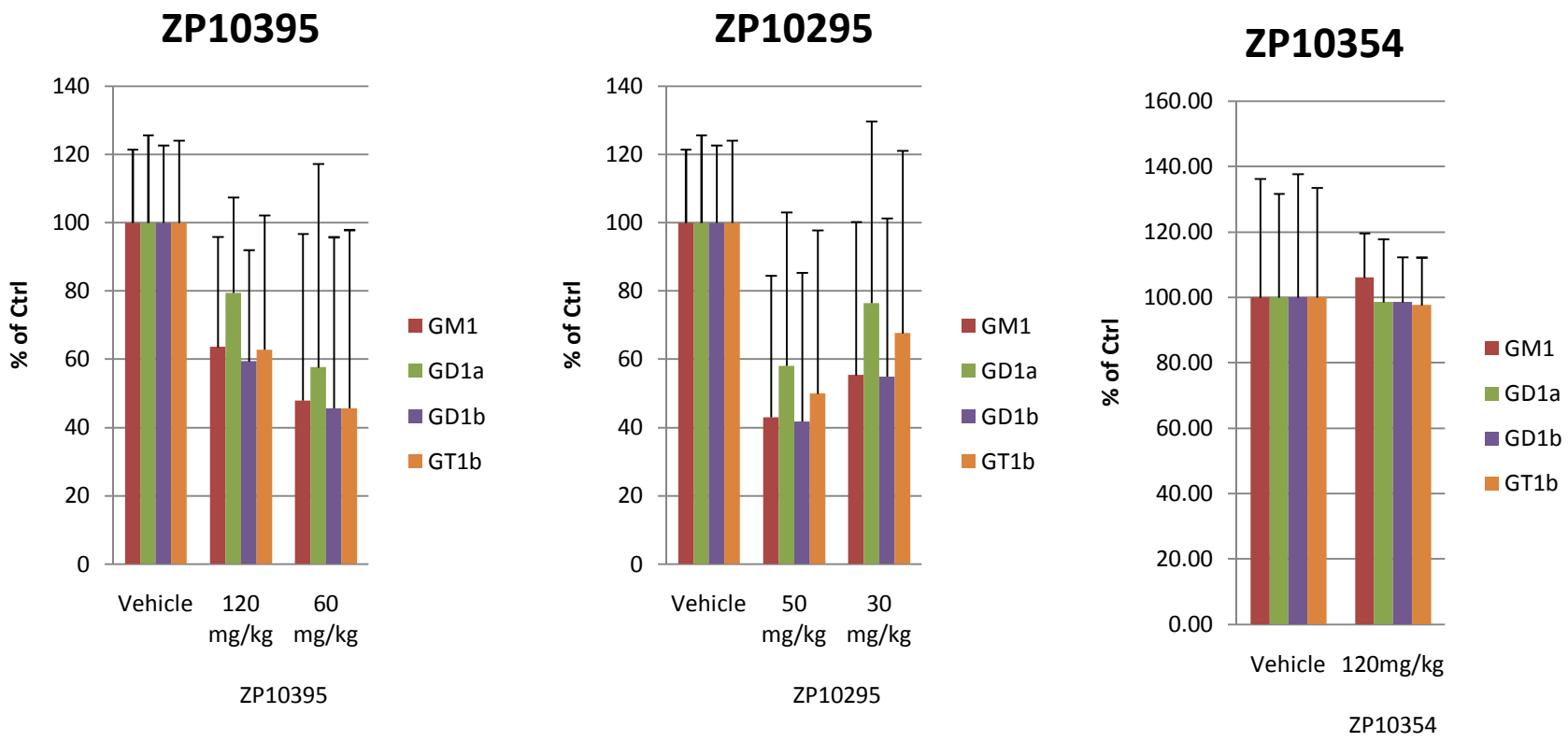
The Ganglioside Inhibitors are CNS Penetrant and Have Acceptable PK for In Vivo Testing



Panel 11. All Three Ganglioside Inhibitor Scaffolds Penetrate the CNS and Have Acceptable PK Properties for In Vivo Testing. The active ganglioside inhibitors were evaluated in mouse PK studies using subcutaneous and oral administration. ZP10395 and ZP10295 scaffolds have excellent BBB penetration and reached blood and CNS levels at or close to the level required to inhibit ganglioside biosynthesis (based on in vitro assays).

In Vivo Inhibition of Ganglioside Biosynthesis

Two of the Top Three Scaffolds are Effective In Vivo



Panel 12. In Vivo Inhibition of Ganglioside Biosynthesis in the Brain. Based on the PK and BBB penetration of the lead scaffolds, an in vivo efficacy study was run in wild type C57Bl6 mice. The mice were dosed at the indicated doses for 40 days and brain gangliosides were quantified by HPLC. ZP10395 and ZP10295 had a significant effect on the ganglioside content in the brain. At this dose, ZP10354 was not effective.

Other Applications - Oncology

Potent Ganglioside Inhibitors Would also Have Therapeutic Applications in Oncology

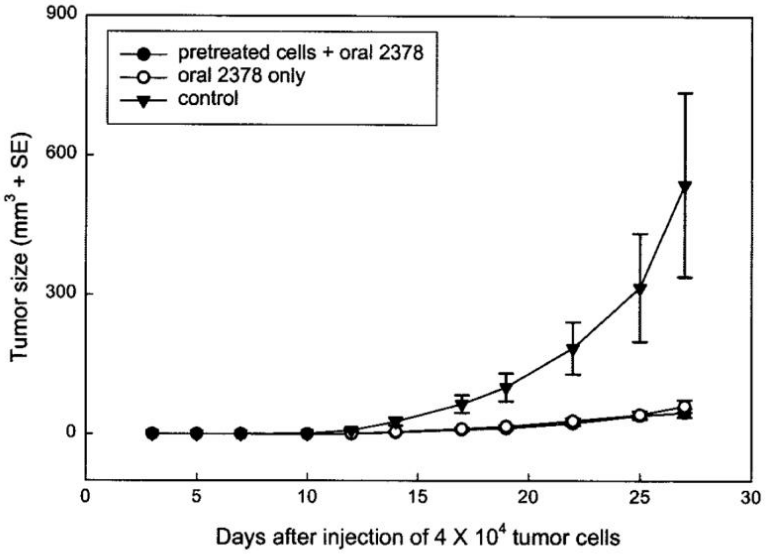


Fig. 3. Effect of p.o. administration of OGT2378 on MEB4 melanoma tumor growth. The mean ± SE tumor volumes are shown.

Figure from: Weiss et al.. **Inhibition of Melanoma Tumor Growth by a Novel Inhibitor of Glucosylceramide Synthase,** CANCER RESEARCH 63, 3654–3658, July 1, 2003.

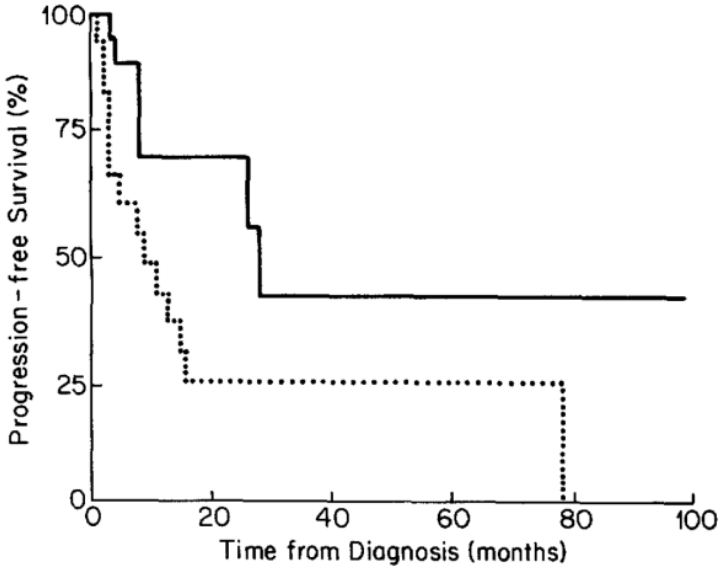


Fig 3. Kaplan-Meier analysis of PFS of neuroblastoma patients. The survival of the quartiles of patients with the lowest (—) and highest (· · ·) G_{D2} levels at diagnosis are compared.

Figure from: Valentino et al. **Shed tumor gangliosides and progression of human neuroblastoma,** Blood, 1990 75: 1564-1567

Panel 13. Oncology is an Example of Other Application for Inhibitors of Ganglioside Biosynthesis. There are a number of alternative medical applications for small molecule inhibitors of ganglioside biosynthesis. One of these is oncology. Several tumor types have been shown to require gangliosides for their aggressive growth in animal models (MEB4 model shown on the left). Additionally, the progression of some human tumor types can be predicted based on tumor ganglioside expression. The progression of human neuroblastoma based on ganglioside levels at diagnosis are shown on the right.

Conclusions and Ongoing Studies

Development of a CNS Penetrant Therapy for Sandhoff and Tay-Sachs Disease

We are preparing to test these compounds in the mouse models of Lysosomal Storage Diseases that involve ganglioside accumulation. These studies will help determine the effects on the lysosomal accumulation of GM2 in the CNS of the Sandhoff mouse model. Future studies will examine the clinical benefit to neurological function and survival.

Simultaneously, we have a significant medicinal chemistry effort focused on designing, synthesizing and testing analogs of these compounds to improve the potency and drug-like properties to support in vivo efficacy at the lowest possible dose.

Additional studies will investigatethe efficacy of these agents in models of Tay-Sachs, GM1 gangliosidosis and Niemann Pick C. We are actively testing the use of these compounds in animal models of other human diseases that involve gangliosides such as cancer.