Pompe disease: how to solve many problems with one solution

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Glycogen storage disease type II (GSDII) is a rare and often fatal neuromuscular disorder caused by a defect in the gene for acid α-glucosidase (GAA), a lysosomal enzyme that metabolizes glycogen to glucose. Since GAA is the sole enzyme that can break down glycogen in the acidic milieu of the lysosome, the enzyme deficiency leads to progressive intralysosomal accumulation of undegraded glycogen and distention of this organelle in multiple tissues. The condition is better known as Pompe disease, named after the Dutch pathologist Johannes Cassianus Pompe, who decades ago provided initial description of a widespread vacuolar glycogen storage from an autopsy report of a 7-month-old girl. The disease can present from infancy to old age with symptoms that depend on the levels of residual enzyme activity. If the enzyme activity is absent or near absent (<1% normal), cardiomegaly and muscle weakness with hypotonia, hepatomegaly, and breathing problems are often noticeable at birth, and death from cardiorespiratory failure usually occurs in the first year of life. This severe end of the clinical spectrum is classified as infantile-onset form (IOPD). Patients with some residual enzyme activity (>1% normal) present in childhood or any time thereafter with proximal muscle weakness and wasting, commonly associated with diaphragmatic and respiratory muscle weakness, eventually leading to respiratory insufficiency, wheelchair-dependency and death from respiratory failure. In these milder late-onset forms (LOPD) cardiac muscle is largely spared.

Pompe disease is one of several lysosomal storage disorders (LSDs) being treated with enzyme replacement therapy (ERT). The concept of ERT for lysosomal disorders is based on the distinctive feature of lysosomal enzymes—the intrinsic capacity of the newly synthesized endogenous enzymes to partially escape the mannose 6-phosphate receptor-mediated lysosomal trafficking pathway and end up in the extracellular space where they can be taken up and targeted to lysosomes of the same cell or neighboring cells, a phenomenon known as "cross-correction" (1). The therapeutic implication of this secretion-recapture mechanism is that the cell can internalize exogenous hydrolases and direct them to lysosomes through the mannose 6-phosphate receptor-mediated endocytosis.

The development and approval of ERT with recombinant human GAA (rhGAA; alglucosidase alfa; Lumizyme®, Genzyme Corp., a Sanofi Company) was a huge leap forward in Pompe disease treatment.

The most reliable effect of ERT has been on cardiac pathology and function, irrespective of the disease severity. This outcome has profoundly changed the natural course of the disease in IOPD patients who survive significantly longer compared to untreated infants. On the other hand, skeletal muscle response was much less impressive. Some patients experienced modest improvement, while others showed no improvement or continued to decline despite the therapy. Furthermore, many surviving IOPD patients remain weak and continue to carry the heavy burden of the disease. Those who lack any GAA protein and develop

high sustained anti-GAA antibody titers (cross-reactive immunologic material-negative; CRIM-negative patients) require immunosuppression and are invariably among the poor responders (2).

As a response to the limitations of ERT, multiple clinical and basic studies have been undertaken to characterize the new evolving phenotype; to understand the pathogenesis of muscle damage and the mechanism of muscle resistance to therapy; and to improve lysosomal targeting of the recombinant enzyme. The new phenotype of long-term survivors with IOPD includes muscle weakness and progressive loss of independent ventilation, hearing loss, arrhythmias, hypernasal speech, dysphagia, and osteopenia (3). The emerging phenotype of LOPD, once considered a limb-girdle myopathy with respiratory muscle involvement, reflects a multisystem disorder involving musculoskeletal, peripheral nervous, vascular, cardiac, and gastrointestinal systems (4). Over the course of the years, the treatment guidelines have changed to adjust to a new reality, and the introduction of newborn screening for Pompe disease allowed for the initiation of ERT within days after birth in infants (5).

The expansion of glycogen-filled lysosomes had long been an accepted mechanism of muscle damage in Pompe disease. It is now understood that the pathogenesis goes well beyond this simplistic model and includes autophagic defect and a buildup of undegraded materials outside lysosomes; defects in calcium homeostasis, mitochondrial abnormalities and oxidative stress; and aberrant mTOR (mammalian target of rapamycin) signaling and excessive proteolysis (6-8). No doubt, the list will continue to grow.

Several attempts have been made to improve the delivery of the rhGAA by increasing the number of mannose 6-phosphate groups to facilitate the uptake and trafficking through the cation-independent mannose 6-phosphate receptor. These new-generation drugs are currently being tested in clinical trials (NCT02782741; NCT02675465). However, even in the best-case scenario, ERT remains costly and requires frequent life-long infusions, not to mention the inability of the therapeutic enzyme to cross the blood brain barrier (BBB) and resolve neurological manifestations. Accumulation of glycogen in the central nervous system (CNS) was shown to contribute to severe muscle weakness, and both skeletal muscle- and CNS-targeting therapies are needed to fully correct the phenotype (9).

As a monogenic recessive disorder with a well-established primary defect and a reasonably well understood pathophysiology, Pompe disease, as well as other LSDs, is an excellent candidate for gene therapy. The therapy could potentially provide clinical benefit greater than the ERT while requiring only a single administration. Indeed, gene therapy has already been a subject of great interest in this illness.

An "ideal" gene therapy for Pompe disease would efficiently clear glycogen in multiple tissues, including the CNS; it would address autophagic defect, the major secondary abnormality in skeletal muscle, and would elicit minimal immune response. The paper by Francesco Puzzo and colleagues, recently published in *Sci Transl Med* 2017 entitled "*Rescue of Pompe disease in mice by AAV-mediated liver delivery of secretable acid α-glucosidase*", largely appears to meet these requirements (10).

The paper describes an extensive preclinical gene therapy study in a mouse model of Pompe disease. As reflected in the title, the authors selected adeno-associated vector (AAV) for the transgene delivery, and the liver as a target organ for the transgene expression. Both choices are well justified and fit comfortably into the current treatment paradigm for Pompe disease.

Among different viral vectors—adenovirus, AAV, retrovirus, and lentivirus—AAV is one of the most commonly used for gene therapy, and has thus far proven to be one of the safest. AAV is an approximately 4.7 kb non-enveloped single-stranded DNA parvovirus with the potential ability of certain serotypes to cross the BBB. AAV is non-pathogenic, capable of infecting both dividing and non-dividing cells, and when designed for therapeutic purposes is not able to replicate. The exogenous DNA of interest (flanked by two AAV-specific palindromic inverted terminal repeats) in the recombinant AAV (rAAV) vectors remains mainly episomal in the nucleus of the transduced cells. The rAAV technology has been successfully tested in preclinical studies and applied in the clinic for a variety of disorders (11,12), including Pompe disease.

A number of studies in a murine model of Pompe disease (GAA-KO) explored muscle-targeted AAV gene therapy, since skeletal muscle is the most disease-relevant tissue. A variety of muscle-specific regulatory cassettes in combination with muscle-tropic AAV serotypes resulted in correction of the local pathology when administered intramuscularly. The improvement in skeletal, cardiac, and respiratory muscle function following systemic administration of AAV vectors showed the feasibility of retrograde transduction of the peripheral and central nervous systems. Direct administration of rAAV2/1 to

the diaphragm of the GAA knockout mice resulted in the local glycogen clearance and increased efferent phrenic nerve activity (9,13). These preclinical studies led to a clinical trial for intra-diaphragmatic injections of rAAV1-containing hGAA driven by ubiquitously expressed CMV (cytomegalovirus) promoter in Pompe patients (14). Collectively, these studies demonstrated a greater efficacy of gene therapy compared to ERT, but the immune response remained a major challenge. Antibody responses against the capsid or transgene were observed in preclinical settings and in the clinical trial.

Liver-directed AAV gene therapy, employed by Puzzo *et al.*, is a well-known way to induce immune tolerance. Because of the tolerogenic properties of the liver, hepatocyte-restricted transgene expression limits the risk of unwanted antibody formation against the transgene product. The mechanism behind the tolerance to the hepatocyte-derived gene product is linked to the generation/expansion of antigen-specific CD4+CD25+FoxP3+ regulatory T (Tregs) cells (15). AAV-mediated liver gene transfer has been successfully used in Pompe disease mouse models.

The formation of neutralizing antibodies and cytotoxic T-cells against the transgene was prevented by using liver-tropic AAV2/8 vectors expressing *GAA* driven by a liver-specific promoter (16). Furthermore, immune tolerance to rhGAA, induced by a low dose liver-directed gene therapy, enhanced the effect of ERT and allowed for repeated infusions of ERT without anaphylaxis (17). A direct comparison of the efficacy of ERT and AAV liver-targeted gene transfer in GAA-KO mice demonstrated that both treatments reduced glycogen in the heart and diaphragm, but unlike ERT, AAV gene therapy did not trigger an immune response. Notably, at a higher AAV vector dose gene therapy induced long-term immune tolerance to the rhGAA (18). This study paved the way for a clinical trial delivering AAV-GAA to the liver to increase immune tolerance to rhGAA; the trial is scheduled to begin in the fall 2018.

The potential of hepatocyte-directed gene transfer is not merely to serve as an addition to ERT, but rather to transform the liver into a "factory" that produces and secretes the transgene product into the bloodstream for systemic cross-correction. The challenge here is achieving a long-term robust expression of the transgene at the levels that are therapeutic, while reducing the vector dose to a minimum to avoid immune response. Unfortunately, in a mouse model of Pompe disease, high doses of the recombinant AAV8 vector (encoding hGAA under the human serum albumin promoter) were required to achieve partial efficacy (19). In the paper by Puzzo *et al.*, the authors seem to find the right balance and accomplish what others could not: whole-body correction of biochemical and functional defects in muscle, CNS and spinal cord, and amelioration of cardiac hypertrophy and respiratory function.

The key to their success appears to be that the engineered transgene product is highly secretable, exhibits superior therapeutic efficacy, and has much lower immunogenicity compared with its native GAA counterpart. The authors put a great deal of effort into designing the expression cassette by replacing the natural secretion signal peptide of the human GAA pro-enzyme, removal of several amino acids, and codon optimization to boost the secretion and render the transgene product less immunogenic. Codon optimization is a technique to achieve optimum expression by matching codon-usage frequency in the transgene to the abundance of transfer RNA (tRNA) in the target tissue/species.

The concept is based on the evidence that the inherent redundancy of the genetic code has a purpose. Preferential usage of particular codons within a reading frame, called codon usage bias, plays an important role in the control of gene expression, protein translation and folding, and interaction with RNA-binding protein, all of which can profoundly affect cellular function (20). Codon changes which do not alter protein sequence, dubbed "silent" mutations, had long been considered inconsequential. This notion is no longer valid; therefore, these changes received a more appropriate name—synonymous mutations—reflecting the meaning of a synonym in language. Synonymous codon changes have been shown to dramatically increase heterologous expression—a phenomenon that is widely exploited in the gene therapy studies (12).

The authors' effort has paid off: AAV vectors optimized for hepatic expression of *GAA* transgenes resulted in high levels of secreted GAA, uptake in peripheral tissues and cross-correction, and low immunogenicity.

Of course, any treatment is associated with some limitations and does not completely eliminate the risk. The authors indicate that the therapeutic efficacy of the liver gene transfer may be partially lost over time as the liver grows, which would be a problem in young pediatric patients with Pompe disease. One should also keep in mind that although the majority rAAV vector genomes remain episomal after transduction, AAV-based vectors are capable

of integration and a low number of random integrations may occur leading to a potential risk of insertional mutagenesis and genotoxicity (21,22). With these caveats, Puzzo *et al.* have brought us closer to a more efficient and less burdensome treatment for those suffering from Pompe.

Finally, there is a postscript to the publication. In a subsequent paper, the authors tested AAV-based gene therapy for another glycogen storage disease, GSDIII, and they used different rAAV vectors including their highly efficient AAV8 vector expressing a secretable acid α-glucosidase (23). GSDIII is a rare disorder of glycogen metabolism caused by mutations in *Agl* gene coding for glycogen debranching enzyme, amylo-1,6-glucosidase,4-α-glucanotransferase. The deficiency of the enzyme leads to excessive accumulation of abnormally structured cytoplasmic glycogen in multiple tissues, primarily in liver and muscle. Most patients have both muscle and liver involvement and present with hepatomegaly, hypoglycemia, growth retardation, progressive myopathy, and cardiomyopathy (GSD type IIIa); in some patients, only the liver is affected (GSD type IIIb) (24). Only symptomatic treatments are available for GSDIII patients. Administration of AAV8-GAA in a mouse model of GSDIII resulted in high levels of GAA activity in the bloodstream and in all tissues, consistent with what had been shown in GAA-KO mice. Intriguingly, these supraphysiological GAA levels significantly decreased glycogen accumulation in liver but not in muscle. Although the mechanism of cytoplasmic glycogen degradation by the lysosomal acid α-glucosidase and the difference in GAA-mediated glycogen clearance in liver versus muscle remain unclear, the results have certainly given us food for thought.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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