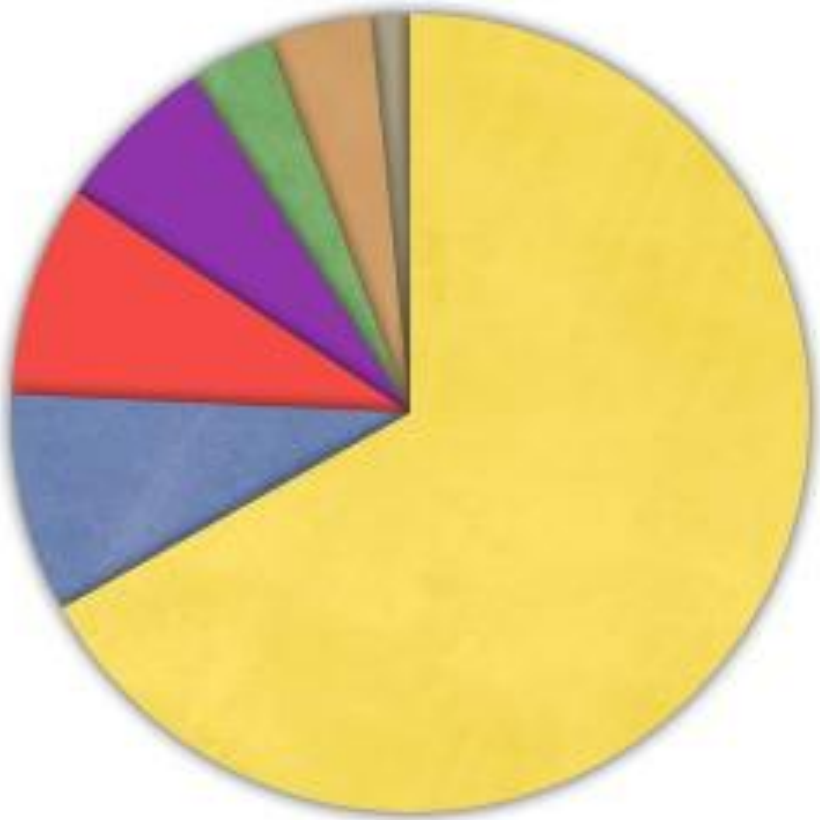
Lecture 10

Gene therapy of monogenic diseases

4th January 2009

2006

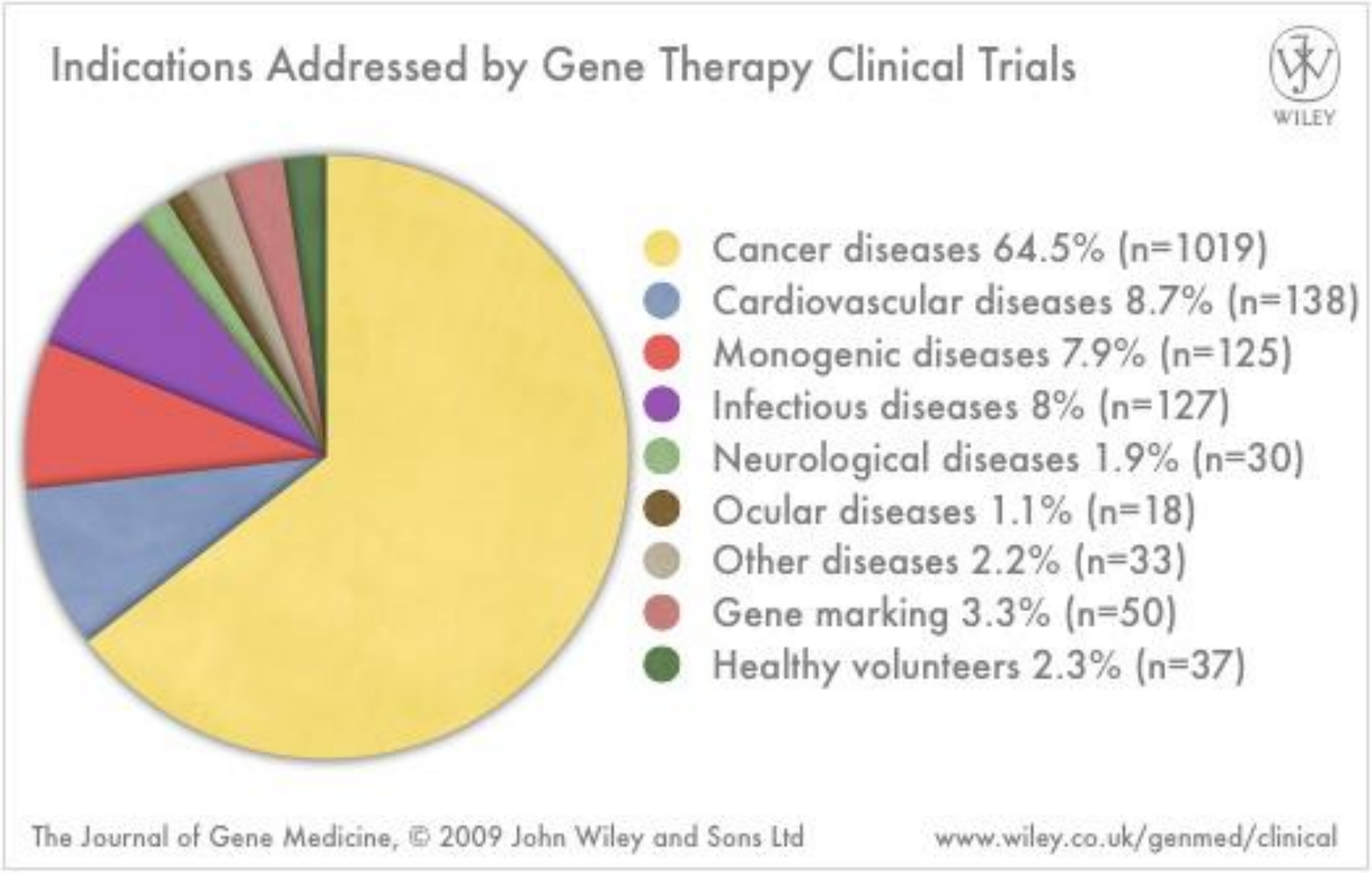
Indications Addressed by Gene Therapy Clinical Trials



- 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)

www.wiley.co.uk/genmed/clinical

2009


Indications Addressed by Gene Therapy Clinical Trials
WILEY
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)
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www.wiley.co.uk/genmed/clinical

Phases of Gene Therapy Clinical Trials

www.wiley.co.uk/genmed/clinical

Phases of clinical trials

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

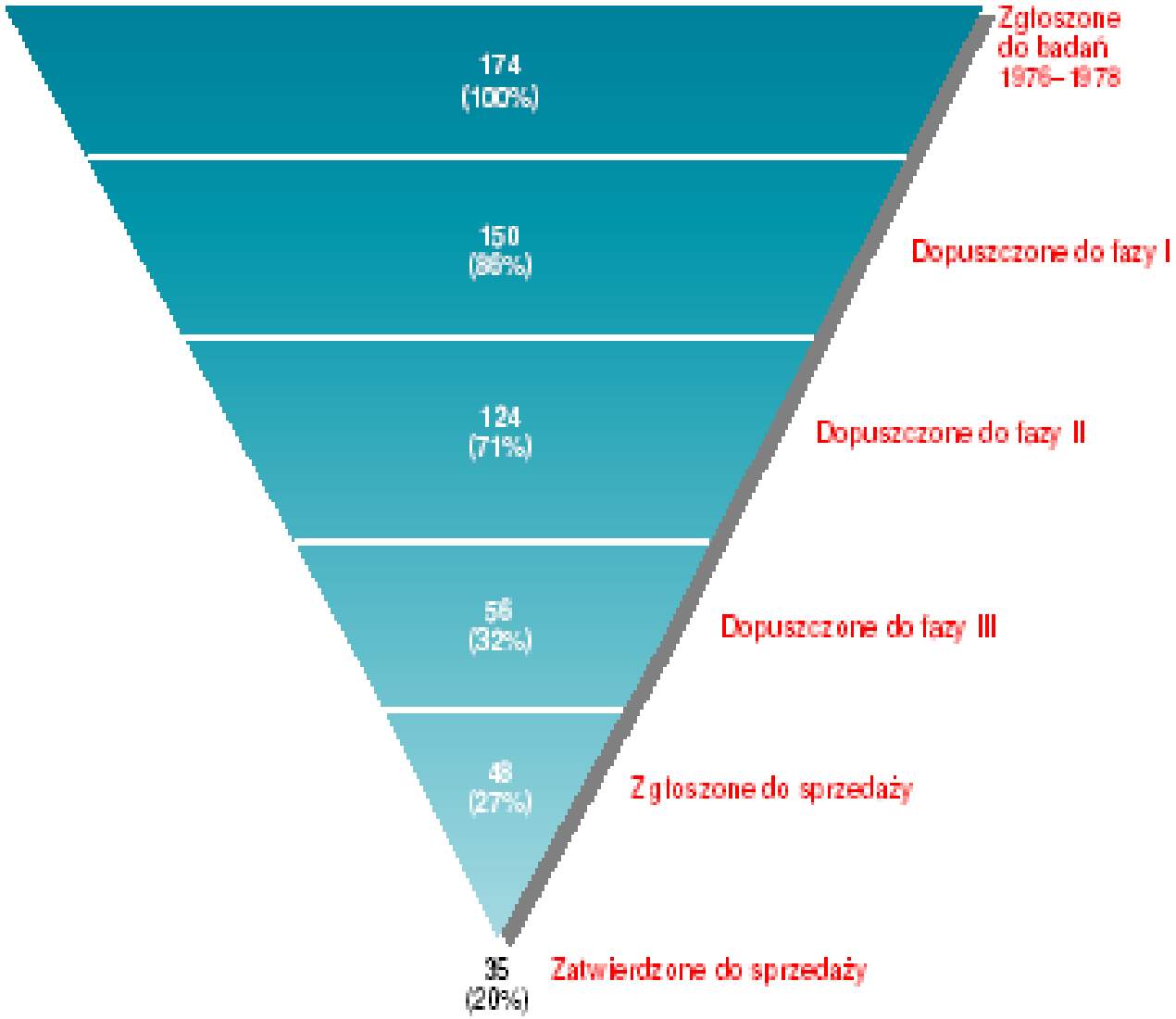
Some Phase II and most Phase III drug trials

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



HEDI NOLAND; Źródło: „SUCCESS RATES FOR NEW DRUGS ENTERING CLINICAL TESTING IN THE UNITED STATES"
A. DiMASI in Clinical Pharmacology and Therapeutics; VII/1995.

Gene therapy of monogenic diseases

Genetic diseases - incidence, mutated genes and detection rate

Disorder	Incidence	Gene	Mutation detection rate
Monogenic			
Cystic fibrosis	1:4000	CFTR	98%
Duchenne muscular dystrophy	1:4000	DMD	~90%
Fragile X syndrome	1:4000	FMR	100%
Huntigton disease	1:5000-10 000	HD	100%
Hemophilia A	1: 10 000	F8C	~90%
Phenyloketonuria	1: 10 000	PAH	99%
Polycystic kidney disease	1:1500	PKD1, PKD2	~15%
Inherited cancer			
Breast-ovarian cancer	1:4000	BCRA1 (80%)	50-65%
		BCRA2 (20%)	35%
Li-Fraumeni syndrome		p53	50%
Ataxia-telangiectasia		ATM	70%
Familial polyposis coli	1:4000	APC	87%
Hereditary non-polyposis coli	1:2000	MLH1 (30%)	33%
		MLH2 (60%)	12%
Cardiovascular disorders			
Familial hypercholesterolemia	1:500	LDLR	60%
Hyperlipidemia		APOE	10%

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 1 Classification of severe combined immunodeficiencies[a]

Disease	Relative frequency[b] %		Affected cells	Gene product
Reticular dysgenesis	4	AR	T, B, NK, myeloid cells, platelets	Unknown
Alymphocytosis	15	AR	T, B	RAG-1, RAG-2
	15	AR	T, B	Artemis
Absence of T lymphocytes and NK	40	X-L	T, NK	γc chain
	10	AR	T, NK	JAK-3
Absence of T lymphocytes	5	AR	T	IL-7 Rα chain
	$\leq$1	AR	T	CD45
	$\leq$1	AR	T	CD3δ
ADA deficiency	10–15	AR	T, B, NK	ADA

[a]Abbreviations: ADA, adenosine deaminase; AR, autosomal recessive; NK, natural killer cells; X-L, X-linked recessive.
[b]Frequency in our experience.

Cavazzana-Calvo M et al., Ann Rev. Med. 2005

Table 1 Clinical trials of gene therapy for childhood immunological diseases[a]

	No. of trials	*No. of subjects*	*Clinical benefit*	*SAE*
ADA (−) SCID (1990–1996)	6	19	0	0
ADA (−) SCID (1999–present)	4	20	19	1
XSCID	3	26	16	4
Jak3 SCID	1	1	—	0
CGD	4	14	4	1
LAD (CD18)	1	1	?[b]	0
Wiskott–Aldrich syndrome	1	2	?[b]	0

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.

[a]Based on presentations at the 10th Annual Meeting of the American Society of Gene Therapy, Seattle, WA, June 2007.

[b]? = outcome not reported.

Kohn, Bon Marrow Transplant 2008

Summary

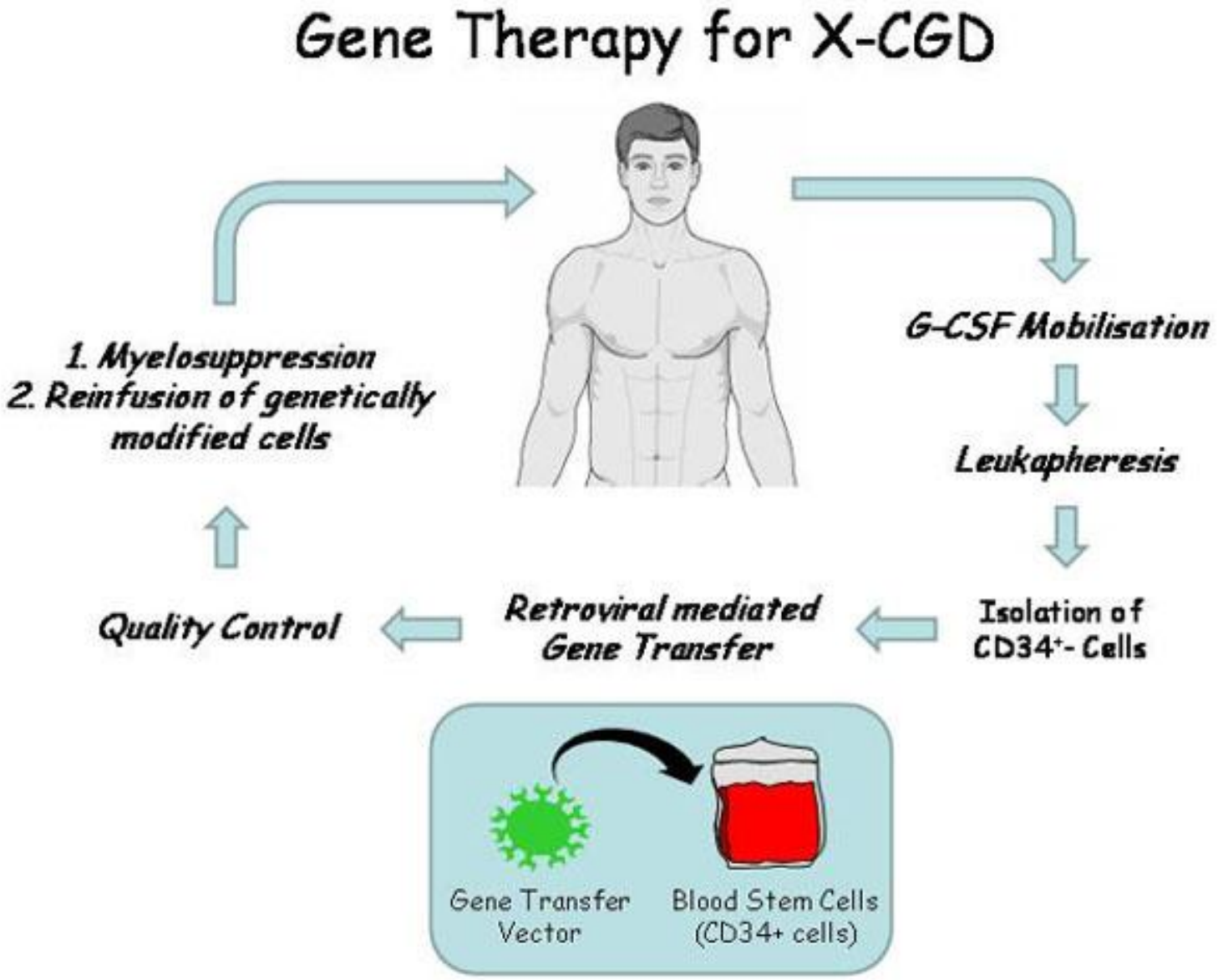
The proof of concept of gene therapy has been demonstrated in patients with SCID

Gene therapy can cure diseases; side-effects happens

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.



Dr Manuel Grez's website

Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of *MDS1-EVI1*, *PRDM16* or *SETBP1*

Marion G Ott[1,16], Manfred Schmidt[2–4,16], Kerstin Schwarzwaelder[3–5,16], Stefan Stein[6,16], Ulrich Siler[7,16], Ulrike Koehl[8], Hanno Glimm[2,3], Klaus Kühlcke[9], Andrea Schilz[9], Hana Kunkel[6], Sonja Naundorf[9], Andrea Brinkmann[8], Annette Deichmann[3,4], Marlene Fischer[2,3,5], Claudia Ball[3–5], Ingo Pilz[3,5], Cynthia Dunbar[10], Yang Du[11], Nancy A Jenkins[11], Neal G Copeland[11], Ursula Lüthi[12], Moustapha Hassan[13], Adrian J Thrasher[14], Dieter Hoelzer[1], Christof von Kalle[2–4,15,16], Reinhard Seger[7,16] & Manuel Grez[6,16]

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 $gp91^{phox}$. 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.

Nature Medicine April 2006

Correction of neutrophil bacteriocidal function by overexpression of gp91phox subunit of NADPH oxidase

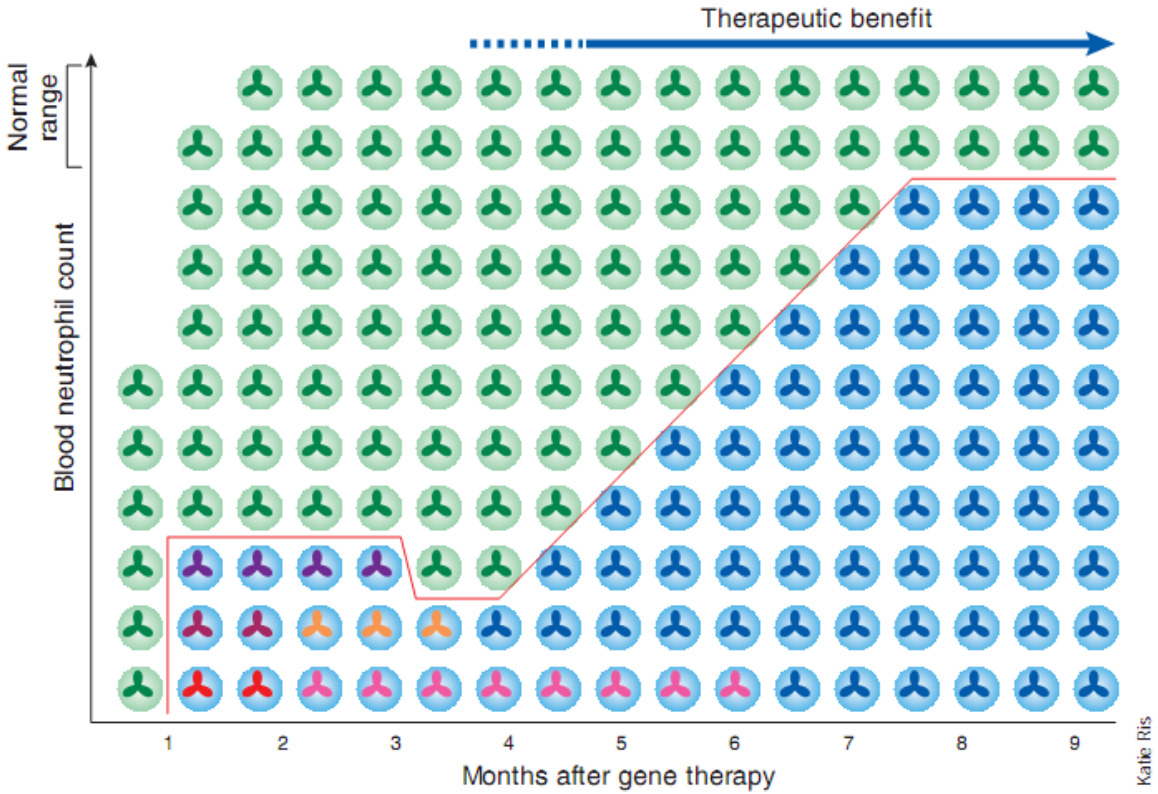


Figure 1 Gene-corrected neutrophils in the blood of an individual with CGD after HSPC gene therapy. Whereas CGD neutrophils lack NADPH activity (green cytosol), the progeny of HSPCs engrafted after gene therapy have reconstituted enzyme activity (blue cytosol) resulting from the presence of a functional 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.

Naldini L, Nature Med. April 2006

Gene therapy for X-linked chronic granulomatous disease

Table 1 Patients developing insertional mutagenesis/uncontrolled clonal expansions

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

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 haematopoïetic 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

Disease targets for gene therapy

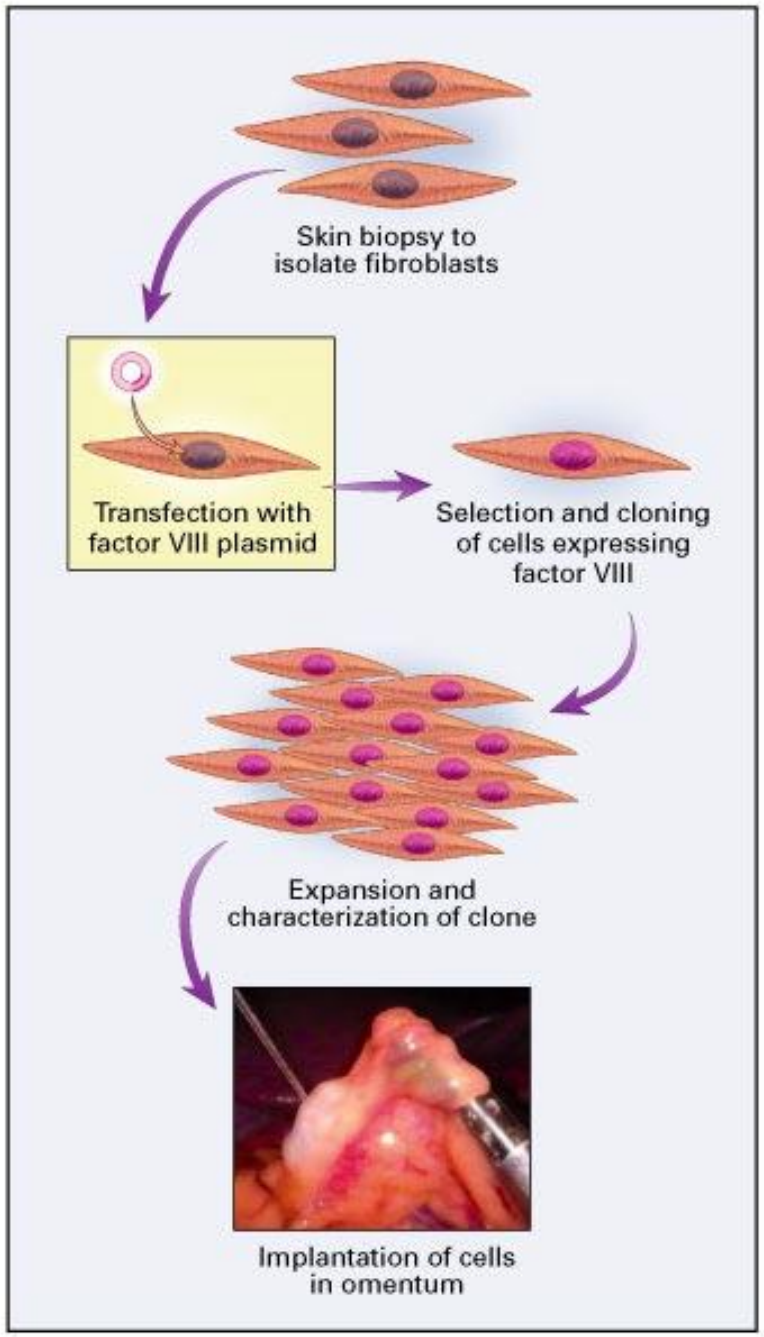
Disease	Gene(s)
Cystic fibrosis	CFTR, α-1-anti-trypsin
SCID	ADA
Gaucher disease	glucocerebrosidase
Hemophilia A	**Factor VIII**
Hemofilia B	**Factor IX**
Familial hypercholesterolemia	LDL-R
Muscular dystrophy	sarcoglycan, dystrophin, utrophin
Ornithine transcarbamylase deficiency	OTC

Hemofilia 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 - factor VIII - 8,8 kb
factor IX - 1,8 kb



Clinical gene therapy for haemophilia A

Steps in human factor VIII gene transfer procedure

Ex vivo – plasmid gene therapy

Roth DA et al., NEJM 2001; 344: 1735

Clinical gene therapy for haemophilia A

TABLE 1. CHARACTERISTICS OF THE SIX PATIENTS.*

CHARACTERISTIC	VALUE
Age (yr)	
Mean	46
Range	20–72
Weight (kg)	
Mean	70
Range	50–91
Pretreatment factor VIII activity <0.8% of normal (no. of patients)	6
Viral exposure (no. of patients)†	
Human immunodeficiency virus	4
Hepatitis A virus	5
Hepatitis B virus	5
Hepatitis C virus	6

*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.*

PATIENT NO.	FACTOR VIII PRODUCTION BY HARVESTED CELLS†	NO. OF CELLS IMPLANTED	TOTAL FACTOR VIII PRODUCTION BY IMPLANTED CELLS‡
	IU/10^6 cells/day		IU/kg/day
1	0.8	100×10^6	1.3
2	4.9	100×10^6	5.4
3	1.9	100×10^6	3.8
4	1.8	400×10^6	10.4
5	1.6	400×10^6	8.4
6	6.7	400×10^6	36.0

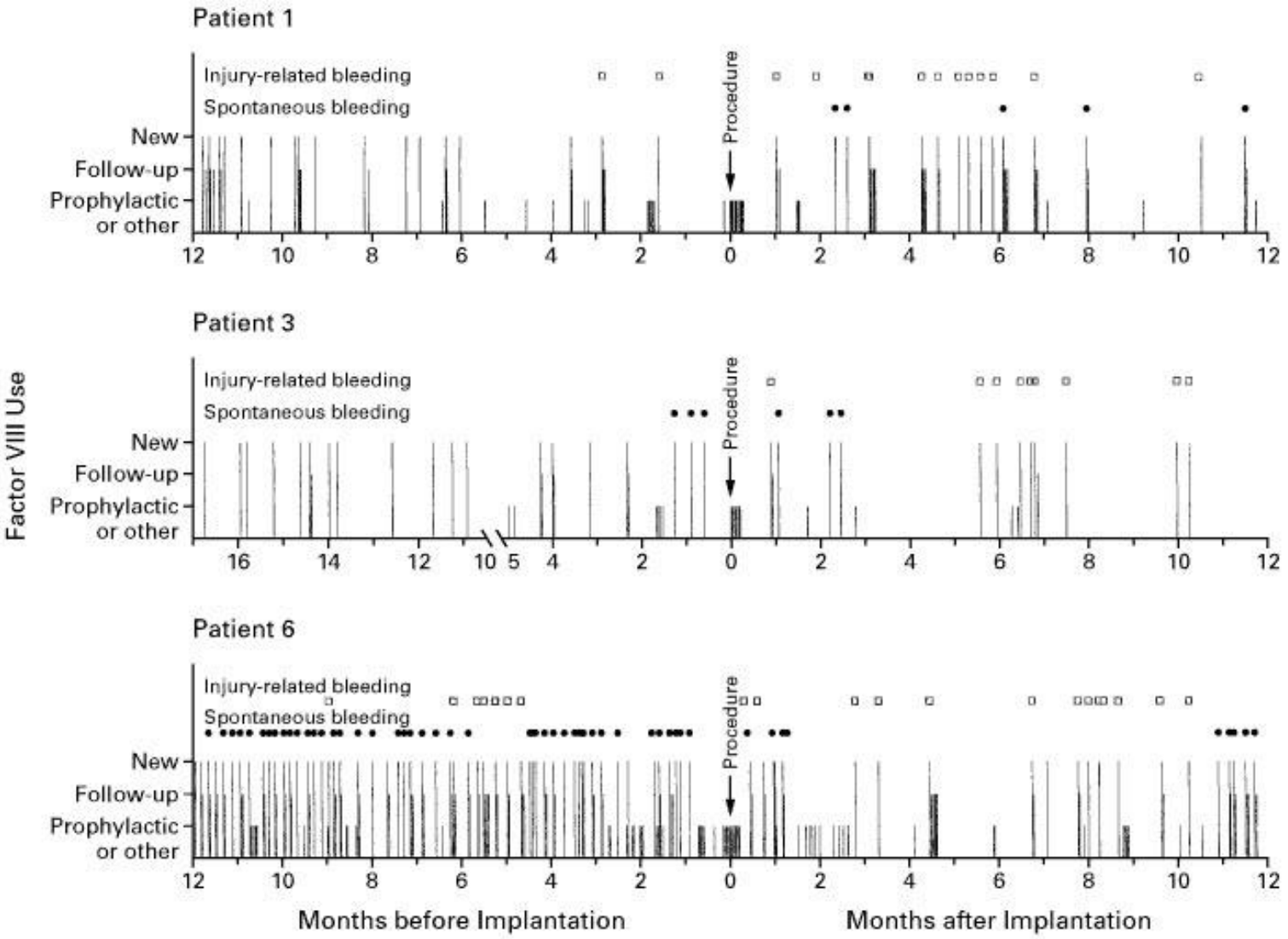
*The conditioned medium of each fibroblast clone was replaced with fresh medium 24 hours before it was assayed for factor VIII expression levels by a human factor VIII enzyme-linked immunosorbent assay.

†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.



Roth DA et al., NEJM 2001; 344: 1735

Retroviral vectors for gene therapy of hemophilia

Blood. 2003 Sep 15;102(6):2038-45. Epub 2003 May 22.

Phase 1 trial of FVIII gene transfer for severe hemophilia A using a retroviral construct administered by peripheral intravenous infusion.

Powell JS, Ragni MV, White GC 2nd, Lusher JM, Hillman-Wiseman C, Moon TE, Cole V, Ramanathan-Girish S, Roehl H, Sajjadi N, Jolly DJ, Hurst D.

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 Jr[1,2,5]

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

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

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

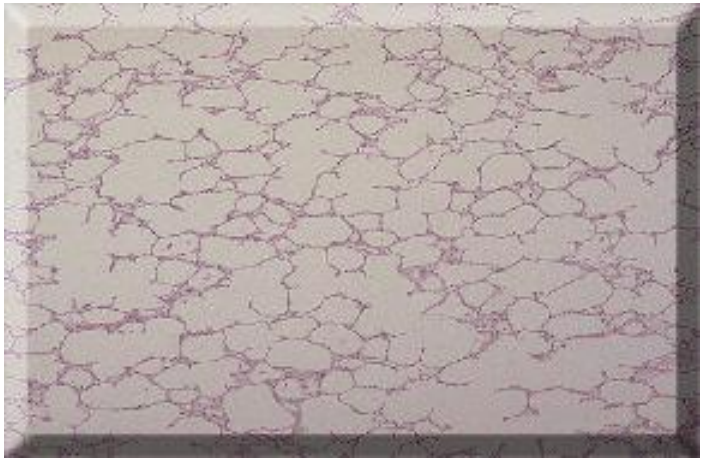
Cystic fibrosis
Mukowiscydoza

- 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

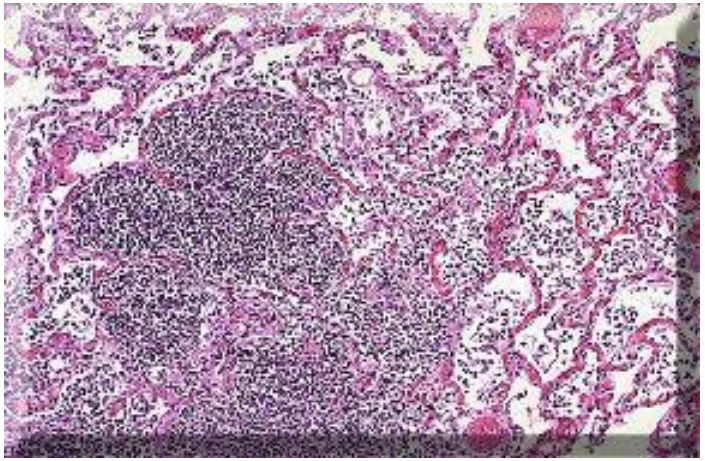
- after asthma, CF is the commonest cause of chronic respiratory distress in childhood; responsible for the majority of deaths

-

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

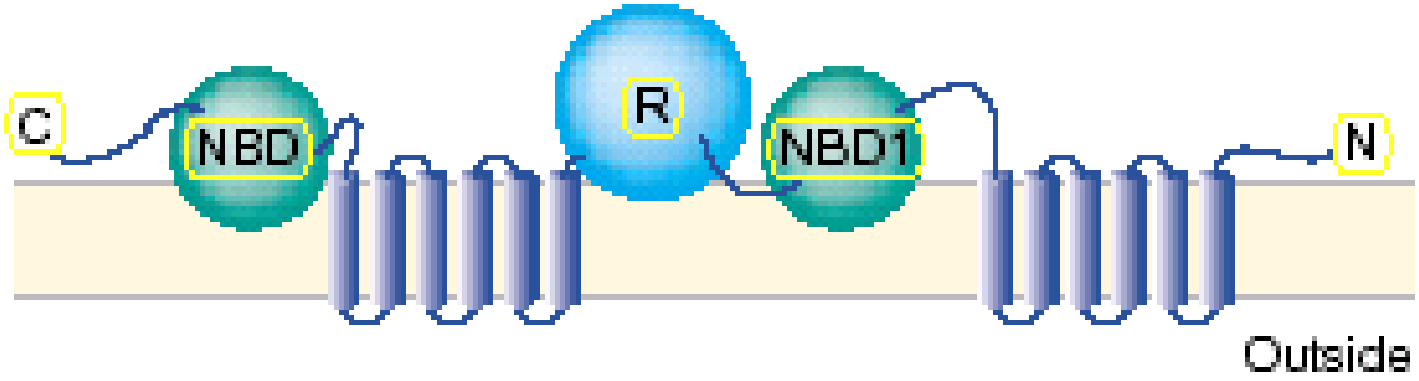
CFTR gene

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.


News & Comment

The Cystic Fibrosis Gene Is Found

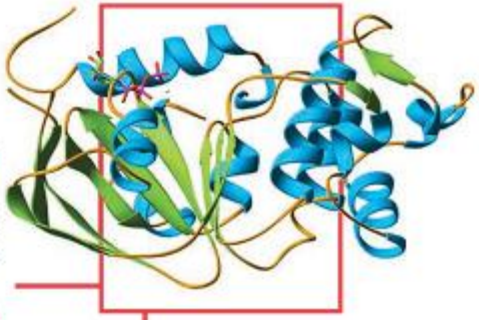
1990: Scientists suggest that protein folding is behind CF.

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.



Science, 19 June 2009

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 TGFß 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:
A gene for type 2 diabetes predisposes children to CF-associated diabetes.

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 unsuccesful
   b) mode of delivery:
      - bronchoscopy
      - aeorosol

Clinical trials of gene therapy in CF

Table 1 Summary of clinical trials of liposome-mediated gene therapy in cystic fibrosis subjects to date

Abbreviation: P. D., potential difference.

Trial/year/reference	Liposome	No. of patients	Target	Route	Efficacy	Side effects
Caplen et al., 1995 [82]	DC-Chol/DOPE	15	Nose	Aerosol	P.D. 20% correction towards normal	None
Zabner et al., 1997 [83]	GL-67™/DOPE	12	Nose	Direct installation	Partial correction nasal P.D. (equivalent to naked DNA)	None
Gill et al., 1997 [84]	DC-Chol/DOPE	12	Nose	Direct installation	Functional evidence in 6/8 treated patients	None
Porteous et al., 1997 [85]	DOTAP	16	Nose	Aerosol	P.D. 20% correction in two patients, no SPQ-positive	None
Alton et al., 1999 [86]	GL-67™/DOPE/DMPE-PEG_{5000}	16	Lungs	Nebulized	Lung P.D. 25% correction towards normal	Mild, flu-like symptoms
Hyde et al., 2000 [87]	DC-Chol/DOPE	12	Nose	Direct installation, repeat dosing	6/10 patients positive after each dose	None
Noone et al., 2000 [80]	EDMPC-Chol	11	Nose	Aerosol	None	None
Ruiz et al., 2001 [88]	GL-67™/DOPE/DMPE-PEG_{5000}	8	Lungs	Nebulized	Vector-specific mRNA in 3/8 patients	Mild, flu-like symptoms

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, *Paraplégie hypertrophique de l'enfance de cause cerébrale*. Because of the intellectual impairment in the affected boys, Duchenne initially thought the condition was cerebral in origin.

Structure of contractile unit – role of dystrophin

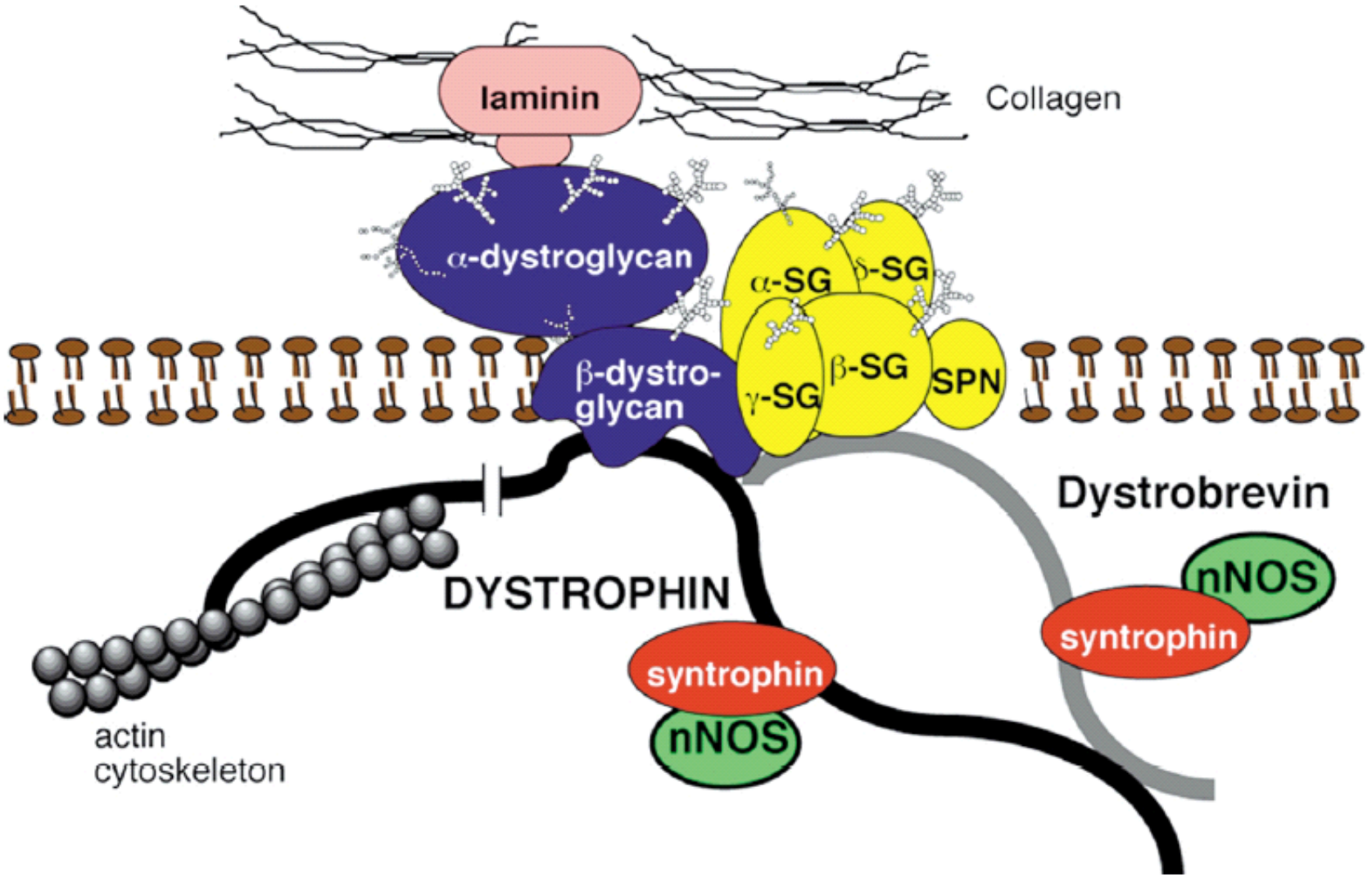


Figure 1. Dystrophin and the dystrophin–glycoprotein complex in muscle. Dystrophin is a cytoskeletal protein that links the γ-actin filaments to the extracellular matrix via the dystroglycan/sarcoglycan complexes. The C-terminal domain of dystrophin binds syntrophin and dystrobrevin, the latter of which 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 that Duchenne

Phase I study of dystrophin plasmid-based gene therapy in Duchenne/Becker muscular dystrophy.

Hum Gene Ther. 2004 Nov;15(11):1065-76

Romero NB, Braun S, Benveniste O, Leturcq F, Hogrel JY, Morris GE, Barois A, Eymard B, Payan C, Ortega V, Boch AL, Lejean L, Thioudellet C, Mourot B, Escot C, Choquel A, Recan D, Kaplan JC, Dickson G, Klatzmann D, Molinier-Frenckel V, Guillet JG, Squiban P, Herson S, Fardeau M.

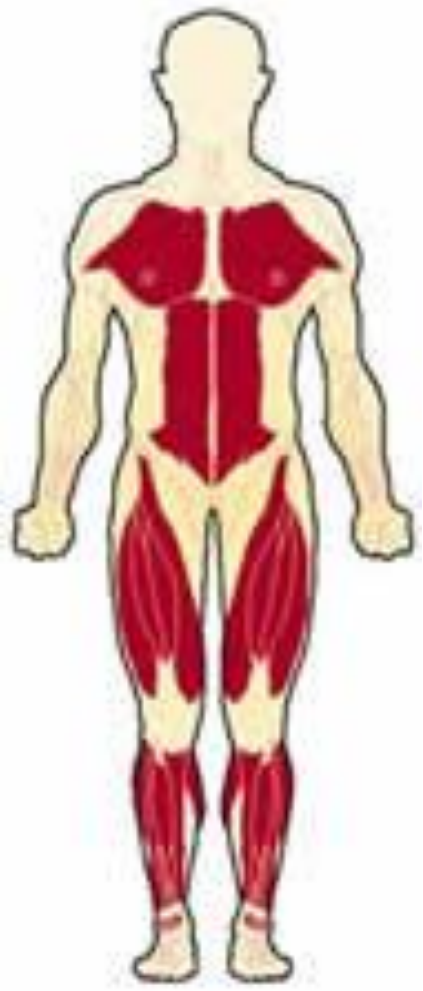
Institut de Myologie, INSERM U582, CHU Pitie-Salpetriere, 75013 Paris, France.

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.

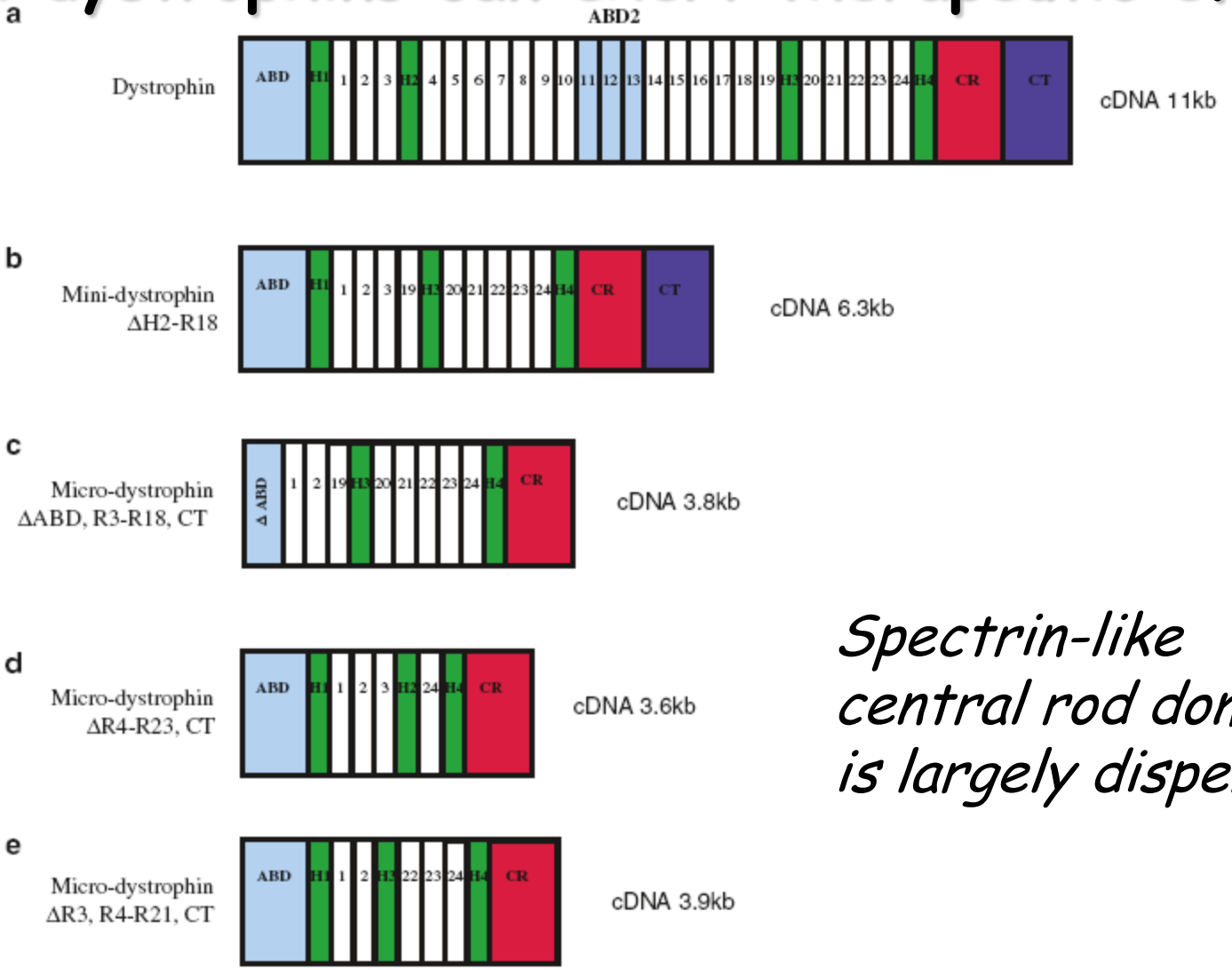
Problems in gene therapy of Duchenne muscular dystrophy

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



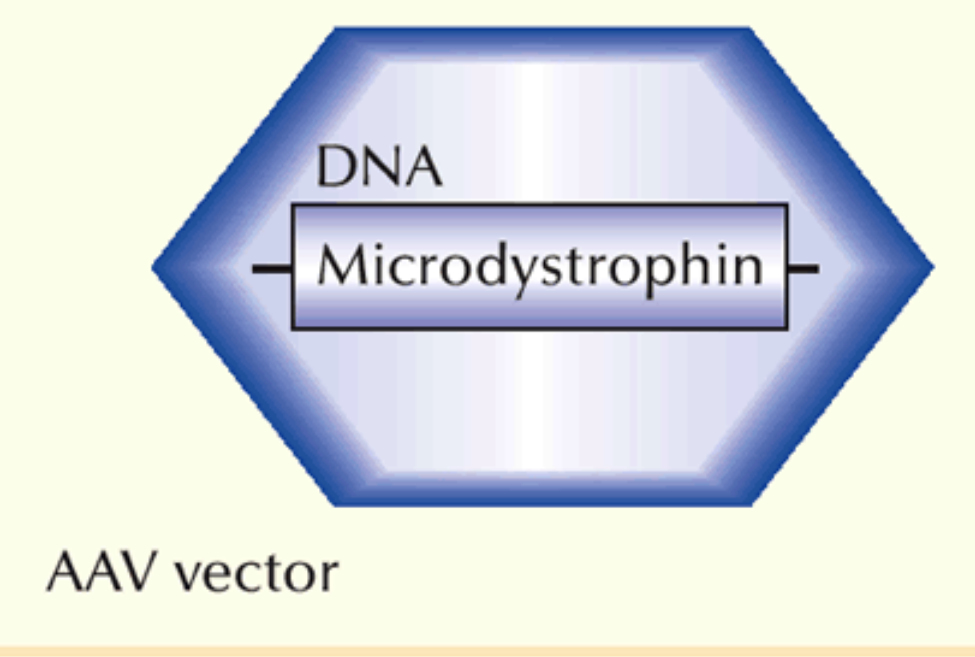
Partial dystrophins can exert therapeutic effect



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.

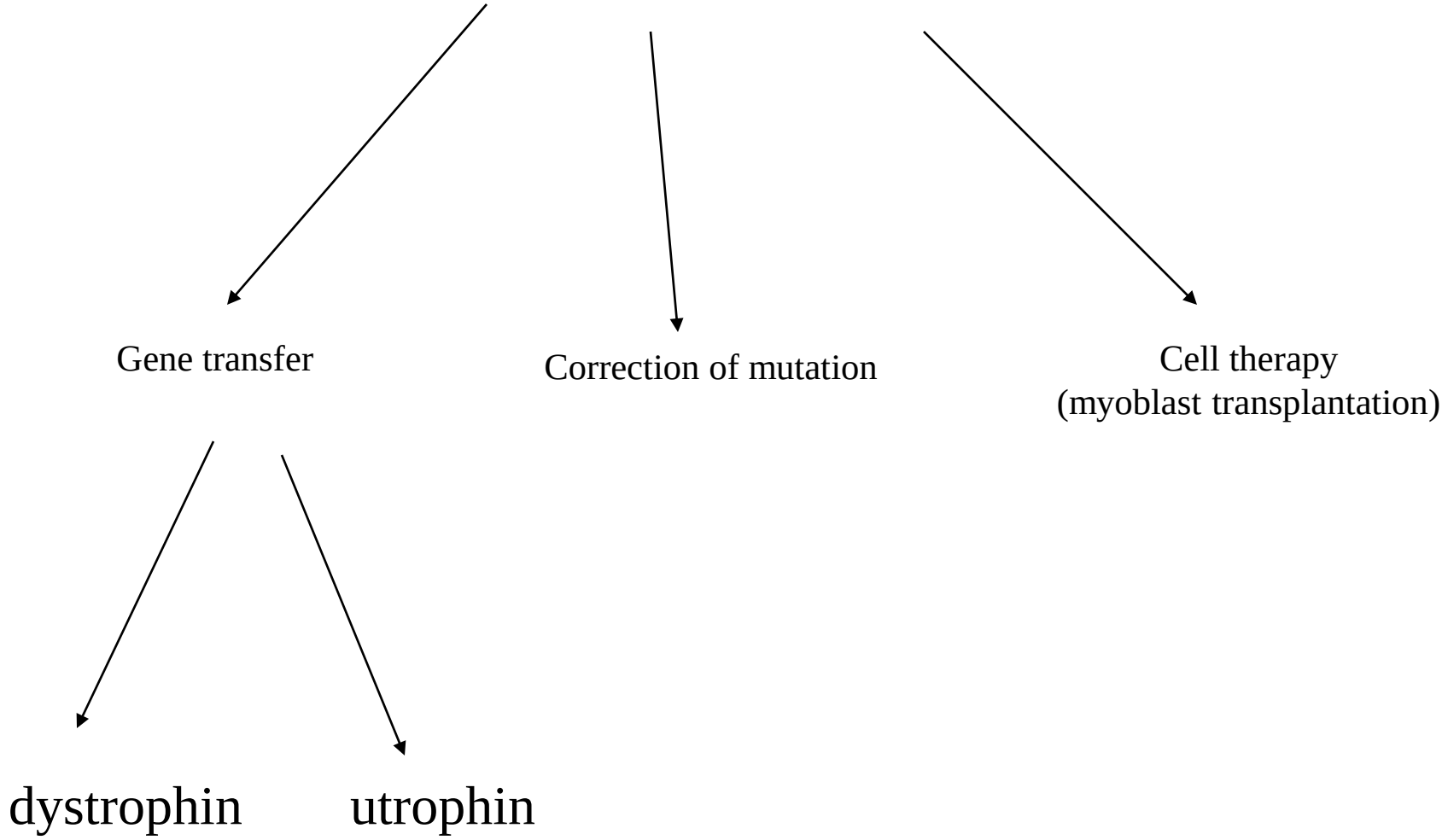
Foster et al., Gene Therapy 2006



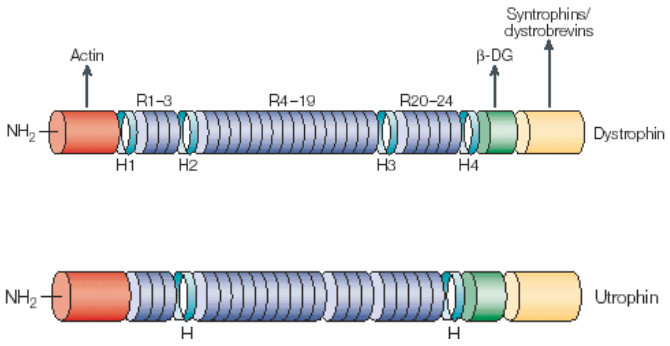
James G Tidball & Melissa J Spencer

Nature Medicine 9, 997 - 998 (2003)

Gene therapy for Duchenne muscular dystrophy



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**

Model	Mutation	Effect	Pathologic/physiologic symptoms	Gene-therapy studies
mdx mouse	Nonsense mutation in exon 23 (3185C>T)	Stop codon introduced, dystrophin synthesis aborted prematurely	Fibre degeneration (particularly from 2–8 weeks), replacement by centrally nucleated regenerating fibres, myopathy less severe later in life, normal lifespan	Used for most strategies owing to its relative experimental simplicity
CXMD/GRMD golden retriever dogs	3′ splice-site point mutation in intron 6 (739–2a>g)	Exon 7 skipped from transcript, frame shift, dystrophin synthesis aborted prematurely	Severely affected, massive muscle degeneration, resembles human DMD best compared with other models	Chimeroplasts, adenovirus-mediated (mini-)dystrophin/ utrophin transfer
HFMD	Deletion of Dp427m and Dp427p promoter regions	No muscle dystrophin expression	Large areas of muscle-fibre degeneration and regeneration, mononuclear infiltraton, hypercontracted fibres	None

Note that further mouse mutants (*mdx*$^{2-5cv}$) that have been generated by 5 *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis are not often used for therapeutic studies[131].
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

2358 Human Molecular Genetics, 2002, Vol. 11, No. 20

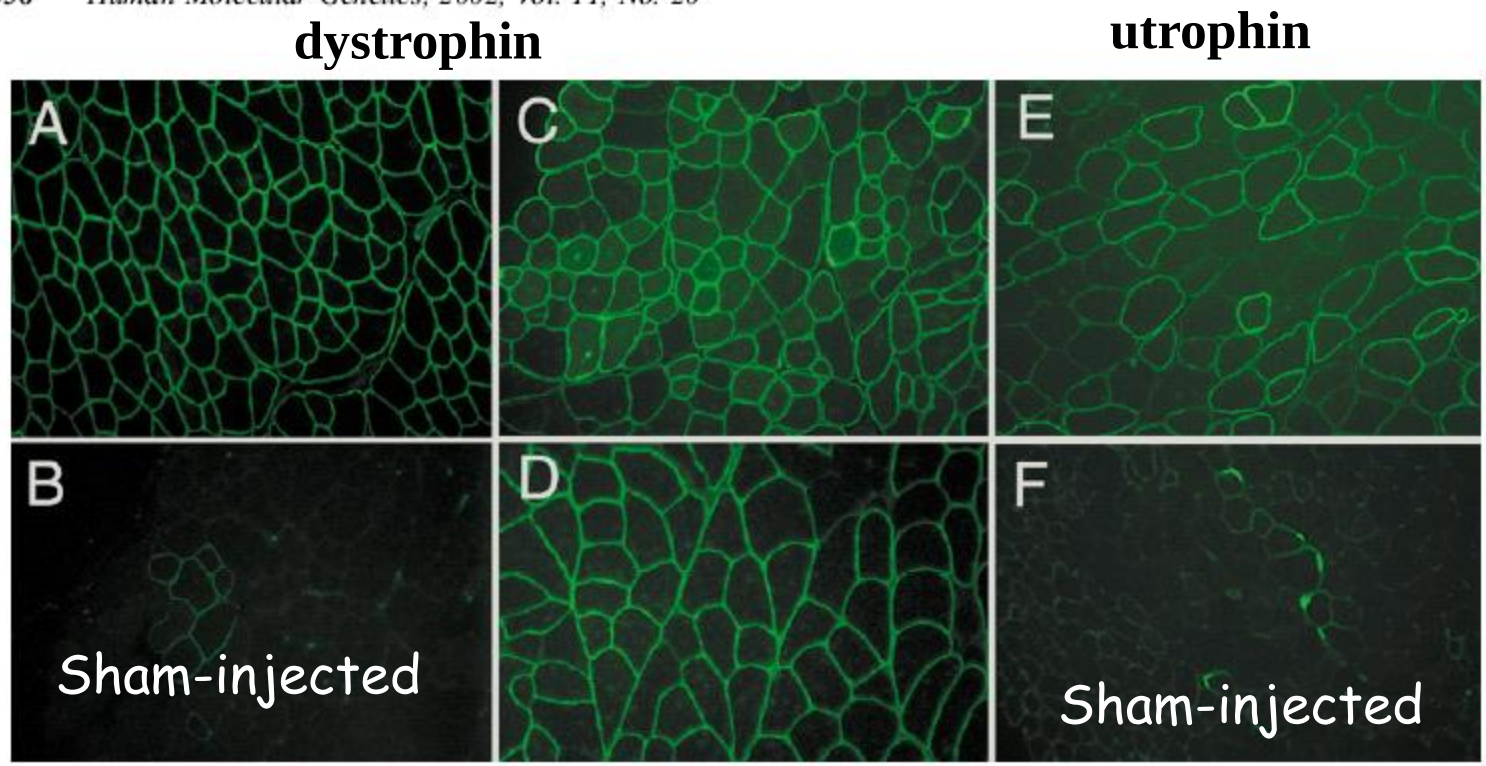


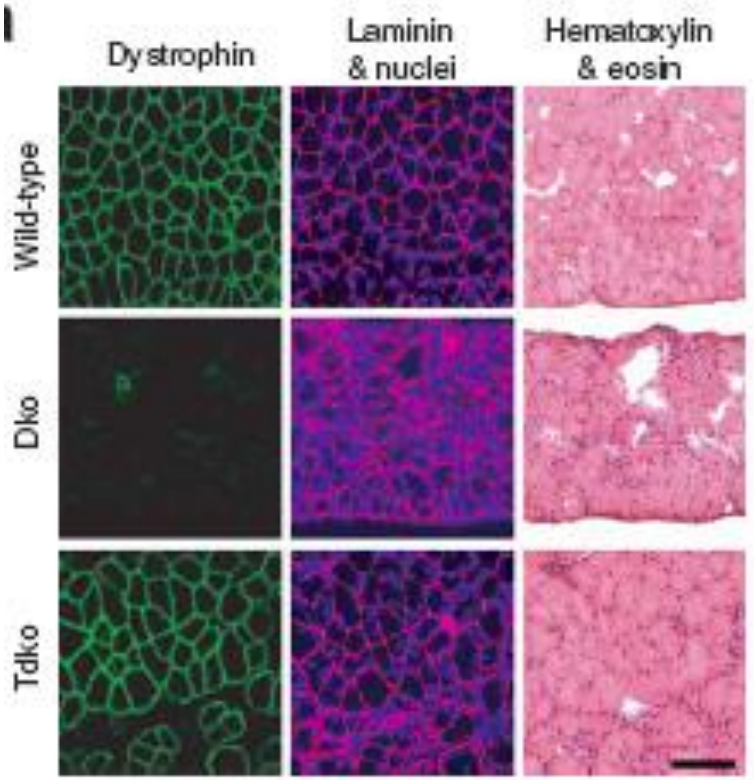
Figure 3. Transduction of adult, immunocompetent *mdx* mouse muscles by dystrophin and utrophin expression vectors. The figure shows cross-sections of C57Bl/10 (**A**) or *mdx* mouse TA muscles that had been (**B** and **F**) sham-injected (no vector), or (**C**, **D** and **E**) injected with viruses expressing different dystrophin or utrophin cDNAs. At intervals following injection, the mice were sacrificed and muscle sections were stained with antibodies against dystrophin (A–D) or utrophin (**E** and **F**). Gutted Ad vectors express full-length mouse dystrophin (C) or mouse utrophin (E) for at least 3 months after injection; (D) AAV vectors express micro-dystrophin for at least 5 months after injection.

Mdx mice – a model of muscular dystrophy

rAAV6-microdystrophin preserves muscle function and extends lifespan in severely dystrophic mice

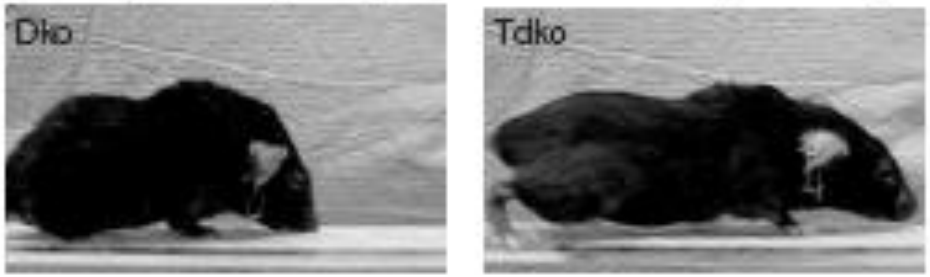
Paul Gregorevic[1], James M Allen[1,2], Elina Minami[3], Michael J Blankinship[1], Miki Haraguchi[1], Leonard Meuse[1], Eric Finn[1], Marvin E Adams[4], Stanley C Froehner[4], Charles E Murry[3] & Jeffrey S Chamberlain[1,2,5]

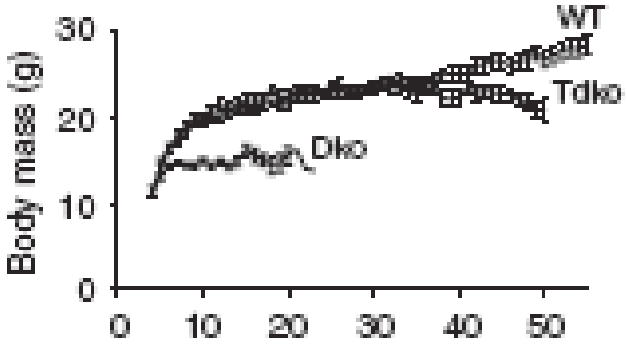
Nature Med., July 2006

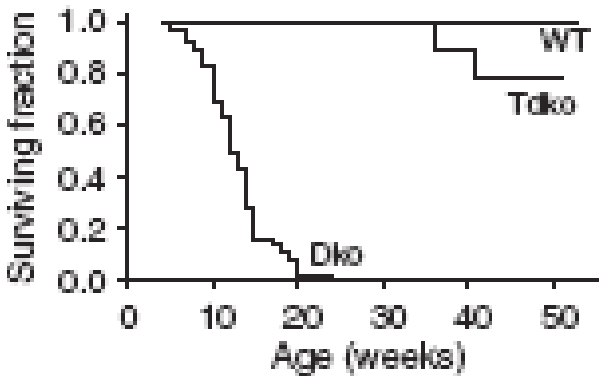


Double KO – dystrophin & utrophin

80% mortality at 15 weeks of age



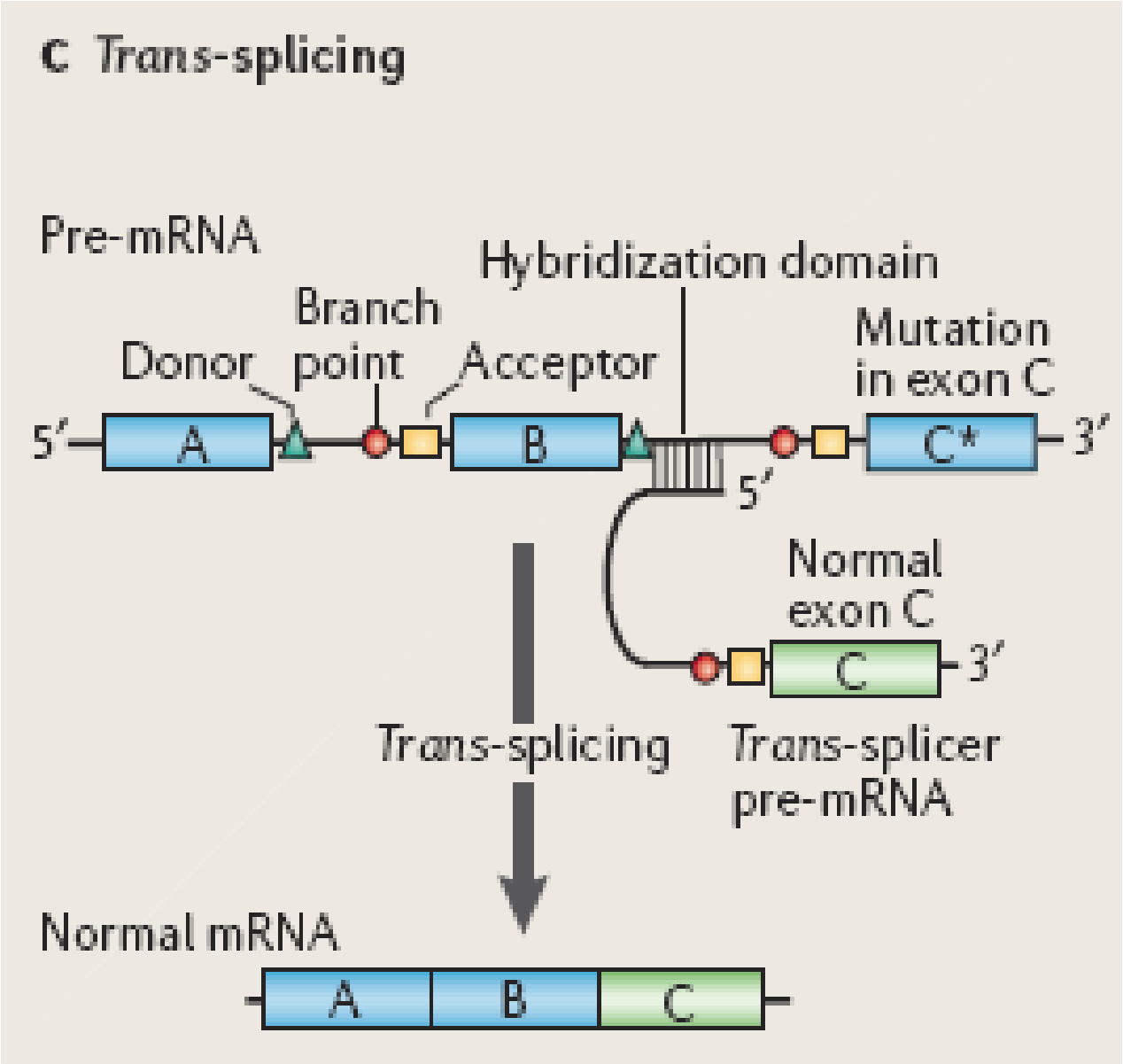




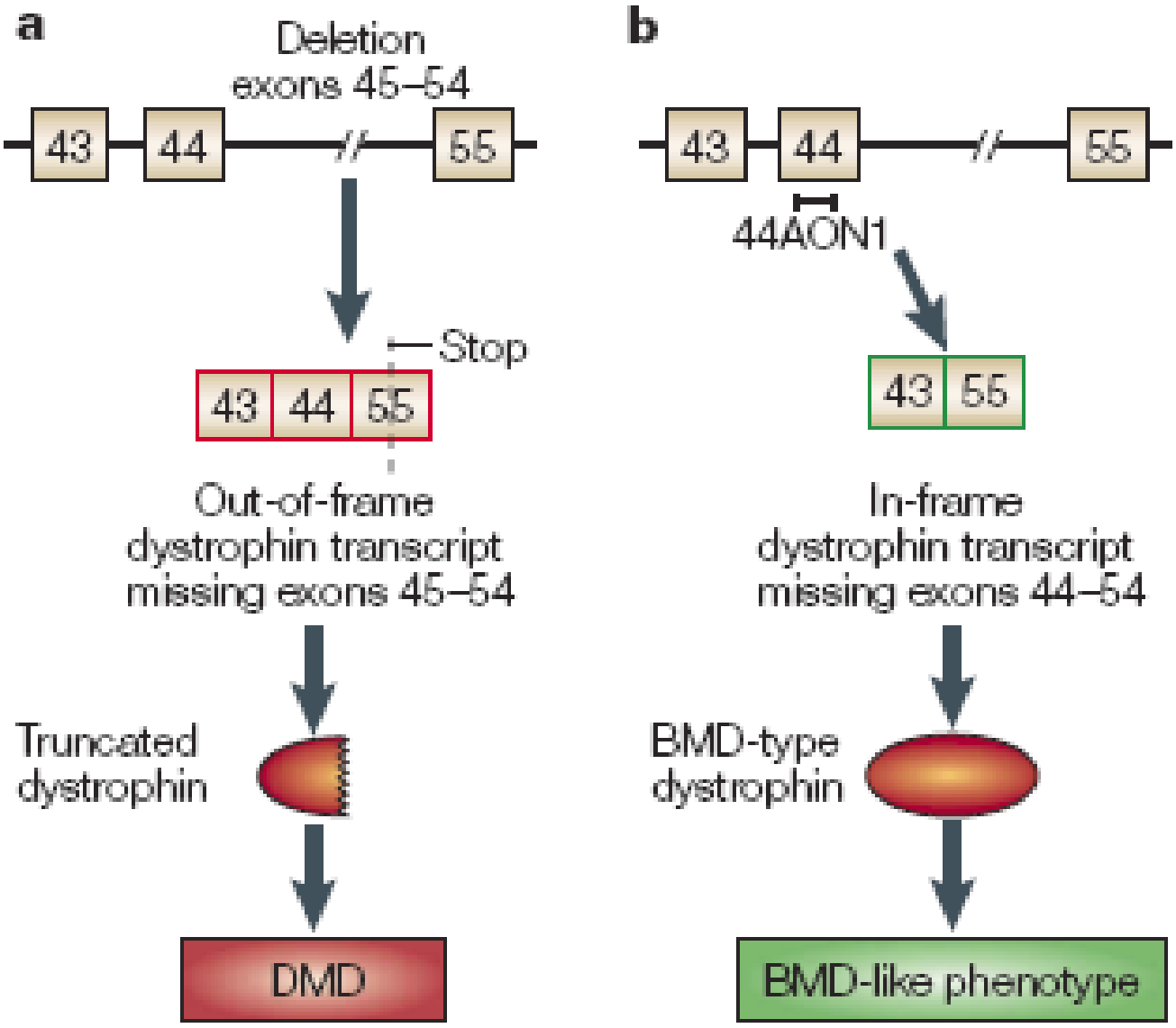
dystrophin in diaphragm after AAV6-delivery

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

Repairing of mutation in gene therapy



Exon skipping as a way to improve conditions in DMD



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Local Dystrophin Restoration with Antisense Oligonucleotide PRO051

Judith C. van Deutekom, Ph.D., Anneke A. Janson, B.S., Ieke B. Ginjaar, Ph.D., Wendy S. Frankhuizen, B.S., Annemieke Aartsma-Rus, Ph.D., Mattie Bremmer-Bout, B.S., Johan T. den Dunnen, Ph.D., Klaas Koop, M.D., Anneke J. van der Kooi, M.D., Ph.D., Nathalie M. Goemans, M.D., Ph.D., Sjef J. de Kimpe, Ph.D., Peter F. Ekhart, M.Sc., Edna H. Venneker, M.D., Gerard J. Platenburg, M.Sc., Jan J. Verschuuren, M.D., Ph.D., and Gert-Jan B. van Ommen, Ph.D.

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 $\alpha 2$.

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.

Published as: *Lancet Neurol*. 2009 October ; 8(10): 918–928.

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[a,b,‡], **Virginia Arechavala-Gomeza**[a,‡], **Lucy Feng**[a], **Sebahattin Cirak**[a], **David Hunt**[c], **Carl Adkin**[a], **Michela Guglieri**[d], **Emma Ashton**[e], **Stephen Abbs**[e], **Petros Nihoyannopoulos**[f], **Maria Elena Garralda**[i], **Mary Rutherford**[h], **Caroline Mcculley**[g], **Linda Popplewell**[j,k], **Ian R Graham**[j,k], **George Dickson**[j,k], **Matthew JA Wood**[l], **Dominic J Wells**[g], **Steve D Wilton**[m], **Ryszard Kole**[n], **Volker Straub**[d], **Kate Bushby**[d], **Caroline Sewry**[a,o], **Jennifer E Morgan**[a], and **Francesco Muntoni**[a,b,*]

[a]The 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.

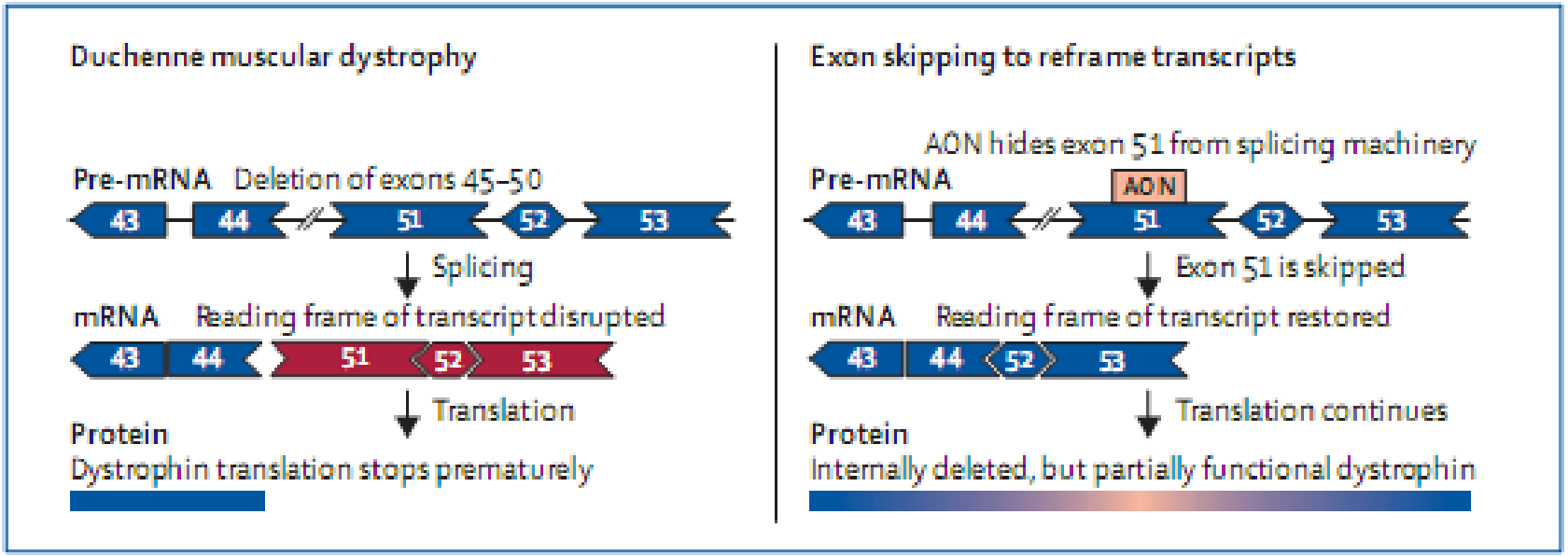


Figure 1: **Antisense-mediated exon skipping to reframe *DMD* transcripts**

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.

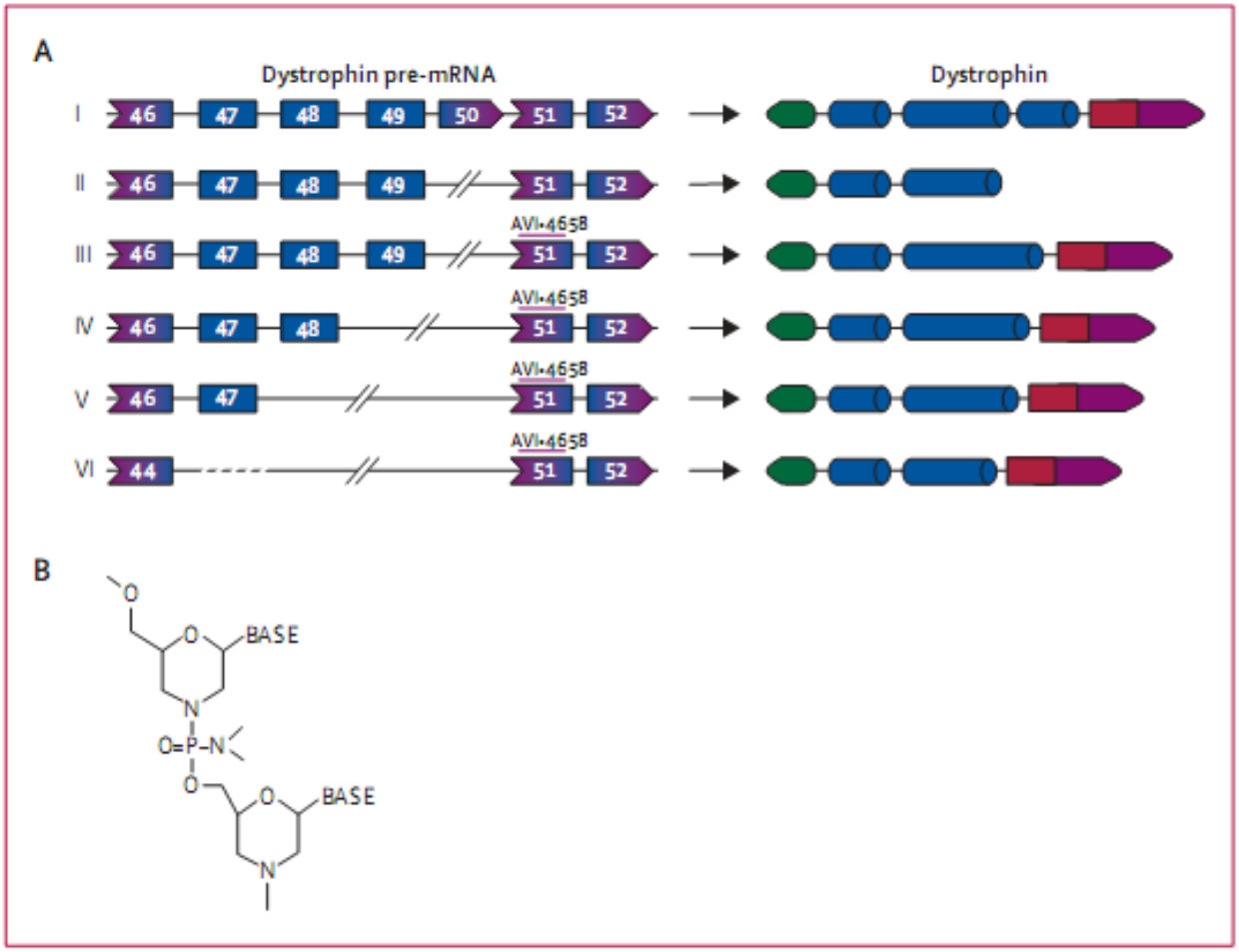


***Figure 1*: Deletions and predicted results of exon skipping in the patients who were studied**
(A) Pre-mRNA transcripts and dystrophin protein products from full length *DMD*, in patients with Duchenne muscular dystrophy, and predicted protein sequences after exon skipping. (I) The normal dystrophin 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.[9,10] (B) Structure of the phosphorodiamidate morpholino modification of the antisense oligomer.

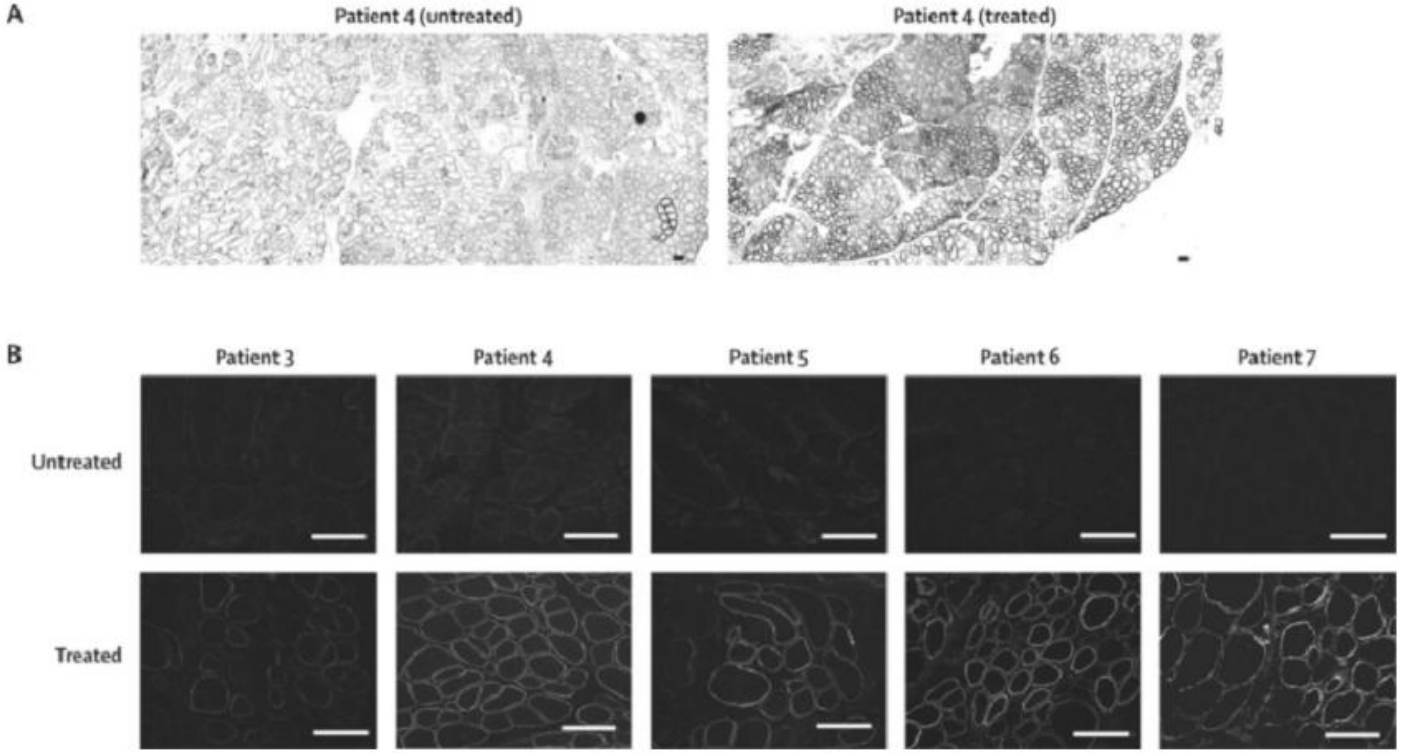
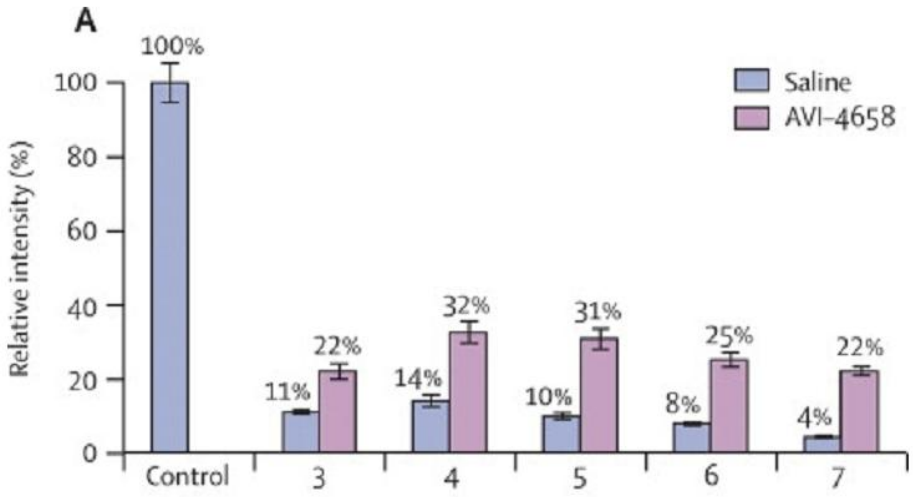
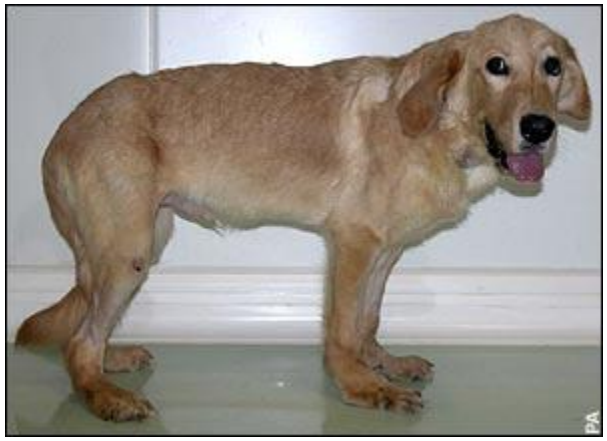


Figure 3.
Dystrophin expression in patients treated with high-dose AVI-4658



Cell therapy in DMD



Azor

Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs

Maurilio Sampaolesi[1,2]*, Stephane Blot[3]*, Giuseppe D'Antona[2], Nicolas Granger[3], Rossana Tonlorenzi[1], Anna Innocenzi[1], Paolo Mognol[4], Jean-Laurent Thibaud[3], Beatriz G. Galvez[1], Ines Barthélémy[3], Laura Perani[1], Sara Mantero[4], Maria Guttinger[5], Orietta Pansarasa[2], Chiara Rinaldi[2], M. Gabriella Cusella De Angelis[2], Yvan Torrente[6], Claudio Bordignon[1], Roberto Bottinelli[2] & Giulio Cossu[1,5,7]

Dog no.	Dog name	Cell treatment	Lentiviral vector	Onset of treatment	Immune suppression (time)	Dystrophin expression	Motility	Outcome of experiment (at time P400)
01A	Ucal	Autologous, gene therapy	CK-μdys-ires-GFP	P118	–	+/−	Loss	Euthanasia (P272)
02H	Vrillie	Heterologous, WT donor	–	P80	CYC A (P78)	+	Loss	Euthanasia (P235)
03H	Valgus	Heterologous, WT donor	–	P75	CYC A (P73)	+++	No decline	Alive and well
04H	Varus	Heterologous, WT donor	–	P75	RAP (P73)	+++	Modest decline	Alive and well
05H	Viko	Heterologous, WT donor	–	P77	RAP + IL-10 (P74)	ND	ND (sudden death)	Myocarditis (P186)
06A	Vaccin	Autologous, gene therapy	MLC1F-μdys	P113	–	++	Major decline	Euthanasia (P326)
07A	Valium	Autologous, gene therapy	MLC1F-μdys	P113	–	ND	Loss	Pneumonia (P245)
08A	Vampire	Autologous, gene therapy	MLC1F-μdys	P113	–	++	Major decline	Pneumonia (P154)
09H	Azur	Heterologous, WT donor	–	P159	CYC A (P157)	++	Restored	Alive and well
10H	Azor	Heterologous, WT donor	–	P159	CYC A (P157)	+++	Restored	Alive and well
11U	Akan	None	–	–	–	–	Loss	Euthanasia (P380)
12U	Vulcano	None	–	–	–	–	Loss	Euthanasia (P376)
13U	Viking	None	–	–	–	–	Loss	Euthanasia (P340)

4 dogs received autologous or heterologous mesoangioblasts transduced *in vitro* with lentiviral vector encoding microdystrophin

Nature, published on line Nov 15, 2006

Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs

Maurilio Sampaolesi[1,2*], Stephane Blot[3*], Giuseppe D'Antona[2], Nicolas Granger[3], Rossana Tonlorenzi[1], Anna Innocenzi[1], Paolo Mognol[4], Jean-Laurent Thibaud[3], Beatriz G. Galvez[1], Ines Barthélémy[3], Laura Perani[1], Sara Mantero[4], Maria Guttinger[5], Orietta Pansarasa[2], Chiara Rinaldi[2], M. Gabriella Cusella De Angelis[2], Yvan Torrente[6], Claudio Bordignon[1], Roberto Bottinelli[2] & Giulio Cossu[1,5,7]

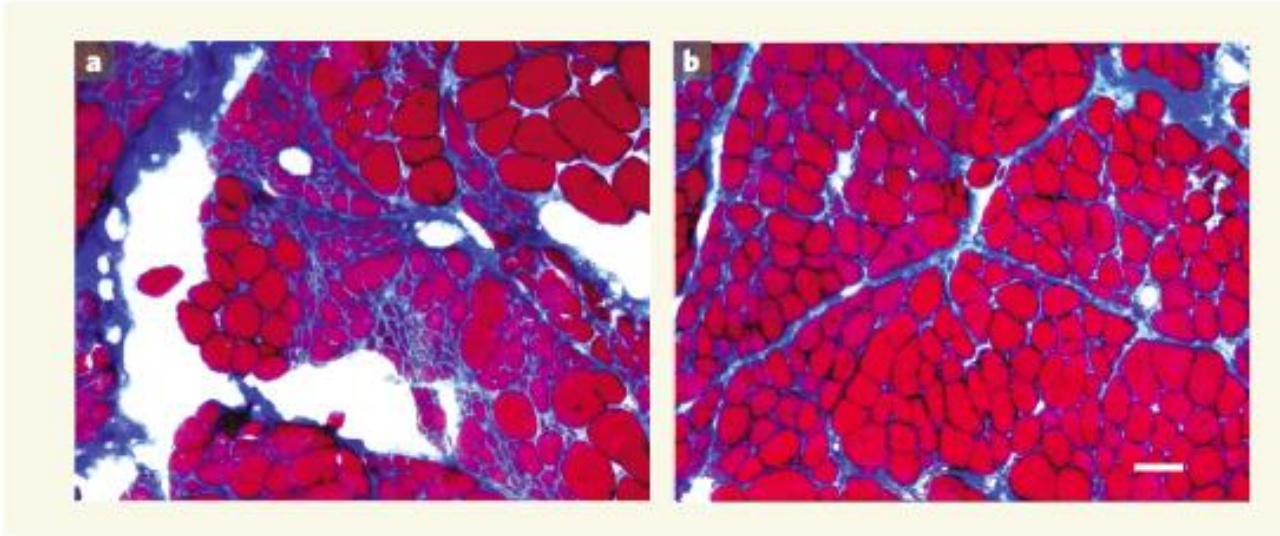


Figure 1 | Stem-cell treatment of dystrophic muscle in dogs[1]. These pictures are stained biopsies of (**a**) untreated dystrophic skeletal muscle and (**b**) muscle after treatment. The latter was both structurally more ordered and functioned better. Scale bar, 200 μm.

Nature, published on line Nov 15, 2006

REVIEW

Gene therapy progress and prospects: Duchenne muscular dystrophy

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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

Selected Companies and Clinics Offering Stem Cell Therapies

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

SOURCE: COMPANY AND CLINIC WEB SITES, INFORMATION PACKAGES, INTERVIEWS, ALSTDF, BIOMARK CRIMINAL INDICTMENT. NA=INFORMATION NOT AVAILABLE.

Be aware of dishonest people!

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* (lecithin-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 1. Clinical trials of *in vivo* ocular gene therapy

Disease	Vector	Transgene	Clinical centers	Phase	NCT number	Refs
Retinoblastoma	Adenovirus	Herpes virus thymidine kinase gene	Texas Children Hospital, Houston, TX, USA	I	Not found	[21]
Age-related macular degeneration	Adenovirus	Pigment epithelium derived factor gene	Wilmer Eye Institute, Johns Hopkins University School of Medicine, Baltimore, MD, USA	I	NCT00109499	[48]
Leber congenital amaurosis	Adeno-associated virus type 2	RPE65 gene	Children's Hospital, Philadelphia, PA, USA; Second University of Naples, Italy	I	NCT00516477	[77]
Leber congenital amaurosis	Adeno-associated virus type 2	RPE65 gene	Moorfields Eye Hospital, London, UK	I	NCT00643747	[76]
Leber congenital amaurosis	Adeno-associated virus type 2	RPE65 gene	Scheie Eye Institute of the University of Pennsylvania, Philadelphia, PA, USA; University of Florida/Shands, FL, USA	I	NCT00481546	[78,80]

Age-dependent effects of *RPE65* gene therapy for Leber's congenital amaurosis: a phase 1 dose-escalation trial

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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 ($1{\cdot}5\times10^{10}$ vector genomes), medium ($4{\cdot}8\times10^{10}$ vector genomes), or high dose ($1{\cdot}5\times10^{11}$ 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.

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Summary – gene therapy of monogenic diseases