Gene therapy PR001 increased GCase activity and improved neuronopathic Gaucher disease phenotypes in mouse models
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Background

Mutations in the GBA1 gene are believed to be the most common etiology of lysosomal storage diseases, with almost 300 mutations reported.1 GBA1 mutations cause Gaucher disease (nGD), an autosomal recessive inherited disorder, and are a major risk factor for Parkinson’s disease. Deficiency in the GBA1 enzyme glucocerebrosidase (GCase), a key lysosomal enzyme required for the normal metabolism of glycolipids, leads to the accumulation of the GCase glycolipid substrates glucosylsphingosine (GluSph) and glucosylceramide (GluCer) and ultimately results in toxicity and inflammation as seen in conditions such as neuronopathic GD (nGD).

Gene therapy utilizes non-replicating viruses as shuttle vectors to deliver engineered DNA cargos to human cells. We have chosen adeno-associated virus (AAV)-based vectors as these have shown substantial promise in achieving stable, long-acting gene expression. Specifically, we have chosen AAV9 for our nGD program as it is uniquely well-suited to deliver gene material to the brain, and has demonstrated efficacy, acceptable safety, and broad brain-wide biodistribution in third-party clinical trials in other disease areas. By expressing functional GBA1 in patients with nGD, we aim to stop disease progression.

Approach

We have developed a gene therapy product candidate, PR001 (AAV9-GBA1). PR001 efficacy was evaluated in two mouse models: (i) a chemical model of GCase deficiency; (ii) a genetic model of nGD. Both models exhibit characteristic phenotypes of nGD: deficient GCase activity, accumulation of the glycolipid substrates of GCase, deficits in motor behavior, and neuropathological changes including angiopathy and microglia, reflecting neuroinflammation. PR001 efficacy and select safety endpoints were examined in these models, and further toxicology studies were performed in nonhuman primates.

CBE Model Establishment

• Conduritol-β-epoxide (CBE) is a pharmacological inhibitor of GCase
• Mice treated with CBE display phenotypes consistent with GCase loss-of-function
• A CBE dose-ranging study was performed to establish a CBE mouse model of nGD
• Mice were dosed with daily intraperitoneal (IP) injections of phosphate buffered saline (PBS) or CBE (25 mg/kg, 37.5 mg/kg, 50 mg/kg) starting at postnatal day 8 (P8)
• Animals were tissue collected on P27-29

Figure 1. CBE Model Validation

![CBE Model Validation](image)

For all graphs: means are presented. Error bars are SEM. *p<0.05; **p<0.01; ***p<0.001.

• CBE treatment led to lethality, a failure to gain weight, and a motor coordination deficit in a dose-dependent manner
• All future CBE model studies used the 25 mg/kg dose as it recapitulated key features of nGD

PR001 Efficacy in the CBE Model

• PR001 efficacy was examined in the CBE model
• Animals were treated with PR001 or excipient at P3 via intracerebroventricular (ICV) injection
• All comparisons were made to the CBE + Excipient group to examine model phenotypes and PR001 efficacy

![PR001 Efficacy in the CBE Model](image)

Animals were weighed daily and subjected to motor behavioral tests prior to tissue collection on day 38-40. n=10 animals/group.

PR001 Persistence in the CBE Model

• A six-month study was performed in the CBE model to assess the persistence of PR001
• Animals were treated with PR001 or excipient at P3 via ICV injection

![PR001 Persistence in the CBE Model](image)

Assays were performed and analyzed as above. n=10-11 animals/group.

• PR001 treatment resulted in sustained GBA1 expression and suppression of lipid accumulation in the CBE model

PR001 Efficacy in the 4L/PS-NA Model

• PR001 efficacy was examined in the 4L/PS-NA model
• This model harbors a pathogenic mutation in GBA1 (V394L) in addition to deficiency of the GCase activator saposin C, resulting in reduced GCase activity
• Animals were treated with PR001 or excipient at 3-4 weeks of age via ICV injection
• All comparisons were made to the 4L/PS-NA + Excipient group to examine model phenotypes and PR001 efficacy

![PR001 Efficacy in the 4L/PS-NA Model](image)

Animals were tissue collected at 18 weeks of age. GCase activity was measured using a fluorometric enzyme activity assay. GCase substrates (GluSph and GluCer) were quantified using UPLC-MS/MS. n=4-5 animals/group.

• PR001 treatment resulted in a significant increase in GCase activity along with decreased lipid accumulation
• The lifespan of these mice is approximately 22 weeks,2 therefore, the long-term effects of PR001 treatment were not tested in this model

Safety and Toxicology Studies

Safety was evaluated in each of these mouse models by H&E staining. The brain and peripheral tissues showed no adverse histopathologic findings or evidence of toxicity due to treatment. Biodistribution and toxicology studies were performed in nonhuman primates. Broad distribution of vector genomes throughout the CNS and periphery was observed. In-life assessments and H&E staining showed no adverse findings or evidence of toxicity due to treatment.

Conclusions

These studies demonstrate that PR001 effectively increased GCase expression, reduced glycolipid substrate accumulation, and reduced inflammation in mouse models of nGD. In addition, PR001 treatment was well tolerated in the mouse models evaluated as well as in the NHP studies. Together, these studies support the clinical development of PR001 for the treatment of patients with nGD.

The current standard of care for GD patients is limited to enzyme replacement or substrate reduction therapies. These do not cross the blood-brain barrier and, therefore, only treat the peripheral symptoms, not the neurological symptoms, of GD. For patients with nGD, PR001 has the potential advantage of treating the neuronal symptoms of the disease.

References


Disclosures

All authors were or are currently employed by Prevail Therapeutics.