Gene therapy of monogenic diseases
Indications Addressed by Gene Therapy Clinical Trials

- Cancer diseases 67% (n=797)
- Vascular diseases 8.9% (n=106)
- Monogeneic diseases 8.6% (n=102)
- Infectious diseases 6.5% (n=78)
- Other diseases 3.4% (n=40)
- Gene marking 4.2% (n=50)
- Healthy volunteers 1.6% (n=19)
Indications Addressed by Gene Therapy Clinical Trials

- Cancer diseases 64.5% (n=1019)
- Cardiovascular diseases 8.7% (n=138)
- Monogenic diseases 7.9% (n=125)
- Infectious diseases 8% (n=127)
- Neurological diseases 1.9% (n=30)
- Ocular diseases 1.1% (n=18)
- Other diseases 2.2% (n=33)
- Gene marking 3.3% (n=50)
- Healthy volunteers 2.3% (n=37)
Phases of Gene Therapy Clinical Trials

- Phase I: 60.3% (n=952)
- Phase I/II: 18.9% (n=299)
- Phase II: 16.3% (n=258)
- Phase II/III: 0.8% (n=13)
- Phase III: 3.4% (n=53)
- Phase IV: 0.1% (n=2)
- Single subject: 0.1% (n=2)
Phases of clinical trials

<table>
<thead>
<tr>
<th>Purpose</th>
<th>PHASE 1</th>
<th>PHASE 2</th>
<th>PHASE 3</th>
<th>PHASE 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>To determine a safe dosage of the drug, how it should be given, and its effects.</td>
<td>To determine whether or not the drug has an effect on the particular disease</td>
<td>To also determine any other effects of the drug.</td>
<td>To compare the new drug (or new use of an existing drug) with the current standard of care.</td>
<td>To continue to study the long-term effects and safety of the new drug or treatment</td>
</tr>
</tbody>
</table>

| Number of Study Participants | Usually 15-30 | Usually less than 100 | 100 to 1,000’s | Varies from 100’s to 1,000’s |

Some Phase II and most Phase III drug trials are randomized, double-blind and placebo-controlled
Clinical trials

The strongest evidence for therapeutic interventions is provided by systematic review of randomized, double-blind, placebo-controlled trials involving a homogeneous patient population and medical condition.

**Randomized:** Each study subject is randomly assigned to receive either the study treatment or a placebo.

**Double-blind:** The subjects involved in the study and the researchers do not know which study treatment is being given.

**Placebo-controlled:** The use of a placebo (fake treatment) allows the researchers to isolate the effect of the study treatment.

Some Phase II and most Phase III drug trials
Evidence-based medicine (EBM)

According to the Centre for EBM:
"Evidence-based medicine is the conscientious, explicit and judicious use of current best evidence in making decisions about the care of individual patients."

EBM integrates best available research evidence with clinical expertise.
Registered drugs are only a portion of all tested
Gene therapy of monogenic diseases
<table>
<thead>
<tr>
<th>Disorder</th>
<th>Incidence</th>
<th>Gene</th>
<th>Mutation detection rate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monogenic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>1:4000</td>
<td>CFTR</td>
<td>98%</td>
</tr>
<tr>
<td>Duchenne muscular dystrophy</td>
<td>1:4000</td>
<td>DMD</td>
<td>~90%</td>
</tr>
<tr>
<td>Fragile X syndrome</td>
<td>1:4000</td>
<td>FMR</td>
<td>100%</td>
</tr>
<tr>
<td>Huntigton disease</td>
<td>1:5000-10 000</td>
<td>HD</td>
<td>100%</td>
</tr>
<tr>
<td>Hemophilia A</td>
<td>1:10 000</td>
<td>F8C</td>
<td>~90%</td>
</tr>
<tr>
<td>Phenylketonuria</td>
<td>1:10 000</td>
<td>PAH</td>
<td>99%</td>
</tr>
<tr>
<td>Polycystic kidney disease</td>
<td>1:1500</td>
<td>PKD1, PKD2</td>
<td>~15%</td>
</tr>
<tr>
<td><strong>Inherited cancer</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast-ovarian cancer</td>
<td>1:4000</td>
<td>BCRA1 (80%)</td>
<td>50-65%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BCRA2 (20%)</td>
<td>35%</td>
</tr>
<tr>
<td>Li-Fraumeni syndrome</td>
<td></td>
<td>p53</td>
<td>50%</td>
</tr>
<tr>
<td>Ataxia-telangiectasia</td>
<td></td>
<td>ATM</td>
<td>70%</td>
</tr>
<tr>
<td>Familial polyposis coli</td>
<td>1:4000</td>
<td>APC</td>
<td>87%</td>
</tr>
<tr>
<td>Hereditary non-polyposis coli</td>
<td>1:2000</td>
<td>MLH1 (30%)</td>
<td>33%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MLH2 (60%)</td>
<td>12%</td>
</tr>
<tr>
<td><strong>Cardiovascular disorders</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Familial hypercholesterolemia</td>
<td>1:500</td>
<td>LDLR</td>
<td>60%</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td></td>
<td>APOE</td>
<td>10%</td>
</tr>
</tbody>
</table>
Severe combined immunodeficiency diseases

**Common characteristic:** occurrence of the block in T cell differentiation, always associated with a direct or indirect impairment of B cell immunity

17 distinct SCID phenotypes have been identified to date

Mutations in 10 genes have been found to cause SCID
<table>
<thead>
<tr>
<th>Disease</th>
<th>Relative frequency&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Affected cells</th>
<th>Gene product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reticular dysgenesis</td>
<td>4 %</td>
<td>AR T, B, NK, myeloid cells, platelets</td>
<td>Unknown</td>
</tr>
<tr>
<td>Alymphocytosis</td>
<td>15 %</td>
<td>AR T, B</td>
<td>RAG-1, RAG-2 Artemis</td>
</tr>
<tr>
<td>Absence of T lymphocytes and NK</td>
<td>40 %</td>
<td>X-L T, NK</td>
<td>γc chain</td>
</tr>
<tr>
<td></td>
<td>10 %</td>
<td>AR T, NK</td>
<td>JAK-3</td>
</tr>
<tr>
<td>Absence of T lymphocytes</td>
<td>5 %</td>
<td>AR T</td>
<td>IL-7 Rα chain</td>
</tr>
<tr>
<td></td>
<td>≤1 %</td>
<td>AR T</td>
<td>CD45</td>
</tr>
<tr>
<td>ADA deficiency</td>
<td>10–15%</td>
<td>AR T, B, NK</td>
<td>ADA</td>
</tr>
</tbody>
</table>

<sup>a</sup>Abbreviations: ADA, adenosine deaminase; AR, autosomal recessive; NK, natural killer cells; X-L, X-linked recessive.

<sup>b</sup>Frequency in our experience.

Cavazzana-Calvo M et al., Ann Rev. Med. 2005
Table 1  Clinical trials of gene therapy for childhood immunological diseases

<table>
<thead>
<tr>
<th></th>
<th>No. of trials</th>
<th>No. of subjects</th>
<th>Clinical benefit</th>
<th>SAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADA (−) SCID (1990–1996)</td>
<td>6</td>
<td>19</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ADA (−) SCID (1999–present)</td>
<td>4</td>
<td>20</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>XSCID</td>
<td>3</td>
<td>26</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>Jak3 SCID</td>
<td>1</td>
<td>1</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>CGD</td>
<td>4</td>
<td>14</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>LAD (CD18)</td>
<td>1</td>
<td>1</td>
<td>?^b</td>
<td>0</td>
</tr>
<tr>
<td>Wiskott–Aldrich syndrome</td>
<td>1</td>
<td>2</td>
<td>?^b</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: ADA = adenosine deaminase; CGD = chronic granulomatous disease; LAD = leukocyte adhesion defect; SAE = serious adverse events possibly related to the procedure; SCID = severe combined immune deficiency; XSCID = X-linked SCID.

^aBased on presentations at the 10th Annual Meeting of the American Society of Gene Therapy, Seattle, WA, June 2007.

^b?= outcome not reported.
The proof of concept of gene therapy has been demonstrated in patients with SCID

Gene therapy can cure diseases; side-effects happen.

Side-effects are inherent feature of each treatment (not only gene therapy!!!)
Chronic granulomatous disease
Chronic granulomatous disease (CGD) is a rare inherited immunodeficiency characterized by recurrent, often life threatening bacterial and fungal infections due to a functional defect in the microbial-killing activity of phagocytic neutrophils. It occurs as a result of mutations in genes encoding a multicomponent enzyme complex, the NADPH oxidase, that catalyses the respiratory burst. The majority of patients have an X-linked form of the disease which is associated with mutations in a membrane-bound component gp91phox. HLA-matched allogeneic hematopoietic stem cell (HSC) transplantation can be curative, but for patients without suitable donors, genetic modification of autologous hematopoietic stem cells is an attractive alternative.
Gene Therapy for X-CGD

1. Myelosuppression
2. Reinfusion of genetically modified cells

G-CSF Mobilisation
Leukapheresis
Isolation of CD34+ Cells

Quality Control

Retroviral mediated Gene Transfer

Gene Transfer Vector
Blood Stem Cells (CD34+ cells)
Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EVI1, PRDM16 or SETBP1

Marion G Ott1,16, Manfred Schmidt2−4,16, Kerstin Schwarzwaelder3−5,16, Stefan Stein6,16, Ulrich Siler7,16, Ulrike Koehl8, Hanno Glimm2,3, Klaus Kühlcke9, Andrea Schilz9, Hana Kunkel6, Sonja Naundorf9, Andrea Brinkmann8, Annette Deichmann3,4, Marlene Fischer2,3,5, Claudia Ball3−5, Ingo Pilz3,5, Cynthia Dunbar10, Yang Du11, Nancy A Jenkins11, Neal G Copeland11, Ursula Lüthi12, Moustapha Hassan13, Adrian J Thrasher14, Dieter Hoelzer1, Christof von Kalle2−4,15,16, Reinhard Seger7,16 & Manuel Grez6,16

Gene transfer into hematopoietic stem cells has been used successfully for correcting lymphoid but not myeloid immunodeficiencies. Here we report on two adults who received gene therapy after nonmyeloablative bone marrow conditioning for the treatment of X-linked chronic granulomatous disease (X-CGD), a primary immunodeficiency caused by a defect in the oxidative antimicrobial activity of phagocytes resulting from mutations in gp91phox. We detected substantial gene transfer in both individuals' neutrophils that lead to a large number of functionally corrected phagocytes and notable clinical improvement. Large-scale retroviral integration site–distribution analysis showed activating insertions in MDS1-EVI1, PRDM16 or SETBP1 that had influenced regulation of long-term hematopoiesis by expanding gene-corrected myelopoiesis three- to four-fold in both individuals. Although insertional influences have probably reinforced the therapeutic efficacy in this trial, our results suggest that gene therapy in combination with bone marrow conditioning can be successfully used to treat inherited diseases affecting the myeloid compartment such as CGD.
Correction of neutrophil bacteriocidal function by overexpression of gp91phox subunit of NADPH oxidase

Figure 1 Gene-corrected neutrophils in the blood of an individual with CGD after HSPC gene therapy. Whereas CGD neutrophils lack NADPH activity (green cytosol), the progeny of HSPCs engrafted after gene therapy have reconstituted enzyme activity (blue cytosol) resulting from the presence of a functional gene replaced by a retroviral vector. Gene-corrected cells differ for the site of vector insertion (nucleus in different color), reflecting the origin from distinct progenitors. Early after gene therapy, many progenitors contribute to the neutrophil pool but become exhausted with time. At later times, gene-corrected cells from a unique progenitor progressively expand until accounting for the majority of circulating cells. In these cells, the vector integrated near a growth-promoting gene which is activated by insertional mutagenesis and confers a growth advantage. Total neutrophil number does not increase with time, suggesting that the expanding cells are subjected to normal control. Because the clinical benefit is dependent on the number of circulating gene-corrected cells (gray line), the expansion enables efficacious and sustained correction of the disease.

# Gene therapy for X-linked chronic granulomatous disease

<table>
<thead>
<tr>
<th>Study</th>
<th>Vector</th>
<th>Complication</th>
<th>Insertion sites</th>
<th>Additional chromosomal abnormalities</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCID-X1 (P4, Paris)</td>
<td>MFG-Ampho</td>
<td>T-ALL, mature T cell</td>
<td>LMO2</td>
<td>CDKN2A del</td>
<td>Died</td>
</tr>
<tr>
<td>SCID-X1 (P5, Paris)</td>
<td>MFG-Ampho</td>
<td>T-ALL</td>
<td>LMO2</td>
<td>Notch mutation</td>
<td>CR</td>
</tr>
<tr>
<td>SCID-X1 (P7, Paris)</td>
<td>MFG-Ampho</td>
<td>Late cortical T cell</td>
<td>CCND2</td>
<td>CDKN2A del</td>
<td>CR</td>
</tr>
<tr>
<td>SCID-X1 (P10, Paris)</td>
<td>MFG-Ampho</td>
<td>T-ALL</td>
<td>LMO2</td>
<td>Notch mutation</td>
<td>CR</td>
</tr>
<tr>
<td>SCID-X1 (P8, London)</td>
<td>MFG-GALV</td>
<td>Late cortical T cell</td>
<td>BMI1, LMO2</td>
<td>Notch 1 mutation, CDKN2A deletion, TCRb/STIL-TAL1 translocation, Monosomy-7</td>
<td>CR</td>
</tr>
<tr>
<td>CGD (Frankfurt)</td>
<td>SFFV-GALV</td>
<td>Clonal myeloid expansions</td>
<td>MDS1-EVI1, PRDM16, SETBP1</td>
<td>Monosomy-7</td>
<td>Transgene silencing, Died sepsis</td>
</tr>
<tr>
<td>CGD (Frankfurt)</td>
<td>SFFV-GALV</td>
<td>Clonal myeloid expansions</td>
<td>MDS1-EVI1, PRDM16</td>
<td>Monosomy-7</td>
<td>Transgene silencing, MUD-HSCT</td>
</tr>
</tbody>
</table>

Abbreviations: ALL, acute lymphocytic leukaemia; CDKN2A, cyclin-dependent kinase inhibitor 2A; CGD, chronic granulomatous disease; CR, complete remission; Evi1, ecotropic viral integration site 1; GALV, Gibbon Ape leukaemia virus; HSCT, haematopoietic stem cell transplantation; LMO2, LIM domain only 2; MDS, myelodysplasia syndrome 1; MUD-HSCT, matched unrelated donor haematopoietic stem cell transplantation; PRDM16, PR domain containing 16; SCID, severe combined immunodeficiency; SETBP1, SET binding protein 1; SFFV, Spleen focus forming virus; TCR, T-cell receptor.

Quasim et al., Gene Therapy 2009
Gene therapy of immunodeficiency diseases

In brief

Progress

- The molecular basis of insertional mutagenesis following gene therapy for severe combined immunodeficiency (SCID)-X1 has been characterized in detail.
- Adenosine deaminase deficiency (ADA) SCID gene therapy has mediated sustained clinical benefit with no evidence of vector related toxicity.
- The mechanism of clonal expansions and transgene silencing following retroviral gene therapy for Chronic Granulomatous Disease has been elucidated.
- Gene therapy for Wiskott–Aldrich Syndrome (WAS) has been initiated.

Prospects

- Other immune and haematological disorders are good candidate disorders. For some patients without human leukocyte antigen (HLA)-matched donors, gene therapy may become a standard of care.
- Optimized combinations of conditioning and effective gene transfer will enhance efficiency and safety of gene therapy protocols.
- Self-inactivating gamma-retroviral and lentiviral vectors are entering clinical testing, and will be used more widely.
- New technologies, including targeted integration and gene editing, hold promise for treatment of inherited immunodeficiencies in the longer term.

Quasim et al., Gene Therapy 2009
Gene therapy for Wiskott–Aldrich Syndrome gets underway

Wiskott–Aldrich Syndrome (WAS) is an X-linked disorder characterized by immune deficiency, thrombocytopenia and eczema, and with age, patients become prone to haematological malignancies and autoimmunity. The WAS gene encodes a cytoskeletal regulation protein in haematopoietic lineage cells, which is important for platelet production and immune cell activation and motility. Current recommendations advocate HSCT if a suitable HLA-matched donor is available.
Other gene therapy trials
<table>
<thead>
<tr>
<th>Disease</th>
<th>Gene(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystic fibrosis</td>
<td>CFTR, α-1-anti-trypsin</td>
</tr>
<tr>
<td>SCID</td>
<td>ADA</td>
</tr>
<tr>
<td>Gaucher disease</td>
<td>glucocerebrosidase</td>
</tr>
<tr>
<td>Hemophilia A</td>
<td>Factor VIII</td>
</tr>
<tr>
<td>Hemophilia B</td>
<td>Factor IX</td>
</tr>
<tr>
<td>Familial hypercholesterolemia</td>
<td>LDL-R</td>
</tr>
<tr>
<td>Muscular dystrophy</td>
<td>sarcoglycan, dystrophin, utrophin</td>
</tr>
<tr>
<td>Ornithine transcarbamylase deficiency</td>
<td>OTC</td>
</tr>
</tbody>
</table>
Hemophilia A and B and gene therapy

1. Factor VIII production is not regulated in response to bleeding
2. The broad therapeutic index of factor VIII minimises the risk of overdoses
3. Delivery of factor VIII into the bloodstream does not require expression of the gene by specific organ
4. Even low levels of the protein can be beneficial

Size of the coding sequences of factor VIII and factor IX

\[ mRNA - \text{factor VIII} - 8,8 \text{ kb} \]
\[ \text{factor IX} - 1,8 \text{ kb} \]
Steps in human factor VIII gene transfer procedure

Ex vivo - plasmid gene therapy

Clinical gene therapy for haemophilia A

Roth DA et al., NEJM 2001; 344: 1735
**Clinical gene therapy for haemophilia A**

### Table 1. Characteristics of the Six Patients.*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>46</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>20–72</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>50–91</td>
</tr>
<tr>
<td>Pretreatment factor VIII activity</td>
<td>6</td>
</tr>
<tr>
<td>&lt;0.8% of normal (no. of patients)</td>
<td></td>
</tr>
<tr>
<td>Viral exposure (no. of patients)†</td>
<td></td>
</tr>
<tr>
<td>Human immunodeficiency virus</td>
<td>4</td>
</tr>
<tr>
<td>Hepatitis A virus</td>
<td>5</td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td>5</td>
</tr>
<tr>
<td>Hepatitis C virus</td>
<td>6</td>
</tr>
</tbody>
</table>

*All six patients were men.

†Viral exposure was determined at the time of enrollment by testing for the presence of antibodies to the viruses listed.

### Table 2. Total Factor VIII Production by Implanted Autologous Fibroblasts.*

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Factor VIII Production by Harvested Cells†</th>
<th>No. of Cells Implanted</th>
<th>Total Factor VIII Production by Implanted Cells‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IU/10⁶ cells/day</td>
<td>IU/kg/day</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.8</td>
<td>100×10⁶</td>
<td>1.3</td>
</tr>
<tr>
<td>2</td>
<td>4.9</td>
<td>100×10⁶</td>
<td>5.4</td>
</tr>
<tr>
<td>3</td>
<td>1.9</td>
<td>100×10⁶</td>
<td>3.8</td>
</tr>
<tr>
<td>4</td>
<td>1.8</td>
<td>400×10⁶</td>
<td>10.4</td>
</tr>
<tr>
<td>5</td>
<td>1.6</td>
<td>400×10⁶</td>
<td>8.4</td>
</tr>
<tr>
<td>6</td>
<td>6.7</td>
<td>400×10⁶</td>
<td>36.0</td>
</tr>
</tbody>
</table>

*The conditioned medium of each fibroblast clone was replaced with fresh medium 24 hours before it was assayed for factor VIII expression levels by a human factor VIII enzyme-linked immunosorbent assay.

†The production of factor VIII at the time of cell harvest, before implantation, is shown.

‡The total factor VIII production of each implanted clone is shown, normalized for the weight of each patient.

Roth DA et al., NEJM 2001; 344: 1735
Bleeding Events and Use of Exogenous Factor VIII in Three of the Six Patients.

Patient 1

Injury-related bleeding
Spontaneous bleeding

Patient 3

Injury-related bleeding
Spontaneous bleeding

Patient 6

Injury-related bleeding
Spontaneous bleeding

Roth DA et al., NEJM 2001; 344: 1735
Phase 1 trial of FVIII gene transfer for severe hemophilia A using a retroviral construct administered by peripheral intravenous infusion.


Division of Hematology and Oncology, Suite 3016, UC-Davis Cancer Center, University of California at Davis, 4501 X St, Sacramento, CA 95817. jspowell@ucdavis.edu

In a phase 1 dose escalation study, 13 subjects with hemophilia A received by peripheral intravenous infusion a retroviral vector carrying a B-domain-deleted human factor VIII (hFVIII) gene. Infusions were well tolerated. Tests for replication competent retrovirus have been negative. Polymerase chain reaction (PCR) analyses demonstrate the persistence of vector gene sequences in peripheral blood mononuclear cells in 3 of 3 subjects tested. Factor VIII was measured in serial samples using both a one-stage clotting assay and a chromogenic assay. While no subject had sustained FVIII increases, 9 subjects had FVIII higher than 1% on at least 2 occasions 5 or more days after infusion of exogenous FVIII, with isolated levels that ranged from 2.3% to 19%. Pharmacokinetic parameters of exogenous FVIII infused into subjects 13 weeks after vector infusion showed an increased half-life (T1/2; P <.02) and area under the curve (AUC, P <.04) compared with prestudy values. Bleeding frequency decreased in 5 subjects compared with historical rates. These results demonstrate that this retroviral vector (hFVIII(V)) is safe and, in some subjects, persists more than a year in peripheral blood mononuclear cells, with measurable factor VIII levels and with increased available FVIII activity (increased T1/2 and AUC) after infusion of exogenous FVIII concentrate.
Long-term correction of inhibitor-prone hemophilia B dogs treated with liver-directed AAV2-mediated factor IX gene therapy

Glenn P. Niemeyer,1,2 Roland W. Herzog,3 Jane Mount,1 Valder R. Arruda,4 D. Michael Tillson,5 John Hathcock,5 Frederik W. van Ginkel,6 Katherine A. High,4 and Clinton D. Lothrop Jr1,2,5

1Scott-Ritchey Research Center, College of Veterinary Medicine, Auburn University, AL; 2Department of Biochemistry and Molecular Genetics, University of Alabama, Birmingham; 3Department of Pediatrics, Division of Cellular and Molecular Therapy, University of Florida, Gainesville; 4Departments of Pediatrics and Medicine and Howard Hughes Medical Institute, University of Pennsylvania Medical Center and The Children’s Hospital of Philadelphia; 5Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL; and 6Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

Preclinical studies and initial clinical trials have documented the feasibility of adenoassociated virus (AAV)–mediated gene therapy for hemophilia B. In an 8-year study, inhibitor-prone hemophilia B dogs (n = 2) treated with liver-directed AAV2 factor IX (FIX) gene therapy did not have a single bleed requiring FIX replacement, whereas dogs undergoing muscle-directed gene therapy (n = 3) had a bleed frequency similar to untreated FIX-deficient dogs. Coagulation tests (whole blood clotting time [WBCT], activated clotting time [ACT], and activated partial thromboplastin time [aPTT]) have remained at the upper limits of the normal ranges in the 2 dogs that received liver-directed gene therapy. The FIX activity has remained stable between 4% and 10% in both liver-treated dogs, but is undetectable in the dogs undergoing muscle-directed gene transfer. Integration site analysis by linear amplification-mediated polymerase chain reaction (LAM-PCR) suggested the vector sequences have persisted predominantly in extrachromosomal form. Complete blood count (CBC), serum chemistries, bile acid profile, hepatic magnetic resonance imaging (MRI) and computed tomography (CT) scans, and liver biopsy were normal with no evidence for tumor formation. AAV-mediated liver-directed gene therapy corrected the hemophilia phenotype without toxicity or inhibitor development in the inhibitor-prone null mutation dogs for more than 8 years. (Blood. 2009;113:797-806)

Table 1. Summary of hemophilia B dogs undergoing AAV-mediated gene therapy

<table>
<thead>
<tr>
<th>Dog</th>
<th>Age*</th>
<th>Rx</th>
<th>Bleed frequency</th>
<th>Inhibitor status</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kudzu</td>
<td>NA</td>
<td>None</td>
<td>6/3.6 y</td>
<td>–</td>
<td>Alive/B</td>
</tr>
<tr>
<td>Brad</td>
<td>9 mo</td>
<td>Liver</td>
<td>0/6.25 y</td>
<td>–</td>
<td>Alive/N</td>
</tr>
<tr>
<td>Semillon</td>
<td>5.5 mo</td>
<td>Liver</td>
<td>0/5.9 y</td>
<td>–</td>
<td>Alive/N</td>
</tr>
<tr>
<td>Beech</td>
<td>12 mo</td>
<td>Liver</td>
<td>1/11 wk</td>
<td>+</td>
<td>Dead/FB</td>
</tr>
<tr>
<td>Sauvignon</td>
<td>5.5 mo</td>
<td>Muscle</td>
<td>6/5.9 y</td>
<td>+</td>
<td>Alive/B</td>
</tr>
<tr>
<td>Wilbur</td>
<td>8.5 y</td>
<td>Muscle</td>
<td>8/6.0 y</td>
<td>+</td>
<td>Alive/B</td>
</tr>
<tr>
<td>Wes</td>
<td>9 mo</td>
<td>Muscle</td>
<td>10/2.4 y</td>
<td>+</td>
<td>Dead/FB</td>
</tr>
</tbody>
</table>

NA indicates not applicable; N, normal clotting; B, abnormal clotting; FB, fatal bleed; mo, months; y, years; and wk, weeks.

*Age at vector administration.
Cystic fibrosis
Mukowiscydoza
Cystic fibrosis
Mukowiscydoza

- most common autosomal recessive disorder in Caucasians
  (1:2000-2500 live births; carrier rate in northern Europeans
  - 1:20-1:25)
in Hawaiians - 1:90000

- after asthma, CF is the commonest cause of chronic respiratory
distress in childhood; responsible for the majority of deaths
Cystic fibrosis

Lung of healthy person

Lung of person suffering from cystic fibrosis
Mukowiscydoza
*Cystic fibrosis*

Complications

- respiratory - chronic bacterial infections; respiratory and cardiac failure
- gastrointestinal tract - more than 85% of children –
  malabsorption due to exocrine pancreatic insufficiency
- infertility - affects 85% males and to a lesser extent females

Median survival remains about 35 years
CFTR gene

*Cystic fibrosis transmembrane conductance regulator*

168 kDa protein; 1480 aa residues

CFTR codes for a chloride ion channel

over 600 mutations produce CF; deltaF508 (D508) is the most common (in exon 10, interferes with ATP binding)
**Cystic Fibrosis Key Dates**

1938: Physician Dorothy Hansine Andersen provides the first clinical description of cystic fibrosis.

1983: Chloride transport is identified as the major defect in CF.

1989: CFTR, the cystic fibrosis gene, is found. Median life expectancy for those with CF is about 29.

1990: Scientists suggest that protein folding is behind CF.

1990: Patients with the same gene mutation don’t share the same severe disease, suggesting a role for other genes or the environment.

1992: Scientists describe the first mouse model of CF, but the mice don’t have lung disease.

1993: A 23-year-old man with CF receives the first dose of gene therapy.

1993: The number of mutations in CFTR surpasses 200. Today, we know of over 1500 different mutations.
Cystic fibrosis key dates

1995: Gene therapy fails to help CF patients.

2001: Two medical professional groups recommend prenatal CF carrier screening.

2005: Researchers show that the gene TGFβ modifies the severity of CF.

2006: The first drug designed to target a CF protein defect, by Vertex Pharmaceuticals, enters clinical trials.

2008: Pigs become the second animal model of CF.

2008: Forty-seven U.S. states are screening newborns for CF.

2008: The United Kingdom launches a new CF gene therapy trial.


2009: The phase 3 clinical trial for one Vertex drug opens after lung function improved in an earlier study.

2009: Median life expectancy for those with CF exceeds 37 years old, thanks to more aggressive and earlier treatment.
Gene therapy for cystic fibrosis

- the most often treated disease with gene therapy
- clinical trials started in 1993

Gene therapy strategies
1. Gene transfer of CFTR with:
   a) various vectors, initially adenoviruses, but it has been found that there are not receptors for Ad on the apical surface of lung epithelium
   - then the most common were AAVs – but unsuccessful
   b) mode of delivery:
      - bronchoscopy
      - aerosol
Clinical trials of gene therapy in CF

<table>
<thead>
<tr>
<th>Trial/year/reference</th>
<th>Liposoma</th>
<th>No. of patients</th>
<th>Target</th>
<th>Route</th>
<th>Efficacy</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caplen et al., 1996</td>
<td>DC-ChoVDOPE</td>
<td>15</td>
<td>Nose</td>
<td>Aerosol</td>
<td>P.D. 20 % correction towards normal</td>
<td>None</td>
</tr>
<tr>
<td>Zabner et al., 1997</td>
<td>GL-67™/DOPE</td>
<td>12</td>
<td>Nose</td>
<td>Direct installation</td>
<td>Partial correction nasal P.D. (equivalent to naked DNA)</td>
<td>None</td>
</tr>
<tr>
<td>Gill et al., 1997</td>
<td>DC-ChoVDOPE</td>
<td>12</td>
<td>Nose</td>
<td>Direct installation</td>
<td>Functional evidence in 6/8 treated patients</td>
<td>None</td>
</tr>
<tr>
<td>Porteous et al., 1997</td>
<td>DOTAP</td>
<td>16</td>
<td>Nose</td>
<td>Aerosol</td>
<td>P.D. 20 % correction in two patients, no SPQ-positive</td>
<td>None</td>
</tr>
<tr>
<td>Alton et al., 1999</td>
<td>GL-67™/DOPE/DMPE-PEG&lt;sub&gt;5000&lt;/sub&gt;</td>
<td>16</td>
<td>Lungs</td>
<td>Nebulized</td>
<td>Lung P.D. 25 % correction towards normal</td>
<td>Mild, flu-like symptoms</td>
</tr>
<tr>
<td>Hyde et al., 2000</td>
<td>DC-ChoVDOPE</td>
<td>12</td>
<td>Nose</td>
<td>Direct installation, repeat dosing</td>
<td>6/10 patients positive after each dose</td>
<td>None</td>
</tr>
<tr>
<td>Noone et al., 2000</td>
<td>EDMPC-Chol</td>
<td>11</td>
<td>Nose</td>
<td>Aerosol</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Ruiz et al., 2001</td>
<td>GL-67™/DOPE/DMPE-PEG&lt;sub&gt;5000&lt;/sub&gt;</td>
<td>8</td>
<td>Lungs</td>
<td>Nebulized</td>
<td>Vector-specific mRNA in 3/8 patients</td>
<td>Mild, flu-like symptoms</td>
</tr>
</tbody>
</table>

Expression limited to 30 days
Dystrofia mięśniowa
Muscular dystrophy
Duchenne muscular dystrophy

Progressive muscle weakness, more proximal
Onset between 2-4 years of age
>95% in wheelchair by 12 years of age
Death between 15-25 years of age
Variable mental retardation
Frequent cardiac involvement
Orthopaedic deformities
Calf hypertrophy
High creatine phosphokinase concentrations
Dystrophin deficiency in muscle
Hereditary,
X-linked disease
Gene Xp21 mutations

In 1861, Duchenne described his first case of the dystrophy that now bears his name, under the title, *Paralgie hypertrophique de l'enfance de cause cerebrale*. Because of the intellectual impairment in the affected boys, Duchenne initially thought the condition was cerebral in origin.
Figure 1. Dystrophin and the dystrophin–glycoprotein complex in muscle. Dystrophin is a cytoskeletal protein that links the $\gamma$-actin filaments to the extracellular matrix via the dystroglycan/sarcoglycan complexes. The C-terminal domain of dystrophin binds syntrophin and dystrobrevin, the latter of which also binds the sarcoglycan complex. Loss of dystrophin results in destabilization and loss of the DGC.
Duchenne & Becker muscular dystrophy

*Duchenne* dystrophy: 1:3000 newborn males

X-linked;
The largest gene described in humans (~1% of X chromosome), extending over 2300 kb and comprising 79 exons

- The size of the gene (2.4 Mb) and mRNA (14 kb) is a serious problem

- In 65% patients the molecular abnormality involves exon deletions

*Becker* dystrophy: 7-10 times less frequent than Duchenne
Phase I study of dystrophin plasmid-based gene therapy in Duchenne/Becker muscular dystrophy.


Institut de Myologie, INSERM U582, CHU Pitie-Salpetriere, 75013 Paris, France.

Nine patients with Duchenne or Becker muscular dystrophy were injected via the radialis muscle with a full-length human dystrophin plasmid, either once with 200 or 600 microg of DNA or twice, 2 weeks apart, with 600 microg of DNA. In the biopsies taken 3 weeks after the initial injection, the vector was detected at the injection site in all patients. Immunohistochemistry and nested reverse transcription-polymerase chain reaction indicated dystrophin expression in six of nine patients. The level of expression was low (up to 6% weak, but complete sarcolemmal dystrophin staining, and up to 26% partial sarcolemmal labeling). No side effects were observed, nor any cellular or humoral anti-dystrophin responses. These results suggest that exogenous dystrophin expression can be obtained in Duchenne/Becker patients after intramuscular transfer of plasmid, without adverse effects, hence paving the way for future developments in gene therapy of hereditary muscular diseases.
Major hurdles include:

1. the need to correct large masses of tissue (40% of the body weight) with minimal damage to the already inflamed and necrotic muscles,
2. Prevent the immune rejection to the therapeutic protein,
3. sustained (if possible, life-long) expression.
4. Size of the gene/cDNA
Partial dystrophins can exert therapeutic effect

Spectrin-like central rod domain is largely dispensable

Figure 1  Structure of full-length and truncated dystrophins. (a) Full-length dystrophin showing all functional domains. ABD, N-terminal actin binding domain; ABD2, internal actin binding domain; CR, cysteine-rich region; CT, carboxy terminus. Spectrin-like repeats are numbered 1–24; hinge regions labelled H1–H4. (b) Mini-dystrophin based on mildly affected BMD patient; (c–e) micro-dystrophin genes engineered for delivery by AAV.

Foster et al., Gene Therapy 2006
Gene therapy for Duchenne muscular dystrophy

- Gene transfer
- Correction of mutation
- Cell therapy (myoblast transplantation)

- dystrophin
- utrophin
Utrophin

- A homologue of dystrophin

- Present in DMD patients

- contains 74 exons, about 1 Mb – so 1/3 of dystrophin, but its transcript (about 13 kb) is almost as large

- Ubiquitously expressed; in muscle its expression is maturation dependent, and it is gradually replaced by dystrophin, therefore it is lacking in DMD

- Upregulation of utrophin by pharmacological treatment or gene transfer may help to improve conditions in DMD
Animal models of DMD

1. Dmdx mice - not fully compatible with human DMD
   (mutation in exon 23 of dystrophin gene; normal lifespan)

2. Golden retriever muscular dystrophy dogs - by 8 months of age dogs walk with most difficulty

Table 2 | Overview of animal models for Duchenne muscular dystrophy gene-therapy studies

<table>
<thead>
<tr>
<th>Model</th>
<th>Mutation</th>
<th>Effect</th>
<th>Pathologic/physiologic symptoms</th>
<th>Gene-therapy studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>mdx mouse</td>
<td>Nonsense mutation in exon 23 (3185C&gt;T)</td>
<td>Stop codon introduced, dystrophin synthesis aborted prematurely</td>
<td>Fibre degeneration (particularly from 2–8 weeks), replacement by centrally nucleated regenerating fibres, myopathy less severe later in life, normal lifespan</td>
<td>Used for most strategies owing to its relative experimental simplicity</td>
</tr>
<tr>
<td>CXMD/GRMD golden</td>
<td>3' splice-site point mutation in intron 6 (739–2a&gt;g)</td>
<td>Exon 7 skipped from transcript, frame shift, dystrophin synthesis aborted prematurely</td>
<td>Severely affected, massive muscle degeneration, resembles human DMD best compared with other models</td>
<td>Chimeroplasts, adenovirus-mediated (mini-)dystrophin/utrophin transfer</td>
</tr>
<tr>
<td>retriever dogs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HFMD</td>
<td>Deletion of Dp427m and Dp427p promoter regions</td>
<td>No muscle dystrophin expression</td>
<td>Large areas of muscle-fibre degeneration and regeneration, mononuclear infiltraton, hypercontracted fibres</td>
<td>None</td>
</tr>
</tbody>
</table>

Note that further mouse mutants (mdx<sup>2–500</sup>) that have been generated by 5 N-ethyl-N-nitrosourea (ENU) mutagenesis are not often used for therapeutic studies. CXMD, canine x-linked muscular dystrophy; DMD, Duchenne muscular dystrophy; GRMD, golden retriever muscular dystrophy; HFMD, hypertrophic feline muscular dystrophy.
Gutless vectors for dystrophin or urotrophin transfer – gutless Ad or AAV vectors

Mdx mice – a model of muscular dystrophy
rAAV6-microdystrophin preserves muscle function and extends lifespan in severely dystrophic mice

Paul Gregorevic¹, James M Allen¹,², Elina Minami³, Michael J Blankinship¹, Miki Haraguchi¹, Leonard Meuse¹, Eric Finn¹, Marvin E Adams⁴, Stanley C Froehner⁴, Charles E Murry³ & Jeffrey S Chamberlain¹,²,⁵

**Double KO – dystrophin & utrophin**
80% mortality at 15 weeks of age

After i.v. delivery of AAV6-dystrophin, the gene was expressed for at least one year
Repairing of mutation in gene therapy

C Trans-splicing

Pre-mRNA

Donor point

Branch

Hybridization domain

Mutation in exon C

3’

Normal exon C

3’

Trans-splicing

Trans-splicer pre-mRNA

Normal mRNA

A B C
Exon skipping as a way to improve conditions in DMD

(a) Deletion exons 45–54

- Out-of-frame dystrophin transcript missing exons 45–54
- Truncated dystrophin
- DMD

(b) 44AON1

- In-frame dystrophin transcript missing exons 44–54
- BMD-type dystrophin
- BMD-like phenotype
Local Dystrophin Restoration with Antisense Oligonucleotide PRO051

BACKGROUND
Duchenne’s muscular dystrophy is associated with severe, progressive muscle weakness and typically leads to death between the ages of 20 and 35 years. By inducing specific exon skipping during messenger RNA (mRNA) splicing, antisense compounds were recently shown to correct the open reading frame of the DMD gene and thus to restore dystrophin expression in vitro and in animal models in vivo. We explored the safety, adverse-event profile, and local dystrophin-restoring effect of a single, intramuscular dose of an antisense oligonucleotide, PRO051, in patients with this disease.

METHODS
Four patients, who were selected on the basis of their mutational status, muscle condition, and positive exon-skipping response to PRO051 in vitro, received a dose of 0.8 mg of PRO051 injected into the tibialis anterior muscle. A biopsy was performed 28 days later. Safety measures, composition of mRNA, and dystrophin expression were assessed.

RESULTS
PRO051 injection was not associated with clinically apparent adverse events. Each patient showed specific skipping of exon 51 and sarcolemmal dystrophin in 64 to 97% of myofibers. The amount of dystrophin in total protein extracts ranged from 3 to 12% of that found in the control specimen and from 17 to 35% of that of the control specimen in the quantitative ratio of dystrophin to laminin α2.

CONCLUSIONS
Intramuscular injection of antisense oligonucleotide PRO051 induced dystrophin synthesis in four patients with Duchenne’s muscular dystrophy who had suitable mutations, suggesting that further studies might be feasible.
Local restoration of dystrophin expression with the morpholino oligomer AVI-4658 in Duchenne muscular dystrophy: a single-blind, placebo-controlled, dose-escalation, proof-of-concept study

Maria Kinali, Virginia Arechavala-Gomez, Lucy Feng, Sebahattin Cirak, David Hunt, Carl Adkin, Michela Guglieri, Emma Ashton, Stephen Abbs, Petros Nihoyannopoulos, Maria Elena Garralda, Mary Rutherford, Caroline Mcculley, Linda Popplewell, Ian R Graham, George Dickson, Matthew JA Wood, Dominic J Wells, Steve D Wilton, Ryszard Kole, Volker Straub, Kate Bushby, Caroline Sewry, Jennifer E Morgan, and Francesco Muntoni

aThe Dubowitz Neuromuscular Centre, University College London Institute of Child Health London, UK.
Background—Mutations that disrupt the open reading frame and prevent full translation of DMD, the gene that encodes dystrophin, underlie the fatal X-linked disease Duchenne muscular dystrophy. Oligonucleotides targeted to splicing elements (splice switching oligonucleotides) in DMD pre-mRNA can lead to exon skipping, restoration of the open reading frame, and the production of functional dystrophin in vitro and in vivo, which could benefit patients with this disorder.

Methods—We did a single-blind, placebo-controlled, dose-escalation study in patients with DMD recruited nationally, to assess the safety and biochemical efficacy of an intramuscular morpholino splice-switching oligonucleotide (AVI-4658) that skips exon 51 in dystrophin mRNA. Seven patients with Duchenne muscular dystrophy with deletions in the open reading frame of DMD that are responsive to exon 51 skipping were selected on the basis of the preservation of their extensor digitorum brevis (EDB) muscle seen on MRI and the response of cultured fibroblasts from a skin biopsy to AVI-4658. AVI-4658 was injected into the EDB muscle; the contralateral muscle received saline. Muscles were biopsied between 3 and 4 weeks after injection. The primary endpoint was the safety of AVI-4658 and the secondary endpoint was its biochemical efficacy. This trial is registered, number NCT00159250.

Findings—Two patients received 0·09 mg AVI-4658 in 900 µL (0·9%) saline and five patients received 0·9 mg AVI-4658 in 900 µL saline. No adverse events related to AVI-4658 administration were reported. Intramuscular injection of the higher-dose of AVI-4658 resulted in increased dystrophin expression in all treated EDB muscles, although the results of the immunostaining of EDB-treated muscle for dystrophin were not uniform. In the areas of the immunostained sections that were adjacent to the needle track through which AVI-4658 was given, 44–79% of myofibres had increased expression of dystrophin. In randomly chosen sections of treated EDB muscles, the mean intensity of dystrophin staining ranged from 22% to 32% of the mean intensity of dystrophin in healthy control muscles (mean 26·4%), and the mean intensity was 17% (range 11–21%) greater than the intensity in the contralateral saline-treated muscle (one-sample paired t test p=0·002). In the dystrophin-positive fibres, the intensity of dystrophin staining was up to 42% of that in healthy muscle. We showed expression of dystrophin at the expected molecular weight in the AVI-4658-treated muscle by immunoblot.

Interpretation—Intramuscular AVI-4658 was safe and induced the expression of dystrophin locally within treated muscles. This proof-of-concept study has led to an ongoing systemic clinical trial of AVI-4658 in patients with DMD.
Figure 1: Antisense-mediated exon skipping to reframe DMD transcripts

Patients with Duchenne muscular dystrophy have mutations in DMD, the gene that encodes dystrophin. The mutations disrupt the open reading frame of dystrophin (in this example, exons 45–50 are deleted). Consequently, protein translation stops prematurely, resulting in a non-functional protein. By use of antisense oligonucleotides that target a specific exon in which there is a mutation that truncates the expression of dystrophin (exon 51 in this example), the reading frame can be restored. This enables the production of an internally deleted but partially functional dystrophin. AON = antisense oligonucleotide.
Figure 1: Deletions and predicted results of exon skipping in the patients who were studied
(A) Pre-mRNA transcripts and dystrophin protein products from full length DMD, in patients with Duchenne muscular dystrophy, and predicted protein sequences after exon skipping. (I) The normal dystrophin gene produces the full length dystrophin product. (II) Patients 1 and 2 had a deletion in exon 50 that disrupts the open reading frame, leading to a truncated and unstable dystrophin. (III) Skipping of exon 51 restores the reading frame, producing a truncated but functional dystrophin that lacks exons 50 and 51. (IV) Patient 7 is missing exons 49 and 50. (V) Patients 3 and 4 are missing exons 48-50. (VI) Patients 5 and 6 are missing exons 45-50. All the truncated dystrophins produced after skipping of exon 51 are missing the hinge 3 region and some of the rod domain but have been associated with the milder BMD phenotype.515 (B) Structure of the phosphorodiamidate morpholino modification of the antisense oligomer.
Figure 3.
Dystrophin expression in patients treated with high-dose AVI-4658

![Graph showing relative intensity of dystrophin expression in untreated and treated patients. The graph compares saline and AVI-4658 treatment groups.](image)
Cell therapy in DMD

Azor
4 dogs received autologous or heterologous mesoangioblasts transduced \textit{in vitro} with lentiviral vector encoding microdystrophin
Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs

Maurilio Sampaolesi¹,²*, Stephane Blot³*, Giuseppe D’Antona², Nicolas Granger³, Rossana Tonlorenzi¹, Anna Innocenzi¹, Paolo Mognol⁴, Jean-Laurent Thibaud³, Beatriz G. Galvez¹, Ines Barthélémy³, Laura Perani¹, Sara Mantero⁴, Maria Gutttinger⁵, Orietta Pansarasa², Chiara Rinaldi², M. Gabriella Cusella De Angelis², Yvan Torrente⁶, Claudio Bordignon¹, Roberto Bottinelli² & Giulio Cossu¹,⁵,⁷

Figure 1 | Stem-cell treatment of dystrophic muscle in dogs¹. These pictures are stained biopsies of (a) untreated dystrophic skeletal muscle and (b) muscle after treatment. The latter was both structurally more ordered and functioned better. Scale bar, 200 µm.

Nature, published on line Nov 15, 2006
REVIEW

Gene therapy progress and prospects: Duchenne muscular dystrophy

K Foster, H Foster and JG Dickson
Centre for Biomedical Sciences, School of Biological Sciences, Royal Holloway University of London, Egham, Surrey, UK

In brief

Progress

- The first dystrophin gene therapy phase I clinical trial for Duchenne/Becker muscular dystrophy is completed.
- Optimized vector cassette design leads to enhanced transgene expression.
- Optimized protocols for local administration of plasmid DNA vectors have achieved therapeutic levels of expression in a single muscle.
- Multiple muscle groups can be transfected efficiently with plasmid DNA by optimized intravenous and intra-arterial approaches.
- Novel adeno-associated virus (AAV) serotypes lead to very efficient systemic transduction of skeletal muscle with truncated microdystrophin cDNAs.
- Expanding the packing capacity of AAV allows delivery of larger dystrophin molecules to dystrophic muscle.
- Improved chemistries of antisense oligonucleotides improve efficiency of exon skipping.
- Alternative chemistries allow efficient systemic delivery of antisense oligonucleotides.
- Two phase I clinical trials for exon skipping in Duchenne muscular dystrophy (DMD) are in progress.
- Development of adjuvant therapies to augment muscle growth.

Prospects

- Regional delivery protocols for plasmid DNA will improve further, with clinical trial planned.
- Enhanced muscle tropism of AAV vectors will lead to lower viral load required for therapeutic levels of dystrophin expression.
- Trans-splicing/overlapping AAV vectors for the delivery of full-length dystrophin will be developed.
- Success of initial clinical trial to be repeated: AAV and antisense – exon skipping trials are ongoing.
- Adjuvant therapies to increase muscle mass to be used in conjunction with dystrophin therapies.
- Effective gene therapy for DMD is on the horizon.
Hope, hypes and cheating

Be aware of dishonest people!

---

<table>
<thead>
<tr>
<th>Company</th>
<th>Location</th>
<th>Conditions</th>
<th>Patients treated</th>
<th>Cost ($)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PATIENTS’ OWN CELLS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Cells4Health</td>
<td>Leuvenheim, the Netherlands</td>
<td>Myocardial infarction, vascular disease, spinal cord injury, stroke</td>
<td>NA</td>
<td>+25,000</td>
<td>Treatment takes place at clinics in Turkey and Azerbaijan</td>
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<tr>
<td>NeuraVita</td>
<td>Moscow, Russia</td>
<td>Neurological diseases and injuries</td>
<td>NA</td>
<td>~20,000</td>
<td></td>
</tr>
<tr>
<td><strong>FETAL CELLS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EmCell</td>
<td>Kiev, Ukraine</td>
<td>More than 50, including neurological disorders, aging, impotence, diabetes, cancer, HIV</td>
<td>Almost 2000 in 13 years</td>
<td>+15,000</td>
<td></td>
</tr>
<tr>
<td>Medra</td>
<td>Malibu, U.S.A.</td>
<td>More than 20, including neurological disorders, depression, autism, sickle cell anemia</td>
<td>More than 1000</td>
<td>NA</td>
<td>Procedures performed in Dominican Republic</td>
</tr>
<tr>
<td>Beijing Xishan Institute for Neuregeneration and Functional Recovery</td>
<td>Beijing, China</td>
<td>Spinal cord injury, ALS, and other neurological conditions</td>
<td>More than 1000 since 2001</td>
<td>20,000</td>
<td>Thousands more on waiting list</td>
</tr>
<tr>
<td>Institute for Regenerative Medicine</td>
<td>St. John, Barbados</td>
<td>More than 40</td>
<td>More than 50 since 2004</td>
<td>25,000</td>
<td>Treatment based on research in the former Soviet Union</td>
</tr>
<tr>
<td><strong>UMBILICAL CORD BLOOD CELLS</strong></td>
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<td></td>
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</tr>
<tr>
<td>Biomark</td>
<td>Atlanta, U.S.A.</td>
<td>ALS, Parkinson’s, muscular dystrophy, and others</td>
<td>At least 23 in 2003</td>
<td>10,000 to 32,000</td>
<td>No longer operative; founders wanted by FBI</td>
</tr>
<tr>
<td>Advanced Cell Therapeutics</td>
<td>Zurich, Switzerland</td>
<td>More than 80</td>
<td>More than 600 in 4 years</td>
<td>25,000</td>
<td>Treatments performed at 12 collaborating clinics worldwide</td>
</tr>
<tr>
<td>Preventive Medicine Center</td>
<td>Rotterdam, the Netherlands</td>
<td>More than 50, including neurological, digestive, and psychological disorders and aging</td>
<td>More than 200 in 2 years</td>
<td>23,000</td>
<td>Also treats patients referred by Advanced Cell Therapeutics</td>
</tr>
</tbody>
</table>

*Source: Company and clinic web sites, information packages, interviews, ALSDF, Biomark Criminal Indictment. NA = Information not available.*
Gene therapy of Leber’s congenital amaurosis
From mouse to human: gene therapy of Leber congenital amaurosis

Leber congenital amaurosis (LCA) is an early-onset and severe inherited retinal degeneration in which rods and cones are non-functional at birth and can be lost within the first years of life [59,60]. LCA is mainly inherited as a recessive trait, which has an estimated prevalence of 1:50,000–100,000. LCA-associated mutations have been reported in 12 genes to date (http://www.sph.uth.tmc.edu/RetNet/), accounting for ~50% of LCA cases. Successful gene therapy has been described in rodents and large-animal models of LCA. Effective gene replacement using rAAV vectors has been reported in rodent models of LCA in which the disease arises owing to deficiency of Rpgrip (encoding the X-linked retinitis pigmentosa GTPase regulator-interacting protein 1) [61] and Lrat (lechthin-retinol acyltransferase) [62] expressed in PRs and RPE, respectively. To date, the most successful example of gene therapy for an ocular disease is gene delivery for LCA arising from mutations in the RPE65 gene, which accounts for 10% of LCA cases. RPE65 encodes the 65-kDa RPE-specific isomerase essential for recycling 11-cis-retinal, the chromophore of rod and cone opsins [60]. rAAV-vector-mediated
<table>
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<th>Disease</th>
<th>Vector</th>
<th>Transgene</th>
<th>Clinical centers</th>
<th>Phase</th>
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<td>RPE65 gene</td>
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Age-dependent effects of RPE65 gene therapy for Leber's congenital amaurosis: a phase 1 dose-escalation trial


Summary

Background Gene therapy has the potential to reverse disease or prevent further deterioration of vision in patients with incurable inherited retinal degeneration. We therefore did a phase 1 trial to assess the effect of gene therapy on retinal and visual function in children and adults with Leber's congenital amaurosis.

Methods We assessed the retinal and visual function in 12 patients (aged 8–44 years) with RPE65-associated Leber's congenital amaurosis given one subretinal injection of adeno-associated virus (AAV) containing a gene encoding a protein needed for the isomerohydrolase activity of the retinal pigment epithelium (AAV2-hRPE65v2) in the worst eye at low (1·5×10^{10} vector genomes), medium (4·8×10^{10} vector genomes), or high dose (1·5×10^{11} vector genomes) for up to 2 years.

Findings AAV2-hRPE65v2 was well tolerated and all patients showed sustained improvement in subjective and objective measurements of vision (ie, dark adaptometry, pupillometry, electroretinography, nystagmus, and ambulatory behaviour). Patients had at least a 2 log unit increase in pupillary light responses, and an 8-year-old child had nearly the same level of light sensitivity as that in age-matched normal-sighted individuals. The greatest improvement was noted in children, all of whom gained ambulatory vision. The study is registered with ClinicalTrials.gov, number NCT00516477.

Interpretation The safety, extent, and stability of improvement in vision in all patients support the use of AAV-mediated gene therapy for treatment of inherited retinal diseases, with early intervention resulting in the best potential gain.
Summary – gene therapy of monogenic diseases