

Minireview

# Pompe disease: Current state of treatment modalities and animal models

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## Abstract

Pompe disease is a rare autosomal recessive lysosomal storage disease caused by deficiency of acid- $\alpha$ -glucosidase (GAA). This deficiency results in glycogen accumulation in the lysosomes, leading to lysosomal swelling, cellular damage and organ dysfunction. In early-onset patients (the classical infantile form and juvenile form) this glycogen accumulation leads to death. The only therapy clinically available is enzyme replacement therapy, which compensates for the missing enzyme by i.v. administration of recombinant produced enzyme. The development of clinically relevant animal models gained more insight in the disease and allowed evaluation of recombinant enzyme therapy. Several therapies are currently under investigation for Pompe disease, including gene therapy. This review gives an overview of the available knockout mouse models, of the *in vitro* and *in vivo* studies performed using recombinant produced enzyme. Furthermore, it describes current therapeutic approaches for Pompe disease as well as experimental therapies like gene correction therapy.  
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Pompe disease is a rare autosomal recessive lysosomal storage disease caused by the deficiency of acid- $\alpha$ -glucosidase (GAA). The disease is characterized by generalized muscle weakness combined with cardiomyopathy, ultimately leading to death of the patient. Pompe disease has been classified into the infantile form and the late-onset form [1]. The severity of the disease is related to the amount of residual enzyme activity. The overall incidence for Pompe disease is estimated at 1 in 40,000 live births and the incidence varies by ethnicity [2,3].

Despite the low incidence, the severity of the disease warrants extensive research into the pathophysiology. So far, several possible therapeutic approaches have been described. Currently, the only available treatment is

enzyme replacement therapy (ERT). The aim of this therapy is to compensate for the missing enzyme by intravenous administration of recombinant GAA. Although symptoms are alleviated, life-long treatment is necessary. Moreover, due to poor uptake of the recombinant enzyme, efficient treatment requires a high dosage which often is associated with an immunogenic response. Currently, ERT results only in a minimal prolongation of survival for patients suffering from the disease. Therefore, further research is necessary to develop a novel therapeutic approach.

## History

Pompe disease or glycogen storage disease type II (GSDII) is an autosomal recessive lysosomal storage disease which has been first described in 1932 by J.C. Pompe. He documented a case of a 7-month-old patient suffering

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from progressive generalized muscle weakness. The patient died from cardiac hypertrophy at eight months of age [4]. G.T. Cori, who discovered the process of catalytic metabolism of glycogen, classified the disorder as glycogen storage disease type II (GSDII) in 1954 [5].

Based on the research of G.T. Cori and on the discovery of the lysosome by C. DeDuve in 1955, H.G. Hers recognized that Pompe disease was caused by the absence of the lysosomal enzyme GAA [6]. With this observation, Pompe disease was the first disease to be classified as a lysosomal storage disease. In 1970, a report was published describing the late-onset form of the disease [7]. Nine years later, the gene responsible for Pompe disease was localized on chromosome 17 and called acid- $\alpha$ -glucosidase (GAA). A first attempt to treat a patient with Pompe disease using bone marrow transplantation failed [8].

The first clinical use of purified human placental GAA was reported in 1973. Low uptake of the administered enzyme and restrictions in clinical trial design prevented a broad application of placental GAA [9]. In 1991, 6-week-old Swiss mice were treated with GAA enzyme isolated from bovine testes. The results proved *in vivo* uptake of GAA in the muscle [10]. Clinical trials were initiated in 1998 with the administration of recombinant human GAA (rhGAA), either produced in milk of transgenic rabbits or in Chinese hamster ovary (CHO) cells. Such ERTs are currently the only available therapies for patients with Pompe disease and trials are still ongoing [11–13] testing modified GAA for improvement of uptake of the enzymes [14].

### The GAA protein

The human GAA gene is localized on chromosome 17, is approximately 28 kb in length and contains 20 exons [15]. The corresponding cDNA is 3.6 kb and encodes for a 952-amino-acid protein. The 110 kDa precursor form of GAA receives cotranslational N-linked glycosylation in the endoplasmic reticulum, and is targeted to lysosomes via mannose-6-phosphate receptors (MPRs) as well as through a mannose-6-phosphate (M6P)-independent mediated pathway by a still unknown receptor [16].

Insertion of M6P residues on lysosomal enzymes is the result of post-translational modification carried out by phosphotransferase and *N*-acetylglucosaminidase located in the Golgi compartment. After addition of M6P residues in the Golgi compartment, the lysosomal enzymes are recognized by cation-independent MPRs and cation-dependent MPRs, which finally translocate the enzymes to the lysosomes [14]. Although most enzymes are taken up by the lysosomes using the M6P-dependent pathway, poorly phosphorylated lysosomal enzymes end up in lysosomes through a M6P-independent pathway [17]. This was proven by a study performed with I-cell disease, which is caused by loss of phosphotransferase. The study revealed that the lysosomal enzyme GAA was present at normal levels in the lysosome, indicating that the unphosphorylated enzyme could be transported to the lysosome via an M6P-indepen-

dent pathway [16]. Moreover, a study using MPR-deficient fibroblasts revealed that enzymes could be transported to lysosomes indicating an M6P-independent pathway [18].

After removal of the signal sequence of the 110 kDa precursor form, proteolytic cleavage occurs in the late endosome and/or lysosome leading to the endosomal intermediate form (95 kDa) and finally to the lysosomal fully active forms (76 and 67 kDa). Both forms of GAA are responsible for the breakdown of glycogen into glucose [19–21].

In the cytoplasm, hydrolysis of glycogen takes place when glucose is needed either to maintain blood glucose levels (by liver cells) or to serve as an energy source (by muscle cells). During times of cellular turnover, glycogen is thus taken up by the lysosomes and broken down into glucose. In Pompe disease, mutations in the gene coding for GAA lead to the lack of adequate activity of GAA resulting in a hampered degradation of lysosomal glycogen. For patients with Pompe disease, the impaired glycogen destruction thus results in a massive glycogen accumulation, leading to lysosomal swelling, cellular damage and organ dysfunction (Fig. 1).

The severity of clinical manifestation in patients is well correlated with the genetic abnormality which determines the amount of residual enzyme activity [22]. Two different phenotypes of Pompe disease have been described. The most severe form is the infantile form diagnosed between birth and 7 months of age with an incidence of 1:138,000 [2,3]. The manifestations are generalized muscle weakness, cardiomegaly, severe hypotonia, mild hepatomegaly, macroglossia, and death usually occurs by cardiorespiratory failure in the first year. Mental development is apparently normal [19,23,24].

The second phenotype is the late-onset form. The late-onset form can be divided into the juvenile form and the adult form. The juvenile form is characterized by onset in the first decade. Typically, there is reduced but residual enzyme activity. Clinical manifestations are mainly skeletal muscle weakness, respiratory muscle involvement and mild hepatomegaly. Cardiac involvement is mild or absent [22,24,25]. The adult form is characterized by onset in the third to sixth decade of life with a predicted frequency of 1:57,000 [3]. The adult form is similar to the juvenile form, however the adult form possess higher levels of residual GAA activity and there is a slower progression of skeletal muscle weakness. In both phenotypes, the respiratory muscles are most strongly affected, leading to death by respiratory failure [19]. More than 200 mutations are known in the GAA gene. For Pompe disease a regular updated mutation database is available at [www.pompecenter.nl](http://www.pompecenter.nl) (maintained by Pompe Center Erasmus MC Rotterdam). In this database also the most common mutations for each phenotype are described.

The nature of a mutation generally can serve as a predictor of a phenotype, and several cases have been described where genotypes correspond to defined phenotypes [19,26]. In recent years, however, an increasing number of

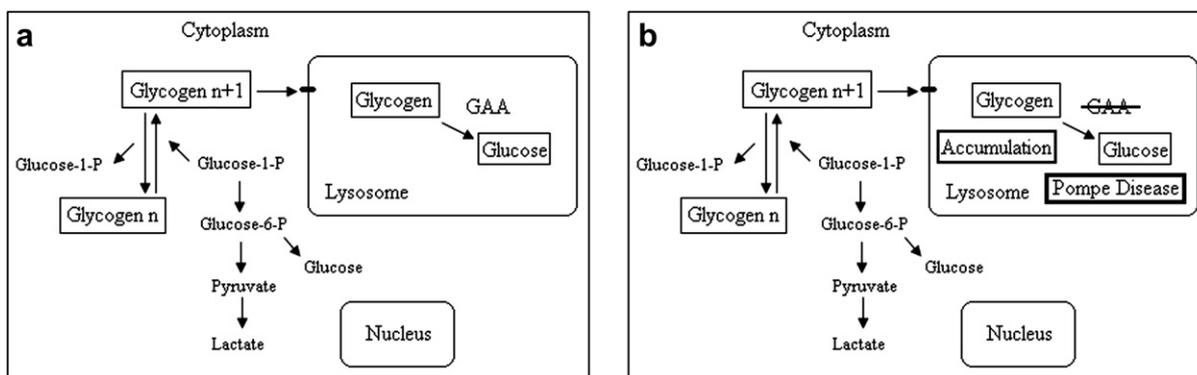


Fig. 1. Pompe disease pathogenesis. In the cytoplasm, glycogen is broken down into glucose when it is needed either to serve as an energy source or to maintain blood glucose levels. During times of cellular turnover, glycogen is taken up by the lysosome. In the lysosome, GAA is the only pathway responsible for glycogen degradation (a). With GAA deficiency (b), substantial glycogen accumulation occurs in the lysosome leading to expansion, cellular damage and organ dysfunction. Adapted from [21].

cases have been reported for which genotype and phenotype do not correlate [19,27,28]. This finding suggests a more complex pathophysiology of Pompe disease.

### Pathophysiology

Low or no GAA-enzyme activity results in massive accumulation of glycogen in lysosomes and cytoplasm. Ultimately, the glycogen storage, particularly in the myocytes of skeletal, cardiac and smooth muscle, is causing contractile dysfunction and muscle weakness [29,30]. Fukuda et al. [29] suggested that the pathological cascade in glycolytic type II muscle fibers comprises a failure of glycogen digestion that results in a local starvation. This lack of glycogen digestion stimulates a strong autophagic response in myoblasts from GAA-KO mice. The autophagic response coupled with the inability of the vesicles to fuse and discharge their contents in the lysosomes, could lead to a continuous autophagic buildup and a profound disorganization of the microtubule structure that may perpetuate the autophagic process.

Based on the ultrastructural changes of the myocyte of Pompe patients as detected by electron microscopy, Thurlberg et al. [30] classified the disease progression into five stages. They described that with increasing accumulation of glycogen, the lysosomes increased in size and number. In the following stages the membranes of the glycogen-filled lysosomes start to rupture. As a consequence of the release of lysosomal glycogen into the cytoplasm, the mitochondrial architecture becomes aberrant. This phenomenon explains the complaints of fatigue of the patients. In the last stage the cells are swollen, due to the uptake of water which leads to dilution of glycogen.

Apart from the deficient GAA activity in cells a few other hallmarks of Pompe disease can be distinguished: in plasma, creatine kinase is elevated up to 10-fold while increased liver transaminase levels are found. Muscle biopsies showed the presence of periodic acid schiff (PAS) positive vacuoles situated in the lysosome and increased acid phosphatase staining [24].

### Animal models

In order to design and evaluate novel treatment strategies, the availability of animal models imitating human diseases is essential. Naturally occurring animal models of Pompe disease include Brahman and Shorthorn cattle, Lapland dog, cats, sheep and a strain of Japanese quail [24]. However, these animal models are less suitable for research on Pompe disease than conventional laboratory animals, because of a long generation time and small litter size (cattle), or evolutionary distance from human (quail) [21]. Therefore, five mouse models have been developed by targeted disruption of the GAA gene to evaluate Pompe treatment strategies in an *in vivo* setting. The first knockout mouse model was generated by targeted disruption of exon 13. Soon after birth, these KO mice developed generalized and progressive glycogen accumulation in lysosomes of liver, heart and skeletal muscle cells. As there was a virtually complete GAA deficiency in all organs especially the heart muscle, this model is representative for the infantile form [31]. Despite the absence of GAA, except in intestine, these mice remained phenotypically normal which might be partially due to their genetic background. A second knockout mouse model was generated in which the exon 6 of the GAA gene was replaced by a cassette containing a *neomycin* resistance gene into exon 6 flanked by *LoxP* sites ( $6^{neo}/6^{neo}$ ). This model has features of both the adult and infantile phenotypes of Pompe disease [32]. GAA activity measurements confirmed the absence of functional enzyme in several tissues. A third mutant mouse model was created by mating  $6^{neo}/6^{neo}$  mice to *Cre*-producing mice. Mating resulted in littermates in which exon 6 of the GAA gene and the *neomycin* resistance gene of  $6^{neo}/6^{neo}$  mice were removed ( $\Delta 6/\Delta 6$ ). This model is similar to the  $6^{neo}/6^{neo}$  model with regard to GAA activity measurements in tissues and tail [32]. Both models showed abnormal lysosomal glycogen storage in heart and skeletal muscle. However, this glycogen accumulation was more marked in the heart than the skeletal muscle. When placed in an open

field performance,  $6^{neo}/6^{neo}$  mice showed weakness in open field and open hang wiring already at 3.5 weeks of age, whereas  $\Delta 6/\Delta 6$  mice showed weakness at a later stage. This demonstrated that the latter model is representative for the milder phenotype [32,33]. In humans, a similar deletion of exon 6 is associated with a relatively mild phenotype [34]. Raben et al. [33] created a similar mouse model by targeting exon 14 of the GAA gene ( $\Delta 14^{neo}/\Delta 14^{neo}$ ) which is comparable to the  $6^{neo}/6^{neo}$  mouse model with regard to clinical course.

Because sequential administration of GAA results in the development of an immune response in the above described immuno-competent mouse models, two other models were generated. The exon 6 KO mouse model therefore was further improved [32] in such a way that mice express low levels of human GAA in the liver, but still retain the disease phenotype. These so-called tolerant knockout mice (tGAA<sup>-/-</sup> mice) are immunological tolerant to injected recombinant enzyme [35,36]. The fifth animal model (GAA-KO/SCID mice) was developed to avoid production of anti-hGAA antibodies after enzyme administration [32,37]. Upon expression of hGAA in these mice, increased enzyme activity was detected due to lack of anti-hGAA antibodies. Moreover, decreased glycogen accumulation was observed in liver, heart and other organs.

## Current therapeutic approaches

### Enzyme replacement therapy (ERT)

Currently, the only clinically available treatment for Pompe disease is enzyme replacement therapy. As early as 1964, a patient with Pompe disease was treated with acid- $\alpha$ -glucosidase which had been isolated from the fungus *Aspergillus niger*. After infusion, a slight increase in GAA activity was detected in the liver resulting in a decrease in lysosomal glycogen. However, the reduction of lysosomal glycogen in the liver could be only obtained after administration of a high enzyme dosage of recombinant enzyme over a long period of time. As the high dosage was associated with serious side-effects, the treatment had to be terminated [38]. It appeared that GAA derived from non-human sources triggered an immune response in humans and only sub-optimal doses can be administered [9,38,39].

In general, the goal of ERT in lysosomal storage diseases is to compensate for the mutant enzyme by the continuous supply of functional enzyme. However, natural sources of suitable GAA are scarce due to the demands of safety, species specificity and efficacy. The administered enzymes used in ERT must be stable and must reach the lysosomes in the target tissues. Furthermore, the enzymes should remain catalytically active after administration and they should not induce an immune response. Because non-mammal sources are not suitable for therapy because of immunogenicity, recent efforts have focused on the production of recombinant human GAA (rhGAA). rhGAA

has been produced in the milk of transgenic rabbits and in Chinese hamster ovary (CHO) cells [40,41].

Several *in vitro* studies compared the effectiveness of rhGAA produced in milk of transgenic rabbits (designated rhGAA<sup>R</sup>) as well in CHO cells (designated rhGAA<sup>C</sup>). Addition of rhGAA<sup>C</sup> to the culture medium of fibroblasts derived from patients with Pompe disease resulted in efficient uptake of the enzyme up to similar intracellular levels as detected in normal fibroblasts [41]. Other *in vitro* studies in deficient fibroblasts demonstrated that the uptake of rhGAA<sup>R</sup> and rhGAA<sup>C</sup> was facilitated by mannose-6-phosphate receptor. Furthermore, the recombinant enzyme was correctly processed into its mature forms and the GAA activity and glycogen content was restored to normal levels [41–43,20,44–46].

Short-term pilot *in vivo* studies in exon 13 knockout mice (Table 1, model 1, Ref. [31]) revealed that administration of rhGAA<sup>R</sup> led to an increase of rhGAA activity in muscle, heart and other organs such as liver. Despite the fact that these studies indicate that rhGAA had reached the tissues of interest, only in the heart and liver a reduction of glycogen content was detectable, which was not the case for the skeletal muscle. Interestingly, long-term treatment with rhGAA<sup>R</sup> led to either normalization or to reduction of the glycogen content in all tissues except brain. Furthermore, the rhGAA<sup>R</sup> was administered as a 110 kDa form and processed into its mature 76 kDa form meaning that rhGAA<sup>R</sup> was delivered into the lysosomes where the 110 kDa form was cleaved [40,42]. Overall, the enzyme administered was well tolerated in the exon 13 KO mice (Table 1, model 1, Ref. [31]), despite the presence of neutralizing antibodies against rhGAA [40].

In tGAA<sup>-/-</sup> mice (Table 1, model 4, Ref. [35]) administration of rhGAA<sup>C</sup> showed that most of the enzyme ends up in the liver. In the treated mice, the GAA activity reached even higher levels in cardiac muscle than in control animals. However, per skeletal muscle type there was an increase in GAA activity, but the levels were lower than in the wild-type animals. These data showed that only partial glycogen clearance in the lysosomes of skeletal muscle could be reached [35,47].

The promising results of these *in vitro* and *in vivo* pre-clinical studies resulted in the start of clinical trials in patients with Pompe disease. In 2000, the first clinical trial in patients with Pompe disease (infantile form) started with administration of rhGAA<sup>R</sup> of 15 and 20 mg/kg body weight, up to 40 mg/kg body weight at a later stage. It was found that the enzyme was well tolerated by the patients. After 12 weeks of administration at 15–20 mg/kg body weight the GAA activity in muscle cells was increased 7- to 30-fold, demonstrating that the administered enzyme can reach the affected tissues. However, this activity was still below normal levels. Importantly, another 12 week period using 40 mg/kg body weight of rhGAA<sup>R</sup> did result in normal levels of GAA activity. Furthermore, improvement in tissue morphology, gained muscle strength, a reduction in heart size, improvement of cardiac

Table 1  
Animal models designed for Pompe disease

Model	Reference	Mice	Abnormality	Phenotype	GAA activity in plasma ( $\mu\text{mol/h/ml}$ )
1	[31]	Disruption of exon 13	Generalized and progressive glycogen accumulation in lysosomes in a.o. heart and skeletal muscle	Early-onset infantile form	Not mentioned
2	[32]	$6^{neo}/6^{neo}$	Abnormal storage of lysosomal glycogen in heart and skeletal muscle	Severe infantile form/adult	Not mentioned
3	[32]	$\Delta 6/\Delta 6$	Abnormal storage of lysosomal glycogen in heart and skeletal muscle	Milder infantile form	Not mentioned
4	[35]	$t\text{GAA}^{-/-}$ mice	GAA deficiency in heart and skeletal muscle	Not mentioned	Not mentioned
5	[37]	GAA-KO/SCID mice	Abnormal lysosomal glycogen storage in heart and skeletal muscle, including lack of murine GAA activity	Adult form	$809.6 \pm 164^*$ wild-type $1653 \pm 231$

Five animal models were developed, by targeted disruption of the GAA gene, to study enzyme replacement therapy in Pompe disease in an *in vivo* setting.

\* The GAA activity in plasma is measured without acarbose as inhibitor of maltase–glucoamylase [78]. This results in high non-specific activity of GAA.

function and improvement in glycogen content was observed [13,48,49]. Another clinical trial initiated in 2005 showed similar results with respect to reduction in heart size and improvement of heart function after administration of rhGAA<sup>R</sup> (40 mg/kg body weight) for a period of 48 weeks [50]. Throughout this study, the patients developed IgG antibodies against the administered enzyme, which was not reported in the first clinical trial [13]. The delicate balance between the benefit of using high rhGAA doses and the increased risk of developing an immune response was nicely illustrated by Hunley and coworkers. They described the development of reversible nephrotic syndrome in a patient with Pompe disease with high anti-rhGAA antibody titers during prolonged, high-dose infusion of rhGAA [51].

In 2001, a phase I/II study was performed in three infants using rhGAA<sup>C</sup>. A dosage of 5 mg/kg body weight was intravenously administered twice a week for 1 year. Results were promising: the enzyme was well tolerated, and there was a decrease in heart size, improvement of skeletal muscle function and a reduction in accumulated lysosomal glycogen [11]. Recently, Kishnani et al. described the results of a clinical study with rhGAA<sup>C</sup> in infants in which a mortality rate of 16.7% is found at 18 months of age [52].

Although ERT using rhGAA<sup>C</sup> is currently the standard treatment modality and the only clinically available therapy, there are some drawbacks. First of all, the production and purification of rhGAA<sup>C</sup> in sufficient amounts are associated with high costs. Secondly, the inability to target the exogenously administered enzyme properly to the affected tissue leads to >80% loss of the administered enzyme. Even though ERT proved to be effective for treatment in cardiac muscle, the effectiveness was not always reached in the skeletal muscle [11,47,49,50,53]. The ineffective response of skeletal muscle fibers type II of GAA-KO mice (Table 1, model 4, Ref. [35]) to treatment with rhGAA<sup>C</sup> has been shown to be due to dysregulation of the autophagic pathway, leading to mistargeting of the rhGAA<sup>C</sup> [29,54]. In

these muscle fibers, rhGAA<sup>C</sup> was kept in the autophagic compartments and therefore was no longer available for glycogen-filled lysosomes in the fiber. Furthermore, mistargeted rhGAA<sup>C</sup> was not capable of resolving the massive autophagic buildup, which led to further expansion during progression of the disease [29,54]. Another obstacle of ERT is that the effectiveness of the therapy depends on frequently repeated and life-long intravenous infusions of sufficient amounts of the GAA enzyme often leading to the induction of an immune response.

An alternative option for ERT could be chemical chaperone therapy. Some mutations found in Pompe disease interfere with transport and stability of the GAA enzyme but not with synthesis and catalytic function. The use of chemical chaperones makes it possible to rescue misfolded or instable proteins from endoplasmic reticulum (ER)-associated degradation. *In vitro* studies using the chemical chaperones deoxynojirimycin (DNJ) and *N*-butyldeoxynojirimycin (NB-DNJ) revealed that in the presence of chaperones, mutant GAA species could be transported from the ER to the lysosomes and that the catalytic function of these mutant GAA species could indeed be partially restored. However, the effect of such chaperones is mutation specific, meaning that chaperone therapy can only be used on a mutation-specific and patient-specific basis [55,56].

A solution for the problems of ERT and for chaperone therapy could be endogenous processing of the enzyme at the natural site. This processing should result in enhanced uptake of the enzyme into the tissues, with the potential to reach the skeletal muscle. Another alternative for ERT and chaperone therapy is gene therapy. Gene therapy works by introducing an active copy of the GAA cDNA into the affected tissues, for example via a modified virus. In this way, gene therapy compensates for the inborn genetic mutation which lies at the basis of Pompe disease. With gene therapy, a longer period of survival is expected compared to ERT and longer intervals between the sequential treatments are to be expected.

## Gene therapy

The aim of gene therapy is to provide a continuous internal source of enzyme. The possibility of gene therapy for Pompe disease has been explored pre-clinically both *in vitro* and *in vivo*. An *in vitro* study using an adenoviral vector transferring the human GAA gene (AdCMV-GAA) showed efficient intracellular accumulation of the precursor form of GAA in GAA-deficient fibroblasts derived from patients with the infantile form of Pompe disease. Furthermore, the transduced GAA-deficient fibroblasts secreted the GAA precursor form efficiently into the culture medium after which it was taken up by recipient cells, leading to a reduction in lysosomal glycogen content in these cells [57]. In addition, it has been shown that GAA-deficient cells can be rescued by neighboring transduced cells through mannose-6-phosphate receptor-mediated uptake of adenovirally expressed GAA [57,58].

An *in vivo* study in GAA-KO mice (Table 1, model 3, Ref. [32]) showed a high-level secretion of the precursor form of GAA after hepatic transduction with an adenoviral vector harboring the human GAA cDNA. This secretion of GAA resulted in systemic delivery and uptake of GAA by skeletal and cardiac muscles resulting in a reduction of glycogen content [59].

In another series of experiments, inducible transgenic mutant cell lines and mutant knockout mice were generated expressing hGAA in the liver, and in skeletal and cardiac muscle. It was found that the liver served as the ideal site for transgene expression and for secretion of the precursor form of hGAA, which was efficiently taken up by skeletal muscle as well as by cardiac muscle. In contrast, hGAA enzyme produced by skeletal muscle was not secreted and there was no uptake by distant cells. By inducing hGAA transgene expression in the mutant knockout mice at different time points, it was shown that therapy was more effective in clearing glycogen in cardiac muscle in young GAA knockout mice compared to older GAA knockout mice (Table 1, model 3, Ref. [32]). Furthermore, established skeletal muscle disease was much more difficult to treat than established cardiac muscle disease [36,60].

In a comparable *in vivo* study, an adenoviral CMV-hGAA vector was intravenously administered to GAA knockout mice (Table 1, model 3, Ref. [32]) resulting in increased plasma GAA levels produced by the liver. However, induction of neutralizing anti-GAA antibodies occurred after administration of the AdCMV-GAA vector. Although skeletal muscles could theoretically serve as a depot organ, able to secrete GAA for use by neighboring non-infected cells, reduction in glycogen content in both injected- and non-injected muscle cells was not achieved as demonstrated by the low GAA plasma levels in the injected muscle due to poor transduction of the Ad vector [61]. Administration of AdGAA in the gastrocnemius of neonates with Pompe disease (GAA<sup>-/-</sup> Δ6/Δ6 mice [32]), however, led to detectable enzyme activity and reduction

of glycogen content in plasma, in both the contralateral gastrocnemius as well as in cardiac muscle [62].

Another viral vector used in gene therapy studies is the adeno-associated virus (AAV) vector. AAV vectors retain only the inverted terminal repeats required for viral replication. *In vitro* studies using AAV vectors resulted in increased GAA activity 14 days post-infection in deficient myoblasts and deficient fibroblasts. Intramuscular and intramyocardial delivery of the same AAV vector in GAA-KO mice (Table 1, model 2, Ref. [32]) yielded nearly normal GAA activities. Skeletal muscle contractility was partially restored, though only in the injected muscle [63]. Because gene therapy approaches containing strong promoters lead to induction of immunologic responses [64,65], tissue restricted expression of GAA could lower the formation of antibodies against hGAA. Therefore, studies were performed using an AAV vector containing a muscle-specific promoter. Intramuscular injection of this vector in GAA-KO mice resulted in high GAA activity in heart and quadriceps, and reduction in glycogen content 24 weeks after injection. A cellular immune response could be prevented whilst the glycogen content was persistently corrected. Administration of an AAV vector containing a liver-specific promoter in GAA-KO mice (Table 1, model 2, Ref. [32]) showed an increase in GAA activity and a decrease in glycogen content in heart and skeletal muscle without activating an immune response [66,67].

Although *in vitro* and *in vivo* studies are promising, a clinical trial for Pompe disease using gene therapy is at least 5–10 years away. First of all, clinical application must await targeted integration techniques. Due to the risk from random insertions, gene therapy has led to unwanted side-effects such as uncontrolled cell growth (leukemia) [68] or induction of apoptosis. Also for the non-integrational adenovirus, one patient died after infusion of E1- and E4-deleted vector containing human Ornithine transcarbamylase (OTC) cDNA in a trial of an inborn error of urea synthesis (Ornithine transcarbamylase) [69].

Besides viral transfection agents used for gene therapy, non-viral transfection agents are available. Non-viral systems are cationic in nature and therefore interact with negatively charged DNA through electrostatic interactions [70,71]. Liposomes are the most promising delivery systems for genes and they are among the most studied non-viral vectors because of their similarity to cell membranes [72–75].

## Conclusion and future perspectives

Increasing insights into the pathophysiology of Pompe disease have led to novel treatment options for Pompe disease. The only clinically available therapy for Pompe disease however is ERT, resulting in only short-prolonged survival of the patient. Gene therapy is still not available in the clinic for Pompe disease due to lack of suitable vectors for long-term and tissue-specific expression. Lack of suitable animal models hampers the design of potent new

therapies. Although five animal models are generated for *in vivo* research, it is not possible to generate one animal model covering the complete disease spectrum due to the differences in mutations found in patients suffering from Pompe disease. Both ERT as well as transient gene therapy only provide the missing enzyme resulting in prolonged survival of the patients without curing the molecular defect responsible for this disease. In this respect, novel strategies of gene correction could offer opportunities to really cure Pompe disease affected patients. This concept is based on restoration of the precise genetic defect via induction of double strand breaks using targeted restriction endonucleases followed by substitution of the affected DNA by wild-type DNA via homologous recombination [76,77]. Correction of the mutated gene, even only in the liver, will contribute to a normal production of GAA, to an efficient uptake of GAA and to less burdening for the patient. When results of such approaches are promising, one can expect that gene correction will also be designed for additional monogenetic inherited diseases.

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