

Surrogate gene therapy for muscular dystrophy

An engineered truncated gene derived from the dystrophin-related protein (utrophin), prevents pathology without an immune response in an animal model of Duchenne muscular dystrophy gene therapy

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DMD is an X-linked muscle wasting disease - affected boys lose ambulation around 12 years and die of respiratory or cardiac problems in their twenties or thirties [1]. DMD is caused by mutations in the gene encoding dystrophin, an essential protein link between the internal cytoskeleton and the extracellular matrix of muscle cells. There is currently no effective treatment. Progress in genetic approaches has led to approved drugs which promote exon skipping or stop codon read-through, but their clinical efficacy needs improvement and they are mutation-specific so not effective in most affected individuals. Gene therapy using the AAV vector holds a lot of promise as it would treat all patients, irrespective of their mutation, by introducing a functional version of the dystrophin gene into muscle. In this issue, Song et al [2] describe an AAV containing a codon optimized truncated gene (μ Utro) derived from the dystrophin related protein, utrophin, and show that this construct prevents dystrophic symptoms in both the *mdx* mouse and canine models of the disease in the absence of an immune response.

There are substantial challenges to gene therapy of DMD using AAV [3] The dystrophin gene is too large for AAV vectors necessitating the use of truncated micro-dystrophin genes (μ Dys) [4]. High doses of this vector are needed to express enough protein to have an effect, and patients need to be pre-screened for antibodies that would block

vector delivery to muscles. In an early trial, some patients showed a mild immune response to dystrophin when expressed from the ubiquitously active CMV promoter [5]. Delivery of AAV- μ Dys in Phase I trials in a small number of patients showed functional improvements but more recently some transient adverse effects have been reported. Various μ Dys designs have been described, but all have been modified from truncated proteins seen in the mildest Becker muscular dystrophy (BMD) patients, thus the therapy would at best convert DMD into a mild BMD. The design of a therapeutic truncated gene which would fit into AAV, maximize function towards normal whilst minimizing the immune response, could potentially transform this therapeutic approach.

Utrophin is a known surrogate for dystrophin as its expression in the *mdx* mouse model for DMD prevents pathology [6] even though it lacks some protein:protein interaction domains present in dystrophin. Neither the functional efficacy of utrophin nor its immunogenicity has ever been tested in large animals, which is a more rigorous test for its use in patients. Song et al [2] made predictions for the optimal way to shorten the rod domain of utrophin, which has been shown to functionally accommodate a variety of deletions [7]. This was done by using partial crystal structures of dystrophin and utrophin and taking into account the deletions of the rod domain in paralogs of utrophin throughout evolution. They also optimized the shortened utrophin DNA sequence with codons known to maximize expression. The codon-optimized construct, μ Utro, is 22-27% divergent from therapeutic truncated utrophin and dystrophin genes reported previously. The μ Utro most closely resembles the deltaR4-R23/deltaCT micro-dystrophin gene currently in the clinical trial from Sarepta [7], and has the same domain structure as the μ Utro previously described by Odom et al [8,9](Fig. 1).

The authors delivered the AAV- μ Utro under the control of the CMV promoter to neonatal *mdx* mice before the onset of disease as a stringent test of efficacy. Their study revealed the vector was able to confer myoprotection throughout muscle development and up to the end of the 4 month study after a single intraperitoneal injection. Furthermore, AAV- μ Utro-treated mice had equivalent grip strength to wild type mice and showed normal behavior in voluntary and downhill treadmill running. This has not been observed previously using either μ Utro or full length utrophin transgenes – these treatments were able to prevent pathology but full function was not restored to wild-type levels, although it has been observed with the latest generation μ Dys vectors in *mdx* mice ([10]).

Although these data provided the hope that treatment with this engineered μ Utro might provide a near normal and not a BMD phenotype in individuals affected with DMD, the efficacy needs to be first tested in the large animal Golden Retriever Muscular Dystrophy (GRMD) dog model of DMD, as its severe phenotype more closely resembles patients than do the mouse models. No cell-mediated immunity was observed against either the vector or the μ Utro in the neonatal dogs and the muscle pathology was prevented. Since boys are usually not diagnosed with DMD before the age of two years, Song et al [2] also carried out delivery of the AAV- μ Utro vector to

juvenile dogs at high vector doses together with prednisone to reduce inflammation. The treated dogs showed complete suppression of ongoing muscle injury. However, given the age of the dogs at vector delivery it should be noted that dystrophic pathology was largely prevented, and any potential reversal of disease phenotype was not examined.

Finally, Song et al [2] compared the immunologic outcomes of delivery of an AAV- μ Dys with that of the AAV- μ Utro in the German Shorthaired Pointer dog which has a fully deleted dystrophin gene. GRMD dogs have a small amount of expressed dystrophin as a result of read-through of the mutation and are therefore not a good test of immunogenicity. A strong systemic cell-mediated immune response to AAV-CMV- μ Dys was observed but not to AAV-CMV- μ Utro. Both of these studies used the ubiquitously active CMV promoter to drive transgene expression. It will be important to observe whether dystrophin immunogenicity is seen when driven from the muscle-specific promoters currently being tested in the clinic ([10]).

Neither full length utrophin nor μ Utro are able to associate with nNOS, a protein important for regulating blood flow to exercising muscles but this may still be compatible with amelioration of pathology since many mild BMD patients lack this nNOS localizing domain [6]. Other differences between dystrophin and utrophin may not be important for clinical benefit. Furthermore, the functional efficacy of AAV- μ Utro in the heart was not tested in this study but the CMV promoter did allow cardiac expression and other studies using μ Utro provide cardiac benefit in the *mdx* mouse [9]. Much has been learned from the AAV- μ Dys work but Song et al [2] present an exciting novel opportunity to potentially restore normal function to DMD patients without a possible immune response, which could simplify repeat administration if needed. In addition, because utrophin and dystrophin can be co-localised at the membrane, BMD patients could also benefit [11]. While current gene therapy trials for DMD are showing encouraging results, a rigorous test of dystrophin immunity has not been performed, and the availability of μ -Utro vectors provides an important alternative approach to treating this devastating human genetic disorder (Fig. 1) .

Competing interests

J.S.C. is a member of the Scientific Advisory Board for Solid Biosciences. K.E.D. has no declared conflicts.

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Fig. 1 Legend:

Utrophin gene therapy for DMD. Structure of clinically relevant dystrophin and utrophin proteins. Full-length dystrophin comprises N-terminal actin-binding domain (ABD); four hinge domains (H), 24 spectrin-like repeats (R) that form the 'rod' domain, an internal ABD, the alpha syntrophin-binding domain, which localizes nNOS (Syn/nNOS), the dystroglycan-binding domain (DgBD), and the syntrophin and dystrobrevin binding domains (SBD, DbBD) which are located in the cysteine-rich (CR) and C-terminal (CT) domains. Below dystrophin is the structure of utrophin, which has only 22 spectrin-like repeats and lacks the alpha syntrophin-binding domain, and the μ Utro design used by Odom et al [8] and Song et al. [2]. The AAV- μ Utro vector was tested in the *mdx* mouse model for DMD, and in golden retriever and German short-haired pointer canine models for DMD, and results are indicated.

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