Novel therapies for Duchenne muscular dystrophy

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The development of therapeutic strategies that overcome the unique problems posed by Duchenne muscular dystrophy (DMD) has led to the development of many contemporary approaches to human disease in general. Various treatment approaches have been explored—such as pharmacological therapies and cell-based, cytokine, and genetic therapies—that are all targeted to specific features of dystrophic DMD muscle pathology. In genetic therapies, the large size of the dystrophin gene has necessitated the development and use of novel functional minidystrophin and microdystrophin genes, muscle-specific promoter systems, and gutted adenoviral vectors. In addition to these well defined viral strategies, plasmid vectors and the upregulation of utrophin (a dystrophin homologue) have potential. Various novel genetic approaches—such as antisense-mediated exon skipping, gene correction, and new cytokine approaches—are also being developed. Together these exciting developments bring an effective treatment for DMD closer than ever before.


Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder for which there is no effective therapy. DMD is the most common muscular dystrophy: it affects 1 in 3500 live males at birth and about a third of cases occur as de novo mutations in the infant. In most cases, the disease is diagnosed on the basis of gait abnormalities at 4–5 years of age. By 8–10 years of age, deterioration of the patient’s condition necessitates wheelchair use. By their early teens, patients are wheelchair bound and, in some cases, neurological and cardiological symptoms are apparent. Progression of muscle degeneration and worsening clinical symptoms lead to death in the late teens or early twenties as a result of respiratory or cardiac failure. In this review, we discuss some of the promising genetic strategies that are currently being developed as potential therapies for DMD.

Molecular pathology of DMD

Dystrophin, the product of the human dystrophin gene (dys), is a 427 kDa protein composed of 3685 amino acid residues. The protein localises to the sarcolemma, where it constitutes 5% of sarcolemmal protein and 0.002% of total striated muscle protein. Dystrophin contains four distinct domains and shows structural homology with spectrin and α-actinin. In myogenic cells, the N-terminal and parts of the helical-rod domain bind to actin whereas the C-terminal and a cysteine-rich domain distal to the rod domain form complexes with syntrophin and β-dystroglycan, respectively (figure 1). The sarcolemmal dystrophin-associated protein complex provides a crucial structural link between the extracellular matrix and the intracellular actin cytoskeleton. Muscles in patients with DMD are deficient in dystrophin. This deficiency causes sarcolemmal instability, which leads to destabilisation of the sarcolemmal dystrophin-associated protein complex. Dystrophin deficiency occurs in patients with Becker muscular dystrophy (BMD), although to a lesser extent. In dystrophic muscle, regeneration gradually fails and the normal cycle of degeneration and regeneration is tipped in favour of degeneration. Cycles of necrosis and repair in dystrophic muscle continue throughout postnatal development until the endogenous satellite-cell pool can no longer compensate for the ongoing muscle-fibre destruction.

Cloning of the complete locus for DMD revealed that more than 50% of human DMD mutations involve large deletions. In myogenic cells, the N-terminal and parts of the helical-rod domain bind to actin whereas the C-terminal and a cysteine-rich domain distal to the rod domain form complexes with syntrophin and β-dystroglycan, respectively (figure 1). The sarcolemmal dystrophin-associated protein complex provides a crucial structural link between the extracellular matrix and the intracellular actin cytoskeleton. Muscles in patients with DMD are deficient in dystrophin. This deficiency causes sarcolemmal instability, which leads to destabilisation of the sarcolemmal dystrophin-associated protein complex. Dystrophin deficiency occurs in patients with Becker muscular dystrophy (BMD), although to a lesser extent. In dystrophic muscle, regeneration gradually fails and the normal cycle of degeneration and regeneration is tipped in favour of degeneration. Cycles of necrosis and repair in dystrophic muscle continue throughout postnatal development until the endogenous satellite-cell pool can no longer compensate for the ongoing muscle-fibre destruction.
deletions at the Xp21.1 locus. The murine and human loci are highly homologous and both DMD and BMD are caused by mutations in the same gene (ie, dys). Dystrophin is encoded by a 2.25 Mbp gene with 79 exons; however, 99.4% of the sequence is composed of introns and the fully processed transcript is only 14 kbp. The cysteine-rich domain is encoded by exons 62–70. The gene is expressed in a cell-specific and developmentally regulated manner. Dystrophin expression is regulated by seven independent promoters, three of which regulate the expression of full-length isoforms, while four intragenic promoters regulate expression of different short isoforms in various tissues. Mutations that affect dystrophin promoter regions and regulate the brain isoforms may be the cause of neurological symptoms in some patients with DMD.

Most dys mutations (roughly 99%) in patients with DMD are large deletions or insertions that result in downstream codon reading frameshifts (58%) or small frameshift rearrangements or point mutations (41%), with the remainder being duplication mutations. All of these mutations affect the correct expression of the cysteine-rich domain. By contrast, the cysteine-rich domain is preserved in patients with BMD mutations, which results in the expression of a partially functional dystrophin protein and, consequently, milder symptoms.

In mdx mice (panel) overexpressing a dystrophin complementary DNA (cDNA) transgene, muscle structure, morphology, and function were similar to that found in non-dystrophic muscle, which suggests that dystrophy can be avoided if functional dystrophin is provided before the onset of irreversible degeneration. Furthermore, only 20–30% of normal human or murine dystrophin expression is required to rescue dystrophic-muscle function in mdx mice.

Not all regions of the dystrophin transcript are equally essential for function of the dystrophin protein. In one family with BMD, mildly affected individuals had a deletion spanning exons 17 to 48 (Δ17–48; figure 2), almost 46% of the dys gene. Although the resultant dystrophin protein lacks the middle of the rod domain, it does retain some function. Indeed, a minidystrophin transgene with this Δ17–48 deletion improves force output when expressed in mdx muscle.

The C-terminal and the cysteine-rich domain are essential for correct assembly of the dystroglycan complex and sarcolemmal function. Two dystrophin isoforms—DP260 in the retina and DP71 in the brain and other tissues—restore the integrity of the sarcolemmal dystrophin-associated protein complex but only DP260, the...
longest of the short dystrophin isoforms, restores some functional integrity in dystrophic muscle.\textsuperscript{33,34} These findings suggest that a better link between the extracellular matrix and actin is necessary for the improvement of dystrophic muscle function than that provided by the DP71 dystrophin isoform.\textsuperscript{34,35}

**Genetic therapies in DMD**

Both viral and plasmid vectors have been used to deliver dystrophin to dystrophin-deficient muscle in vivo (figure 3). The efficiency with which these vectors express dystrophin, or other transgenes, in muscle cells is dependent on various factors. These include transgene size, delivery, expression, persistence, immune response, maturation dependent loss of muscle-fibre transducibility (for viral vectors), and inefficient passage of vectors through the extracellular matrix.

**Truncated dystrophin genes**

The expression of microdystrophin transgenes that contain at least four dystrophin-derived spectrin repeats and three hinge regions in muscle from \textit{mdx} mice results in good functional correction compared with microdystrophin transgenes that contain three repeats and two hinge regions.\textsuperscript{36} However, expression of these microdystrophin transgenes did not result in restoration of sarcolemmal neuronal nitric oxide synthase (nNOS) localisation, which has been observed in earlier studies\textsuperscript{33,37} and has been implicated in the amelioration of dystrophic phenotype.\textsuperscript{38}

The success of minidystrophin and microdystrophin transgenes in improving force output and other features of the dystrophic \textit{mdx} phenotype is promising. There are, however, several issues that need to be addressed before the expression of truncated dystrophin can be used to treat patients with DMD. First, these constructs need to be delivered efficiently throughout the dystrophic muscle. Second, they need to be tested in larger dystrophic muscles (eg, in the golden retriever dog model; panel). Finally, despite its relatively mild phenotype compared with DMD, BMD is a severely disabling disorder and therefore strategies that aim to deliver the full-length \textit{dys} may be advantageous.

**Tissue-specific promoters**

Expression of full-length \textit{dys} cDNA under the control of a muscle-specific (murine muscle creatine kinase [MCK] promoter) can completely prevent the development of a dystrophic phenotype in transgenic \textit{mdx} mice\textsuperscript{27} with no apparent side-effects. Unlike constitutive promoters,\textsuperscript{39} muscle-specific promoters do not elicit a host immune response and express up to 600 times more transgene in muscle than in non-muscle tissue.\textsuperscript{40,41} Targeted transgene expression via muscle-specific promoters is a good platform for vector-mediated therapeutic delivery of \textit{dys} to dystrophic muscle.

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**Figure 3. Schema for genetic and cellular therapy development in DMD.** Gene and cell therapies currently under consideration for DMD are depicted with the stages necessary for their application to DMD.
Recent and ongoing clinical trials for DMD

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<th>Duration</th>
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**Plasmid vectors**

Both full-length and 6-3 kb Becker-like human dys cDNA constructs can be expressed by plasmid DNA (pDNA) in mdx myofibres for up to 6 months42,43 and can improve DAG assembly in mdx muscle.44 Furthermore, full-length dystrophin expressed by pDNA improves resistance to calcium influx in cultured mdx myotubes45 and restores expression of sarcolemmal nNOS in the muscle of mdx mice.46 The Δ17–48 minidystrophin construct also restored sarcolemmal nNOS, but ineffective expression of constructs due to the production of antibodies against the dystrophin protein has been observed in some cases.47 This problem may eventually be overcome by use of inducible pDNA expression or muscle-specific promoters.

Square-wave electroporation—with long-duration electric pulses of moderate field strength—improves intramuscular expression of pDNA transgenes by more than two orders of magnitude compared with conventional protocols that do not use electrotatfer.48,49 Alternatively, systemic injection of pDNA transgene results in expression of full-length dys cDNA in about 40% of mdx mouse diaphragm fibres.49 These studies provide a strong basis for phase I trials to establish the safety of full length dys cDNA plasmids in patients with DMD that are currently underway (table).50–52

**Viral vectors**

Recombinant adenovirus expressing dys cDNA can efficiently transduce skeletal and cardiac muscle in mdx mice.50 Fibres that express dystrophin can be detected for up to 6 months after transduction with a Becker-like minidystrophin construct and host fibres show some resistance to the degenerative process.51 Effective transgene persistence is, however, compromised by immune rejection of cells that express the transgene, vector toxicity, poor tropism, and the age of the host muscle (maturation effect).

The maturation effect is attributed to the age-related loss of αvβ3 and αvβ5 integrins, which mediate adenovirus internalisation, and Coxsackie-adenoviral attachment receptors.52 However, these receptors are not

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the only determinants of the maturation effect. Tropism of recombinant adenovirus may be improved by upregulation of target internalisation molecules or by modification of fibre proteins on the capsid surface. Retargeting of recombinant adenoviral vector to alternative (muscle-specific) cell-surface receptors and delivery of recombinant adenoviral vector by use of myoblasts transduced ex vivo, although promising, are not significantly better than direct adenoviral-mediated dys transgene expression in dystrophic muscle.

As is the case with pDNA transfection in vivo, the extracellular matrix is yet another barrier to efficient muscle transduction by adenovirus. Permeabilisation of the basal lamina may, therefore, improve adenoviral transduction in muscle.

The removal of immunogenic genes from the 35 kb viral genome of adenoviral-vector systems has the dual effect of maximising the transgene size capacity and avoiding an immune response against the viral-gene product (figure 4). First-generation adenoviral vectors lack the early stage genes E1 and E3, which are necessary for activation of viral replication, and can therefore accommodate up to 6.5 kb of transgene. Second-generation adenoviral vectors contain further deletions in the E2 and E4 regions and can accommodate up to about 10 kb. Third-generation adenoviral vectors, only the tandem repeats of the adenoviral genome are retained. These “gutted” adenoviral vectors can accommodate 28–30 kb of transgene including full-length dystrophin cDNA to dystrophic muscle with a reduced risk of immune rejection that may improve the vectors the most promising of the current strategies for the treatment of DMD. A recent study showed efficient transduction of immunocompetent muscle from 1-year-old mdx mice by a gutted adenoviral-mediated muscle-specific expression of full-length human and murine dys transgenes. In injected mdx muscle expressed full-length murine dystrophin resulting in a 40% increase in muscle function. There was, however, an immune response to transgene expression: in this study, this response was strongest against the first generation vector dys transgene, with the murine dys transgene showing a lower CD4+ and CD8+ T-cell response compared with the human dys transgene.

Gutted adenoviral systems driven by the MCK promoter have great potential for dys transgene delivery to dystrophic muscle. Further challenges to their use as therapeutic vectors in human beings include production capacity and delivery throughout the muscles.

Adenoassociated vectors
Adenoassociated virus is a 4680 bp DNA virus that can be grown both in its non-replicative form (an integrated provirus on human chromosome 19) or in its replicative form as a lytic genome. Recombinant adenoassociated virus
does not have the ability to integrate into host genome, but can nonetheless persist for long periods in host mice. It is a non-replicative virus, except in the presence of adenovirus infection. Adeno-associated vector systems have a limited transgene capacity of up to 5·2 kb; however, the generation of partially functional microdystrophin cassettes partly compensates for this disadvantage. Although these vectors were initially thought not to provoke an immune response, there is recent evidence of immune rejection of muscle fibres transduced by recombinant adeno-associated viral vectors under the control of cytomegalovirus promoters or even muscle-specific MCK promoters.

The potential of adeno-associated viruses to effectively deliver a transgene to dystrophic muscle is nevertheless evident by successful delivery of a minidystrophin gene to muscle in mdx mice. An adeno-associated system has been used successfully in clinical trials to deliver sarcoglycans and may ultimately be more applicable to muscle disorders involving genes that are smaller than dys.

Retroviral vectors
Recombinant retroviral vectors are generated from small RNA-based viruses, such as the Moloney murine leukaemia virus, and replicate via reverse transcription in actively dividing host cells. Retroviral vectors can hold up to 7·8 kb of transgene and integrate into the host-cell genome. Although the insert capacity of retroviral vectors does not allow delivery of full-length dystrophin to muscle, these vectors can deliver minidystrophin transgenes, such as the Δ17-48 Becker-like construct. The transduction efficiency of the minidystrophin retroviral vectors in host muscle is low because retroviral vectors can only transduce cells that are undergoing mitotic division. Only 6% of fibres expressed the minidystrophin transgene in injected muscle, although this can be improved by myotoxin-induced muscle regeneration. Implantation of mitotically inactivated cells producing retroviral vector resulted in minidystrophin expression in up to 18% of fibres, with restoration of α-sarcoglycan expression in nude/mdx and mdx mice. Retroviral vectors are not likely to be used as a direct potential therapy for DMD due to their low transduction efficiency and limited insert capacity.

Lentiviral vectors
Lentiviral vectors are derived from a small family of retroviruses, which includes HIV, feline immunodeficiency virus, and equine immunodeficiency virus. Lentiviral vectors can transduce postmitotic cells, including neurons, hepatocytes, and muscle fibres. Their stable transduction of postmitotic cells makes recombinant lentiviral vectors useful for the delivery of minidystrophin transgenes to dystrophic muscle. Transgene expression lasts for up to 2 months with no associated immune response. The lack of an immune response most likely results from the use of low virus titres compared with other viral systems. Lentiviral and retroviral systems are most suited to use ex vivo, and with improved transduction efficiency and understanding of pathogenicity risks, and retain some potential as therapies for DMD.

Miscellaneous viral vectors
Although research into viral gene therapy for DMD mainly focuses on adenoviral, adeno-associated, and retroviral vectors, several additional viral systems may be applicable to DMD. These include herpes simplex virus, Epstein-Barr virus, and chimeric adeno-retrovirus. The investigation of these vectors in DMD is at an early stage compared with the application of adeno-, adeno-associated, and retroviral vectors.

Corrective gene therapy
Targeted corrective gene conversion therapies
The introduction of construct of homologous DNA containing a non-homologous sequence into mammalian cells in vitro induces specific genetic transformations in the host chromosomal DNA. The size of the non-homologous sequence in the introduced construct does not affect gene targeting efficiency, but the extent of homology between the target sequence and the introduced sequence has a direct influence on targeting efficiency. High targeting efficiency (1 in 150 cells) can be achieved by limiting the amount of sequence disruption in the targeted gene to less than 20 nucleotides. In early transgenics applications, researchers used large DNA constructs encoding selection cassettes and large tracts of non-homologous sequence. Comparatively, targeted corrective gene conversion methods use sequences with greater homology to disrupt single nucleotides or small polynucleotide tracts of the target gene. As a result, gene conversion is up to four times greater, with a lower potential for non-specific mistargeting, than other transgenics applications such as plasmid and viral vectors systems.

Targeted corrective gene conversion is an attractive therapeutic strategy for DMD if the DNA can be delivered to the muscles efficiently (figure 3). Expression of the corrected locus is regulated by an endogenous promoter and therefore the potential for an immune reaction is minimal. Although this technique is most applicable to point mutations, 65% of...
Novel therapies for DMD

**Small fragment homologous replacement (SFHR)**

Single stranded SFHR involves the application of PCR amplicons to correct mutant loci in vitro or in vivo (figure 5). Whereas oligonucleotides normally contain up to 70 bp, PCR amplicons for SFHR contain in excess of 100 bp of the wild-type sequence. SFHR was first used to correct the most common mutation found in cystic fibrosis—a three-nucleotide deletion (ΔF508)—in epithelial cells in vitro. The replacement of three nucleotides by SFHR can restore the dys reading frame by insertion or deletion of a single base-pair. Although little is known of the mechanisms involved in SFHR-mediated gene correction, similarities probably exist in the nuclear trafficking and processing of SFHR amplicons with that of pDNA. In contrast to oligonucleotide transport, which mainly involves free diffusion, energy-dependent karyophilic and potocytotic processes mediate the nuclear transport of DNA larger than 320 bp (ie, SFHR amplicons). The actual corrective mechanisms of this technique may involve recombination with or without mismatch correction mechanisms.

A 603 bp PCR amplicon can correct the dystrophin locus in about 20% of cells cultured from mdx mice, although the subsequent viability of myogenic cells remained impaired. The use of lipofection to directly deliver the amplicons to dystrophic muscles from mdx mice did result in some correction (<1%) but higher efficiency is necessary to achieve a biologically significant result. A compromise between correction and subsequent cell viability can be achieved by simple measures, such as multiple application or alternative delivery methods.

The issue of amplicon delivery in vivo is a major challenge; cell-mediated delivery by use of non-muscle cells via systemic or intramuscular routes is likely to be difficult, but might be possible. The successful delivery of pDNA by electroporation and intravascular arterial occlusion suggests that direct SFHR-mediated gene correction in vivo may be greatly improved by these methods. Although promising, SFHR-mediated gene therapy is in the early stages of development and further work is necessary to establish its viability and therapeutic potential in DMD.

**Chimeraplasty**

Chimeraplasts are hybrid RNA–DNA molecules that promote gene conversion via intranuclear DNA mismatch repair mechanisms. Short complementary lengths of deoxyribonucleotides are protected from exonucleolytic digestion by complementary lengths of 2’-O-methyl ribonucleotide analogues (figure 6). Correction efficiency is dependent on the annealing of both the DNA and RNA moieties of the chimeraplast at the target site during gene transcription. A mismatch, mediated by the corrective transformational nucleotide on the DNA component strand at the target site, induces mismatch repair activity and replacement occurs at the target site. Up to 30% correction has been observed in vitro; however, results vary substantially, probably because of differences in intragenomic targets and transfection efficiency. Intracytoplasmic nuclear transport of chimeraplasts probably involves different mechanisms to those for larger corrective DNA species. The DNA component of the chimeraplast causes allelic replacement; in fact, the DNA component alone has been shown to induce more efficient correction than the whole chimeraplast. This is likely to be due to the modified ribonucleotides, which may disrupt the targeting of the DNA motif.

In the mdx mouse model, chimeraplasty resulted in restoration of low expression of normal dystrophin in muscle in vivo. Although correction was found in about 1% of fibres in injected muscles, correction was observed in 10–20% of fibres actually penetrated by chimeraplasts. This was subsequently confirmed in myogenic precursor cells in vitro, in which dystrophin expression was 2–20% that of wild-type dystrophin expression. In the golden retriever dog model of muscular dystrophy, a chimeraplast designed to introduce a dinucleotide corrective conversion at the dys locus successfully corrected the mutation but was not able to effect a double-nucleotide conversion. Correction of the mutation was reported in up to 10% of transcripts from injected muscle over 48 weeks and corrected protein was observed at the sarcolemma by use of immuno-histochemistry. The lack of correction of the second nucleotide in this study was proposed to result from double mismatch-processing function of the intranuclear enzyme systems that cause chimeraplast-mediated correction. This failure of chimeraplast-mediated correction at the dys locus could affect the application of chimeraplast to the restoration of frame-shift deletions in DMD. Although chimeraplast-mediated insertion of a single purine nucleotide has been reported, the applicability and efficacy of chimeraplasty may differ between specific loci.

**Oligonucleotide-mediated exon skipping**

Antisense oligoribonucleotides composed of 2’-O-methyl ribonucleotide analogues do not undergo extensive
degradation by endogenous ribonucleases in the cell. In cultured cells, 2′-O-methyl ribonucleotide analogues bind to a homologous sequence and cause a disruption (“exon-skipping”) of primary transcript processing. In myotubes from mdx mice, binding of 2′-O-methyl ribonucleotide analogues to the intron 22/exon 23 splice junction of the primary dystrophin transcript resulted in excision of the mutation-containing exon 23 from the fully processed transcript. The generation of a Becker-like dystrophin by antisense-induced exon skipping resulted in a patchy sarcosomal distribution of dystrophin similar to that seen in the muscle of patients with BMD. These results are supported by work showing that exon skipping is able to restore dystrophin to the sarcolemma from dystrophic muscle (figure 3). The success of this approach is, however, greatly compromised by the host immune response, both in mdx mice and in patients with DMD. Although immunosuppression with ciclosporin, FK506, and antibodies alleviated muscle rejection, subsequent human trials of muscle-derived precursor cells were unsuccessful. In these trials, dystrophin-positive fibres were accounted for by transcriptional (revertant) variants of the mutant dystrophin transcript.

**Muscle derived precursor cells**

Delivery of normal dystrophin by the transplantation of nondystrophic muscle derived precursor cells results in some recovery of normal function in dystrophic muscle. The identification of specific muscle derived precursor cell types with an improved capacity to remodel dystrophic muscle has been a major focus in myogenic stem-cell research. Muscle derived precursor cells can be isolated using a “preplate” method, in which non-myogenic cell types are progressively filtered out of the heterogeneous cell population, or from single-muscle-fibre explants. In mice, preplated cells were isolated by magnetically assisted cell sorting on the basis of stem cell antigen 1 and m-cadherin defined quiescent satellite cells on single muscle fibres. Studies of clonal populations of preplated cells and cells isolated from single muscle fibres have shown that these cells were stem cell-like, were able to efficiently remodel mdx muscle, and expressed several tissue type outcomes. A "side population" of preplated cells (Sca-1+, CD54+, CD43+) isolated by fluorescence-activated cell sorting was shown to remodel both muscle and bone marrow with wild-type dys loci from donor cells. Mdx muscle can be remodelled by intramuscular or intraarterial injection of non-dystrophic donor cells. Studies in single explanted muscle fibres have shown that transgenic stem cells expressing lacZ contributed to the syncytial structure of the recipient muscle and to the satellite cell pool and were undifferentiated for at least 1 month after transplantation.

It is difficult to establish whether the stem-cell like and remodelling capacities of muscle derived precursor cells extracted by the preplate method originate from the same population of cells as observed in studies of side populations on single fibres. There is general agreement that preparations of muscle derived precursor cells are heterogeneous and contain at least two cell populations. In one study, irradiated dystrophic mdx mouse muscle showed a lack of regenerative capacity induced by myotoxin administration. This finding has led to a renewed emphasis on muscle derived precursor cell transplantation therapy (table) as a potential treatment for DMD or as a vehicle for the autologous delivery of functional dys loci.
Search strategy and selection criteria
Data for this review were identified by searches of Medline using the search terms “dystrophin”, “gene therapy”, “Duchenne”, “mdx”, “DMD”, “utrophin”, and “muscular dystrophy” on the NCBI Pubmed database. These terms were used singly and in combination to maximise the numbers of relevant papers obtained. The main database was then supplemented by references obtained from several reviews and key papers from RK’s private collection and with references obtained by searches for specific authors involved in various aspects of the cited studies.

Non-muscle stem cells
A few systemically injected bone-marrow cells can be induced to enter muscle after regeneration induced by injury.106,107 The myogenic regenerative capacity of donor bone marrow probably resides in the mesenchymal component of the stromal bone marrow cells.108 Systemic administration of wild-type bone marrow cells to mdx mice via the tail vein leads to some segregation of the donor cells and subsequently to the expression of non-dystrophic dys loci in dystrophic cardiac and skeletal muscles.109,110 Systemically injected liver-derived stem cells are able to infiltrate heart muscle after ischaemia—reperfusion injury.111 Cells isolated from adipose tissue have multiple, including myogenic, expression profiles.112 The mesenchymal identity of such multipotent cells is supported by recent work indicating that clonal cells isolated from bone marrow, brain, and muscle display similar antigenic profiles under fluorescent-activated cell sorting analyses.113 These cells have neuronal, osteogenic, myogenic, and haemopoietic expression profiles, and may provide alternatives for cell-based delivery of non-dystrophic loci to dystrophic muscle.

Alternative approaches
Utrophin,114 α7β1 integrin,115 myoprotective or myoprotective cytokine factors (eg, leukaemia inhibitory factor134 and insulin-like growth factor-1 135), and inhibition of myostatin136 may be useful in the support of functional improvement in dystrophic muscle. It is unclear, however, whether such factors will provide long-term benefits since regeneration in dystrophic muscle mediated by myogenic precursor cells may exhaust key cell types126 and the regenerated muscle will inevitably be dystrophic. A better understanding of the dystrophic process, possibly by temporal gene-expression profiling of dystrophic muscle, may reveal new factors that can modulate the clinical severity of DMD phenotype.137

Utrophin
Utrophin is a 395 kDa ubiquitous protein homologue of dystrophin that colocalises with α-bungarotoxin at acetylcholine receptors in the neuromuscular junctions of non-dystrophic adult muscle.138 Utrophin is translated from a 13 kb transcript on chromosome 6 (q24) in human beings and chromosome 10 in mice.139 Utrophin expression has a widespread sarcosomal distribution in human and mouse dystrophic muscle.140,141 Utrophin deficiency in mdx mice exacerbates the mdx phenotype to produce a model that more accurately represents human DMD.142

Utrophin upregulation may compensate for structural deficiency in dystrophic muscle.143 This has been shown in muscle from mdx mice by the expression of a truncated utrophin transgene.144 Interleukin 6 induces overexpression of utrophin in neonatal mdx muscle.145 Utrophin expression can also be upregulated in mdx muscle by L-arginine and in mdx myotubes by L-arginine, nitric oxide, and hydroxyurea.146 If a factor can be identified that controls and maintains utrophin upregulation in dystrophic muscle, this approach may be of use as a potential treatment for DMD.

Conclusion
Several exciting options are currently being explored in the search for an effective therapy for DMD. There are still, however, major challenges facing all of the strategies discussed in this review. In particular, the likelihood that gentamicin, upregulation of utrophin or α7β1 integrin, and targeted corrective gene conversion are likely to induce changes at non-target loci needs to be considered. The targeting capacities of the various therapies are also important: strategies that target existing muscle fibres will eventually lead to replacement by dystrophic satellite cells. Strategies involving muscle derived precursor cells will probably require ablation of existing muscle before they can exert any therapeutic influence. Thus a multifactorial approach will probably be required for successful therapy.

The safety of any novel treatment for a disease must be assessed; this is particularly important in the case of viral-gene therapy, as shown by two recent cases. A human trial of adenoviral vector for metabolic ornithine transcarbamylase deficiency resulted in the death of one of the 18 trial participants due to therapy-related liver and lung failure 48 h after viral-vector injection. The second, more recent, example was the development of leukaemia-like symptoms in one of nine infants in a trial of recombinant murine Moloney leukaemia virus retroviral vector for the treatment of severe combined immunodeficiency disease.147,148 Until this tragic event, and in contrast to the death related to adenoviral-vector therapy, retroviral vector therapy was the only example of successful viral-gene therapy in human beings. Both events were the result of the inherent natures of the respective viral systems used.

These failures highlight the importance of solid scientific data from in vitro studies and animal models. Although systems may be developed and assessed in the mdx mouse model, testing in larger models, such as golden retriever dog model, may help to identify potential problems in applicability to patients with DMD. Current clinical trials of gentamicin, plasmid and myogenic precursors, and other factors (table) are the result of extensive work in these areas and other trials will follow. From the positive data generated thus far, viral-gene therapy—most likely via muscle-specific gutted adenoviral vectors expressing full-length dys—is currently the most effective potential therapeutic approach for DMD. Nevertheless the studies we have described in this review highlight the exciting and novel strategies in this area of research. At least some of these strategies will inevitably evolve into effective therapies, if not for DMD then perhaps for other human disorders.
4 Koenig M, Monaco AP, Kunkel LM. The complete sequence of dystrophin predicts a rod-shaped protein is a major product of the Duchenne Koenig M, Monaco AP, Kunkel LM. The complete sequence of dystrophin predicts a rod-shaped protein is a major product of the Duchenne


