HHS Public Access

Author manuscript

Curr Opin Neurol. Author manuscript; available in PMC 2024 October 01.

Published in final edited form as:

Curr Opin Neurol. 2023 October 01; 36(5): 464–473. doi:10.1097/WCO.0000000000001187.

Current avenues of gene therapy in Pompe disease

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Abstract

Purpose of the review: Pompe disease is a rare, inherited, devastating condition that causes progressive weakness, cardiomyopathy and neuromotor disease due to the accumulation of glycogen in striated and smooth muscle, as well as neurons. While enzyme replacement therapy has dramatically changed the outcome of patients with the disease, this strategy has several limitations. Gene therapy in Pompe disease constitutes an attractive approach due to the multisystem aspects of the disease and need to address the central nervous system manifestations. This review highlights the recent work in this field, including methods, progress, shortcomings, and future directions.

Recent findings: Recombinant adeno-associated virus (rAAV) and lentiviral vectors (LV) are well studied platforms for gene therapy in Pompe disease. These products can be further adapted for safe and efficient administration with concomitant immunosuppression, with the modification of specific receptors or codon optimization. rAAV has been studied in multiple clinical trials demonstrating safety and tolerability.

Summary: Gene therapy for the treatment of patients with Pompe disease is feasible and offers an opportunity to fully correct the principal pathology leading to cellular glycogen accumulation.

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Conflicts of interest

RCS has received research support and received consultancy fees from Maze Therapeutics and is a member of the Medical Advisory Board for Little Warrior Foundation. MSG has research support, research compounds, or consultancy fees from Maze Therapeutics, Valerion Therapeutics, Ionis Pharmaceuticals, PTC Therapeutics, and the Glut1-Deficiency Syndrome Foundation. RCS and MSG are co-founders of Attrogen LLC. MC has received research support from Sanofi, Friedreich Ataxia Research Alliance (FARA), Amicus, AavantiBio, Lacerta, Provention Bio, Sarepta, Duchenne Research Fund, Muscular Dystrophy Association (MDA), GoFAR, Cydan, Audentes. MC has received consulting fees from AavantiBio, Reata, Lilly, Avexis and Gilbert foundation, SwanBio and PCT Therapeutics. BJB has received research support from SolidBio, ProventionBio, Barth Syndrome Foundation. BJB has received consulting fees from AavantiBio, Amicus Therapeutics, Rocket Pharma, Pfizer, Sanofi, and Sarepta Therapeutics. MC and BB are co-founders of Ventura, LLC.

Further work is needed to overcome the limitations related to vector production, immunologic reactions and redosing.

Keywords

Pompe disease; gene therapy; adeno-associated virus; lentivirus

INTRODUCTION

Pompe disease is an autosomal recessive condition secondary to mutations in the acid-α-glucosidase (GAA) gene, responsible for lysosomal glycogen degradation[1]. Pompe disease has a predicted genetic prevalence of ~1:10,000–30,000 based on newborn screening data but historically this ranged between 1:35,000 and 1:138,000, with a carrier frequency of 1:77[2, 3]. The disease results in pathologic accumulation of glycogen primarily in cardiac, skeletal and smooth muscle, and it was once considered a muscle disease, however, there is growing evidence of the impact in endothelial cells and motor neurons with glycogen deposition in the central nervous system (CNS), progressive neuro-degeneration, vasculopathy and cognitive impairment, highlighting its multisystemic impact[4–6]. Similarly, the commonly seen respiratory dysfunction is caused by disruption of the proximal and distal airways structure and function, in addition to the well described muscular weakness[4, 7, 8]. Pompe disease is classified as infantile onset (IOPD) and late onset (LOPD)[9]. Classical IOPD is characterized by cardiomegaly, hypotonia and cardiorespiratory failure during the first year of life, and non-classical IOPD has a less severe phenotype[9, 10]. LOPD is subdivided into childhood or juvenile, and adult-onset, both characterized by myopathy that presents later in life and the lack of cardiomyopathy[9, 11].

Enzyme replacement therapy (ERT) was approved by the Food and Drug Administration (FDA) in 2006. The process of ERT involves intravenous administration of recombinant human alglucosidase alfa (rhGAA)[12, 13]. Early ERT impacts cardiomyopathy in IOPD patients but they continue to present skeletal and smooth muscle dysfunction with associated weakness[14]. rhGAA has limited efficiency due to preferential uptake of rhGAA by the liver, suboptimal binding of mannose-6-phosphate (M6P) to the cation-independent M6P receptor (CI-M6PR) and abnormal trafficking of M6P in the lysosomes[15]. Avalglucosidase-alpha, a synthetic oligosaccharide that includes M6P residues linked to rhGAA to enhance the affinity for CI-M6PR, was approved in 2021 for LOPD, with ongoing trials in IOPD[16–18]. Another approach is the use of small molecule chaperones to improve the bioavailability of rhGAA. Chaperones assist in the folding of rhGAA to prevent premature degradation, while retaining enzyme activity[19]. Cipaglucosidase alfa + miglustat uses this approach. A phase III clinical trial in LOPD patients concluded that the combination was not superior to standard ERT based on 6-minute-walk-test[20].

In addition to the aforementioned limitations, ERT requires biweekly infusions, does not cross the blood brain barrier (BBB) and can result in immunological reactions towards rhGAA[21–23]. Therefore, there is a need for novel interventions for patients with Pompe

disease. Herein, we discuss the recent advancements in the field of gene therapy, as well as other recent strategies.

GENE THERAPY FOR POMPE DISEASE

A functional gene is introduced to substitute for the mutated gene, enabling endogenous production of GAA, which then undergoes natural posttranslational modifications for efficient trafficking to the lysosome[24]. It is crucial to target the right cells by selecting an appropriate vector capsid serotype, promoter, and route of administration[25].

1. Adeno-associated viral vectors in Pompe disease

Adequate selection of the gene promoter and adeno-associated virus (AAV) serotype is key in developing a gene therapy product. Some studies focus on the skeletal and cardiac symptoms of the disease using ubiquitous promoters, such as human cytomegalovirus (CMV) or the hybrid CMV enhancer/chicken β-actin (CBA). CMV and CBA promoters offer quick, strong, and long-lasting expression throughout the body[26, 27], however expression of GAA in other cell types, such as antigen presenting cells can cause adverse events. Conversely, a variety of muscle specific promoters are utilized to restrict expression to striated muscle, showing minimal expression in non-muscle tissues and potentially reducing the immune response to the transgene[28]. The desmin promoter is expressed in all cells with intermediate filaments and provides selective expression in motor neurons, skeletal and cardiac muscles. The synapsin promoter targets principally neurons and the liver-muscle promoter (LiMP) provides expression in non-dividing muscle cells and hepatic tissues[27, 29, 30].

1.1. Intravenous AAV-derived gene therapy—Several studies have tested AAV serotypes with increased muscle tropism to target and correct pathology in Pompe disease, either intravenously or directly into the muscle[29–49]. A single intravenous injection of an rAAV1-GAA vector restored GAA activity in the Pompe mouse model and exhibited a long-lasting corrective effect on cardiac and skeletal muscle resulting in improvement in force mechanics of the soleus and diaphragm[32]. In a separate study, an injection of rAAV-PHP.B, a rAAV9 variant, resulted in therapeutic levels of GAA activity while decreasing glycogen in skeletal and myocardial muscles resulting in improved gait and reduced peripheral neuropathy[50].

AAVB1-DES-hGAA, a vector with high affinity for muscle and CNS, and AAV9-DES-hGAA were utilized in $Gaa^{-/-}$ mice. AAVB1 treatment resulted in weight gain, forelimb strength, and higher levels of transduction in the diaphragm, tongue base, and thoracic spinal cord when compared to AAV9. Both groups displayed above-average GAA activity and reduced glycogen accumulation in the heart, diaphragm, tongue, gastrocnemius, and lung[45].

A study in mice and non-human primates using AT845, an AAV8 vector where GAA expression is driven by the murine muscle creatine kinase (MCK) promoter/enhancer and expressing a codon-optimized GAA, resulted in high enzyme levels, leading to dose dependent functional improvements and glycogen clearance. Unfortunately, higher doses

in cynomolgus macaques led to immune responses and cardiac abnormalities requiring euthanasia. It was later determined that the immune responses were largely due to a xenogenic anti-GAA immune response[38**]. AT845, was used in a phase 1/2 trial in four LOPD patients, three subjects discontinued ERT and functional outcomes were stable 51 weeks later[51]. The study was recently on clinical hold due to neuropathy in one subject[52, 53].

A highly potent AAV variant, AAV.cc47 was recently tested in $Gaa^{-/-}$ mice. Mice received intravenous 1.3e14vg/kg with a single stranded genome encoding GAA driven by the CBh promoter. This resulted in GAA levels of 67% of wild type mice, compared to 26% in AAV9-GAA treated mice[46*].

Eleven rhesus macaques received AAVhu68 tagged with an insulin-like growth factor 2 variant (vIGF2) peptide to increase uptake (AAVhu68-vIGF2-hGAA). Five animals had immune responses, including dose independent T-cell-mediated myocarditis. Toxicity was associated with a major histocompatibility complex class I haplotype in three animals[47**].

Remarkably, the liver can secrete high levels of engineered proteins and can serve as a depot for secretion of rhGAA[54]. The liver can promote immune tolerance and help prevent immune reactions caused by transgene products, provided the gene is expressed only in liver cells[55]. AAV8 vectors in pre-clinical studies have shown GAA expression and secretion through hepatocytes, with evidence of reduced muscle glycogen content and improved functional tests in mice[29, 36, 56–59]. A study in a canine model demonstrated persistent correction of GAA activity two years after concurrent systemic- and liver-targeted vector delivery of rAAV9-DES-hGAA and rAAV9-LSPcoGAA in association with anti-thymocyte globulin and methylprednisolone. This strategy supports the use of dual vector to achieve GAA tolerance[60*].

Two current clinical trials are focused on liver-directed gene therapy by creating a liver depot for GAA production[61, 62]. The trial by SPARK therapeutics uses an AAVRh74 derived capsid in a phase 1/2 trial. (NCT04093349). Smith et al showed the preliminary results in three subjects receiving AAV8-LSPhGAA at 1.6×10^{12}vg/kg. The authors demonstrated safety, however, the lack of glycogen lowering suggested that despite increased GAA activity in skeletal muscle, the efficacy was not sufficient to replace ERT[61**].

1.2. Central nervous system delivery of AAV-derived gene therapy—

While intravenous administration of AAV leads to widespread gene transfer in the neonatal CNS, this approach is not easily translatable to LOPD since it would require high vector doses to treat the CNS manifestations[63, 64]. Intrathecal, intra-cisterna (ICM), and intracerebroventricular (ICV) routes are considered to treat these manifestations[65].

A single intrathecal dose of AAV9-CAG-hGAA or AAVrh10-CAG-hGAA to $Gaa^{-/-}$ mice led to low glycogen levels in the CNS and partial improvement of muscle strength but no changes in muscle glycogen. Furthermore, AAV9 treatment restored cardiac GAA levels and improved cardiomyopathy[66]. Intrathecal administration of AAV5-GAA at the C3-C4 level

in Gaa$^{-/-}$ mice to target the phrenic nerve nucleus area decreased intraneuronal glycogen content and improved ventilation, even without enzymatic activity in the diaphragm[67].

1.3. Other AAV-derived gene therapy approaches—Intrapleural rAAV9-GAA to Gaa$^{-/-}$ mice resulted in improvement of ejection fraction in cardiac magnetic resonance, greater relative inspiratory burst amplitude during baseline conditions, and increased efferent phrenic burst amplitude. The effects were achieved by retrograde transport to motoneurons[42].

Intra-diaphragmatic administration of rAAV1-CMV-hGAA in nine IOPD subjects at two different doses (1.0×10^{12} and 5.0×10^{12}vg) demonstrated no adverse events related to the product. Anti-capsid and anti-transgene antibody response was observed in all, except for those who received concomitant immunomodulation with sirolimus and rituximab[34]. Subjects from this cohort participated in inspiratory muscle conditioning demonstrating benefits to diaphragmatic function, particularly in subjects with higher neuromuscular function[68].

Recently, an AAV9 product encoding an excitatory Designer receptor exclusively activated by designer drugs (DREADD) (AAV9-hSyn-hM3D(gq)-mCherry) was injected to the posterior tongue of GAA$^{-/-}$ mice. Lingual electromyography (EMG) showed significant increases in tonic and phasic inspiratory activity after DREADD administration. mCherry expression was confirmed in hypoglossal motoneurons in all mice, confirming retrograde movement of AAV9. This approach could address dysphagia, dysarthria and sleep disordered breathing in patients with Pompe disease[69*].

Figure 1 lists the most relevant ERT and AAV clinical trials to date.

2. Lentiviral vectors in Pompe disease

Hematopoietic stem and progenitor cell (HSPC) mediated lentiviral gene therapy (HSPC-LVGT) is an attractive approach for the treatment of Pompe disease. The method involves transplantation of *ex-vivo* gene-modified autologous HSPCs to overexpress the needed transgene[70–73]. HSPC-LVGT has been used in other disorders, including B-thalassemia, Wiskott-Aldrich syndrome, and adrenoleukodystrophy[74–76].

HSPC-LVGT demonstrated long-term engraftment and continuous supply of GAA after one intervention in *Gaa*$^{-/-}$ mice with improvement of cardiac and motor function. However, it did not achieve glycogen reduction to normal levels and required a high vector copy number (VCN), which can increase the genotoxicity risk as previously seen with early design gammaretroviral vectors[77, 78]. Currently, third generation self-inactivating LV vectors are used to decrease these risks[79]. Attention has been drawn to the modification of the LV vector to improve receptor affinity, like in the case of IGF2[80]. Liang et al. created a vector that contains a codon-optimized GAA sequence fused to codon-optimized human IGF2 (LV-IGF2.GAAco) leading to correction of glycogen accumulation, autophagy, motor function and brain glycogen content at a much lower VCN[80*]. A similar approach has been used in other preclinical studies to create engineered GAA coding sequences, distinct peptide tags and codon optimizations. The use of LVGT with glycosylation-independent lysosomal

targeting tags increased secretion and reduced glycogen, myofiber and CNS vacuolation in tissues, but maintained low GAA enzyme activity[81**].

Moreover, HSPC-LVGT can limit immunoglobin G (IgG) responses through tolerance induction against the transgene product—one of the key benefits of this technique[71]. It can also allow for complementary ERT, resulting in enhanced glycogen elimination from skeletal and cardiac muscles. In this approach, the existence of GAA-expressing HSPC-derived cells in the thymus indicated the establishment of central immune tolerance[82].

Figure 2 summarizes the AAV and LV-derived products based on tissue tropism.

Challenges and potential solutions in gene therapy

1. Vector production: rAAV production methods are distinguished by cell line type, substrate for cell growth and precursor materials, however, these are often not suitable for large scale production. Chemical transient transfection is popular due to simplicity and availability of raw materials but has limited yield per cell and requires good manufacturing process (GMP) grade plasmids[83–85]. New technology has emerged to scale transient transfections, including packed bed reactors[86–88], conversion to suspension HEK293 lines[89], AAV process intensification[90*, 91*] and the use of novel chemical additives as transfection reagents [84, 89, 92-96*]. Non-plasmid DNA sources such as doggybone DNA and novel transfection reagents could facilitate manufacturing significantly, along with the use of certain chemical additives to boost rAAV production[95, 97–99].

Viral infection platforms, using insect baculovirus (rBV) or Human Herpes Simplex Type I virus (rHSV1) offer high yields per cell and are used in clinical trials[100, 101]. rHSV1 coinfection modifies AAV genome-end recombination, emphasizing the need for improvement of rHSV-1 production[102*]. The rBV system's limitations include loss of AAV particle infectivity, insect virus contamination of cell lines, differences in post-translational modifications of the final product, which produce a negative impact on potency [101, 103] Meanwhile, the rHSV system appears more versatile in producing highly infectious AAV regardless of serotype[92, 93, 104].

Packaging/producer cell lines (PCL) are unique systems that stably incorporate viral and cargo genes into the cell lines prior to GMP manufacturing. Advantages of PCLs include scalability to 2,000L, compatibility with existing biologics production infrastructure, and consistent batch-to-batch performance[105–107]. However, PCLs require an established cell line development effort, and require wild-type Ad propagation as a process-related impurity[105–107].

rAAV manufacturing related impurities can result in significant immunogenicity. Research is needed to ensure effective development of rAAV vectors, while minimizing process and product derived impurities and ensuring safety[91, 93].

2. Immune responses to AAV, and vector re-administration: One major obstacle for successful gene therapy is the immunological response to the vector capsid and transgene

product affecting safety and duration of effect[108]. These reactions can involve innate, cellular and humoral responses[109, 110*].

AAV re-administration has been an important concern especially for IOPD since transgene expression is expected to decrease due to somatic growth and vector dilution[111]. Several strategies to prevent anti-AAV antibody production and to allow for redosing have been proposed including plasmapheresis or antibody cleavage[85, 112]. Only one approach has been tested clinically and consisted in the use of sirolimus and rituximab to demonstrate that two consecutive intramuscular administrations of AAV vectors is possible (NCT02240407) [62].

OTHER TREATMENT APPROACHES

Multiple additional approaches have been reported to date[16, 20, 113–119] Recently, progress has been made in the following:

1. *In-utero* ERT:

A fetus with CRIM-negative IOPD received *in-utero* ERT starting at 24 weeks of gestation, postnatal immune tolerance was started and ERT continued. The child had normal cardiac and motor function at 13 months of age[120**].

2. Glycogen-synthase-1 inhibitors:

Glycogen production is regulated by glycogen synthase (GYS1), which can be inhibited by the phosphorylation of S641 and S645 through the mTORC1 pathway[121, 122]. A recent phase I/II clinical trial (NCT05249621) evaluated an oral GYS-inhibitor in healthy subjects and showed good tolerance and reduced glycogen in peripheral blood mononuclear cells[123].

3. Fusion proteins for targeted delivery of GAA:

These retain enzymatic function and bind to effector proteins that traffic to the lysosome. This approach was done using CD63 and Integrin-subunit-alpha 7 (ITGA7). α-hCD63$_1$IgG:GAA and α-ITGA7IgG:GAA internalized in a CI-M6PR independent mechanism with the former being more effective. Similar findings happened when using a single-chain fragment variable (scFv) instead of IgG. In a second step, the same study used AAV2/8 viruses encoding α-hCD63$_1$scFv:GAA driven by a liver-specific promoter and showed higher GAA levels compared to AAV-GAA treated mice[124*]. A similar strategy was used to create VAL-1221, a fusion protein comprising the Fab portion of a cell penetrating-antibody and rhGAA, tested in a 3-month phase I/II study in 12 adults with LOPD. The study showed dose dependent improvements but no significant changes in PD markers[125].

Figure 3 summarizes all possible strategies in PD treatment

CONCLUSIONS

ERT significantly improves the outcomes of patients with Pompe disease. ERT targets cardiomyopathy and skeletal muscle pathology but has limited effect in other tissues. Gene therapy strategies have the potential to treat multiple systems, including the CNS. Newer viral vectors allow for enhanced expression, specificity and increased enzyme activity based on preclinical and clinical studies. However, gene therapy continues to face major challenges related to vector production, immunological response and redosing.

Financial support and sponsorship

This work was supported by the following grants: R01 HL139708–02 and U01-NS116752–01A1(MC, BJB). R35 NS116824 (MSG) and P01 NS097197 (MSG). NIH grant 1R01AG066653, 1R01AG078702, 1R01CA266004, V-Scholar Grant (RCS).

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Key Points

- Gene therapy for Pompe Disease uses primarily Adeno-associated viral vectors and lentiviral vectors
- Recombinant viral vector production has multiple challenges
- Redosing of gene therapy in Pompe disease is limited by immunological responses

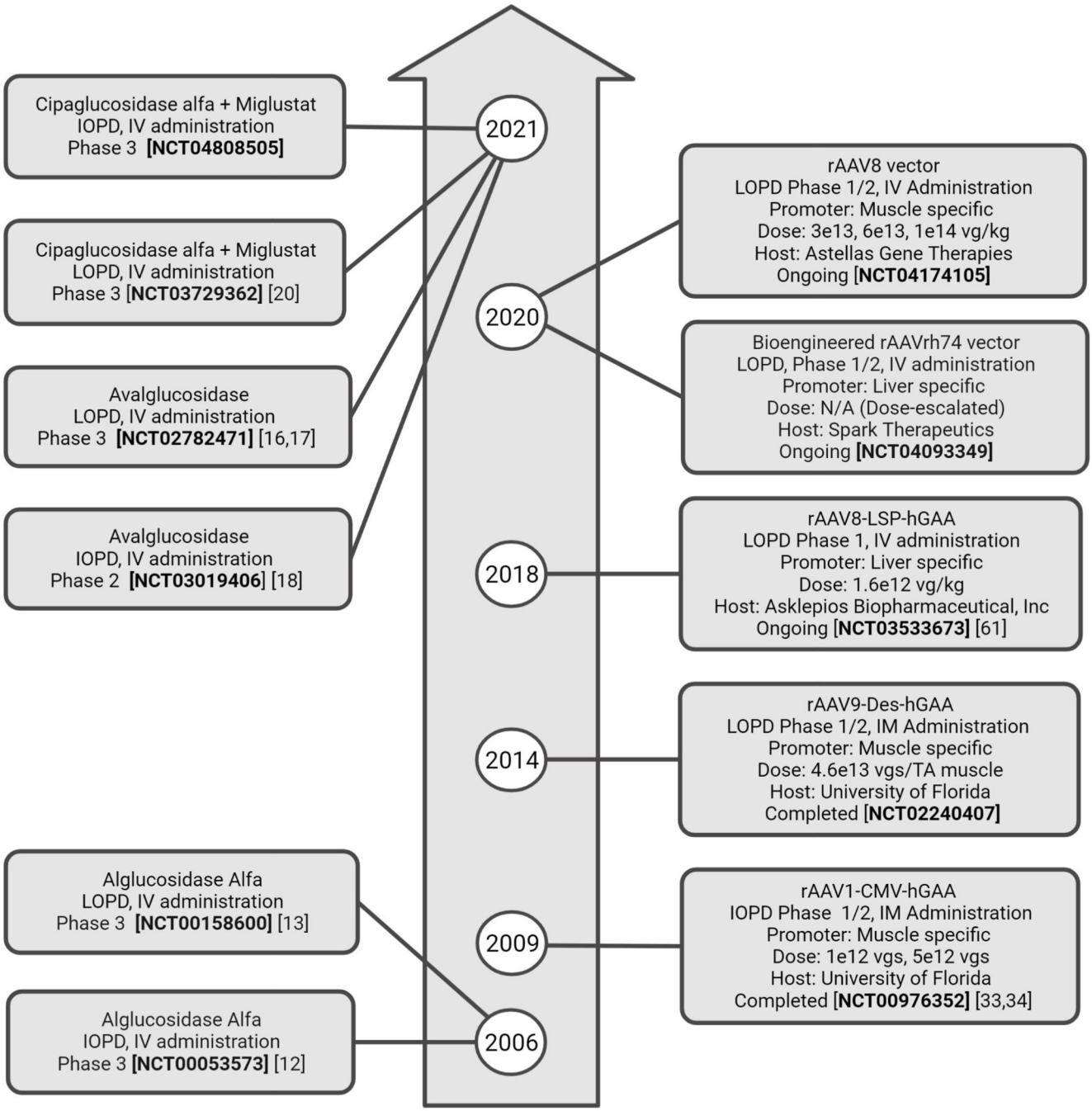

Figure 1.

Timeline of the most relevant clinical trials using enzyme replacement therapy (left) and Adeno-associated viral vectors (right). IOPD: Infantile-onset Pompe disease. LOPD: Late-onset Pompe disease. IV: intravenous. IM: Intramuscular. TA: tibialis anterior. vgs: vector genomes

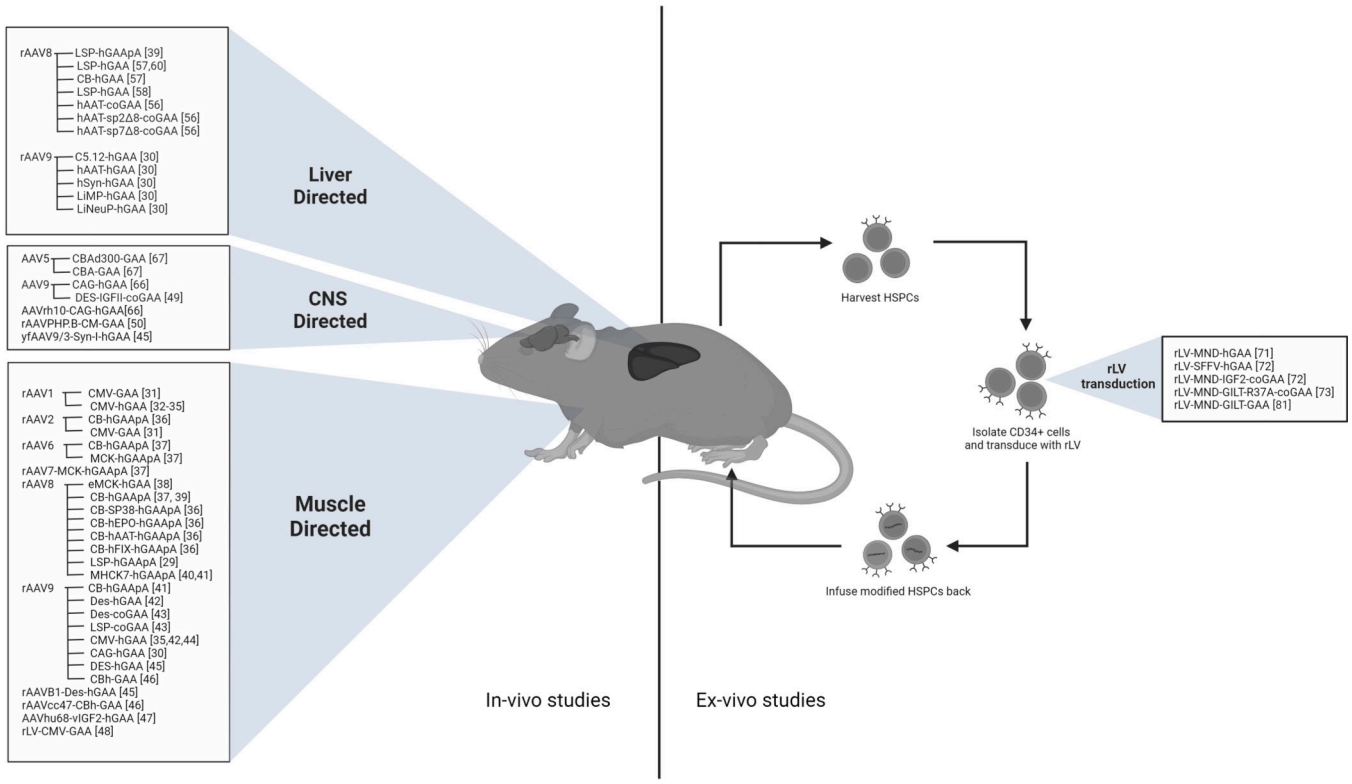

Figure 2.

Summary of all in-vivo and ex-vivo vectors and promoters reported based on tissue tropism. CNS: Central nervous system. HSPC: Hematopoietic stem and progenitor cell

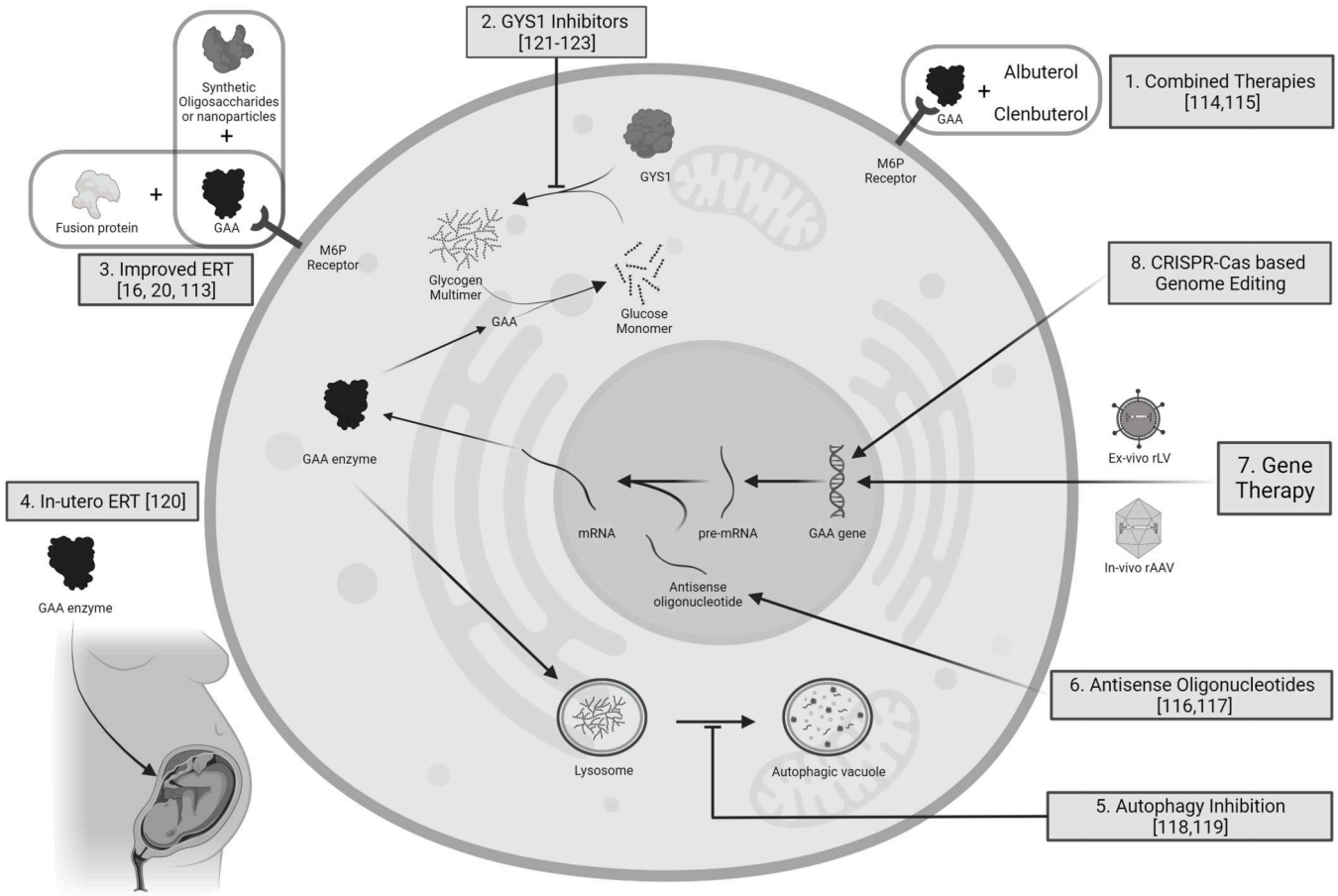

Figure 3.

Preclinical and clinical strategies to treat Pompe disease. ERT: enzyme replacement therapy, GAA: acid alfa glucosidase. M6P: Mannose-6-Phosphate. GYS 1: Glycogen synthase 1