Gene therapy of monogenic diseases

Examples

Lecture 10

20 December 2011

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.

Duchenne & Becker muscular dystrophy

Duchenne dystrophy: 1:3500 newborn males

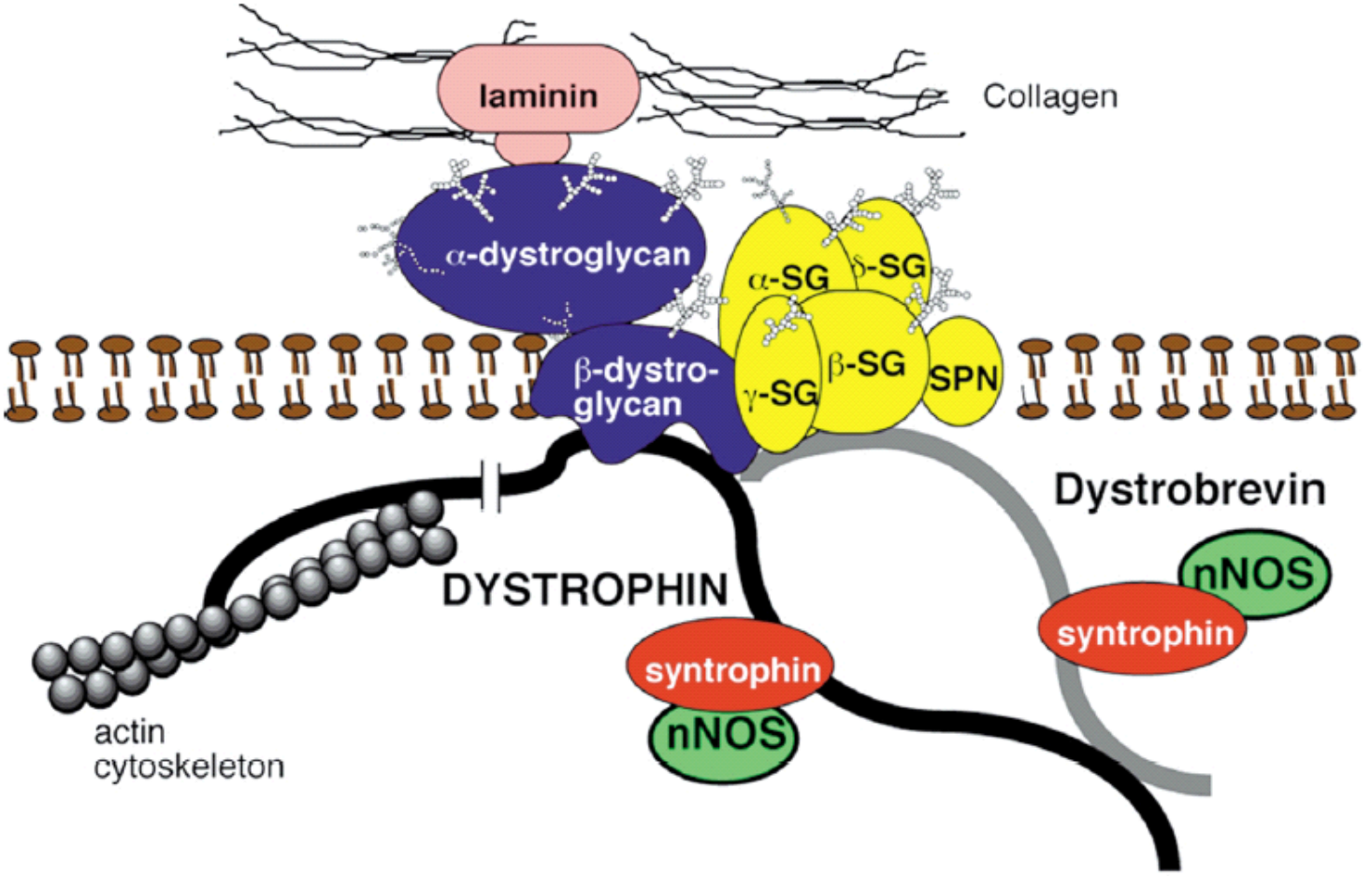
X - linked;
The largest gene described in humans (~1% of X chromosome), extending over 2300 kb and comprising 79 exons

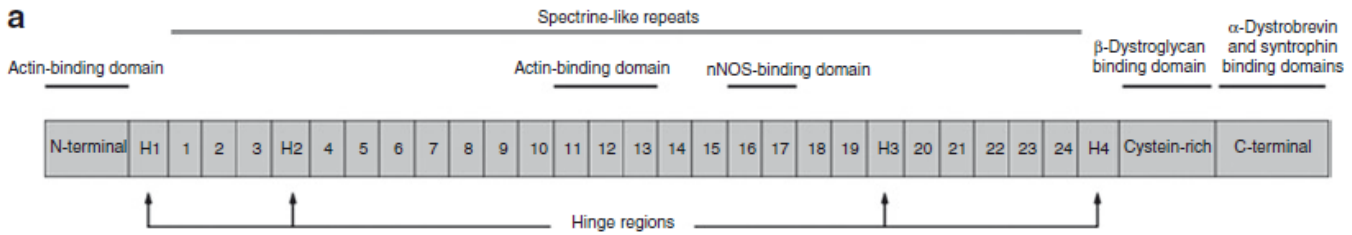
- The size of the gene (2.4 Mb) and mRNA (14 kb) is a serious problem

- in 65% patients the molecular abnormality involves exon deletions

Becker dystrophy: 7-10 times less frequent that Duchenne

Structure of contractile unit – role of dystrophin





Approaches to treat Duchenne muscular dystrophy

1. stop-codon read-through:
   1.1. gentamycin (binds to 40S ribosome subunit)
   1.2. ataluren PTC124) (binds to 60S subunit)

2. Exon skipping

3. Modification of DMD gene with meganuclease or zinc finger nucleases

4. Utrophin overexpression/restoration

5. Myostatin inhibition

6. Gene therapy with dystrophin gene
   6.1. plasmid
   6.2. lentivirus
   6.3. AAV

7. Cell transplantation

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

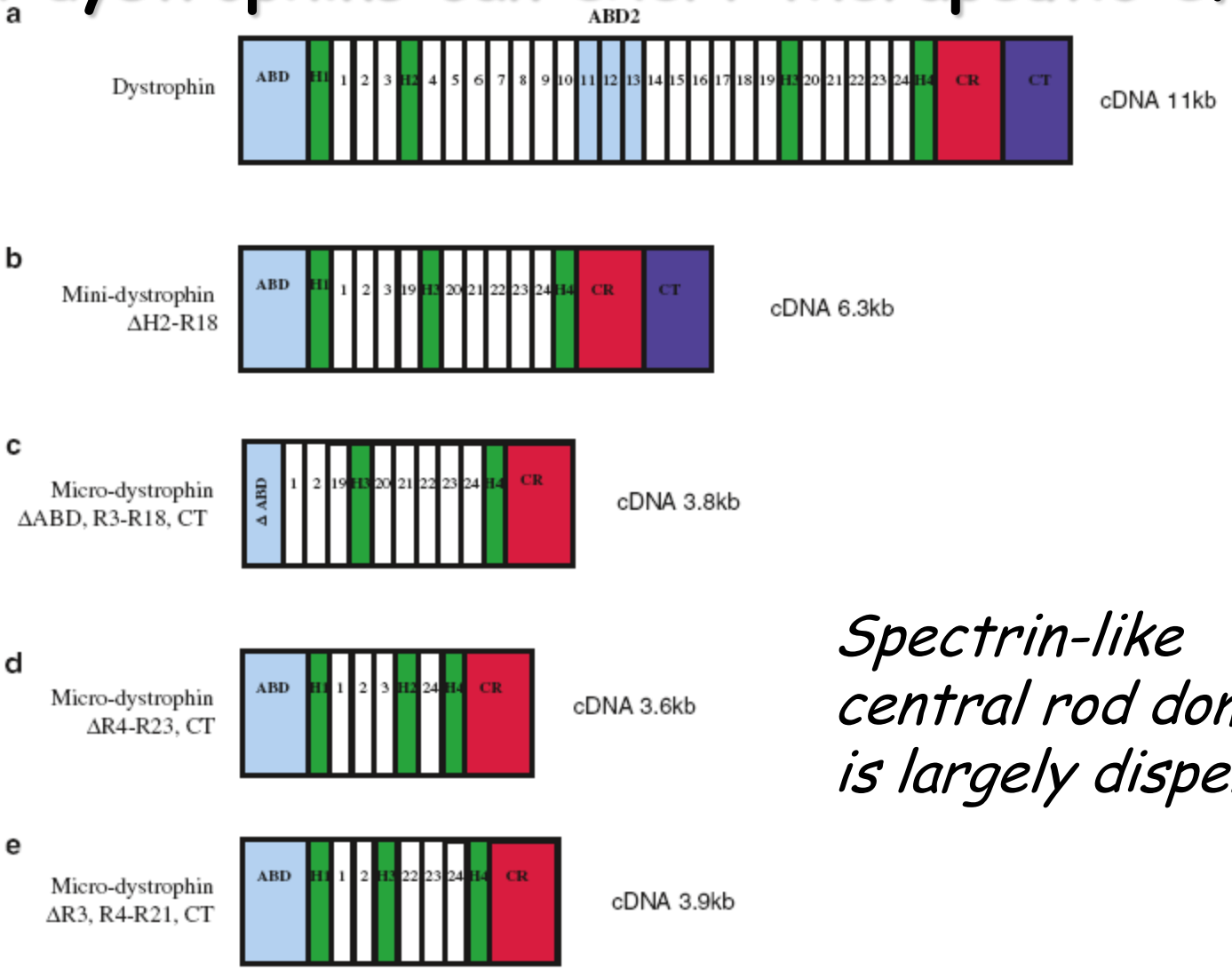
Hum Gene Ther. 2004 Nov;15(11):1065-76

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.

Partial dystrophins can exert therapeutic effect

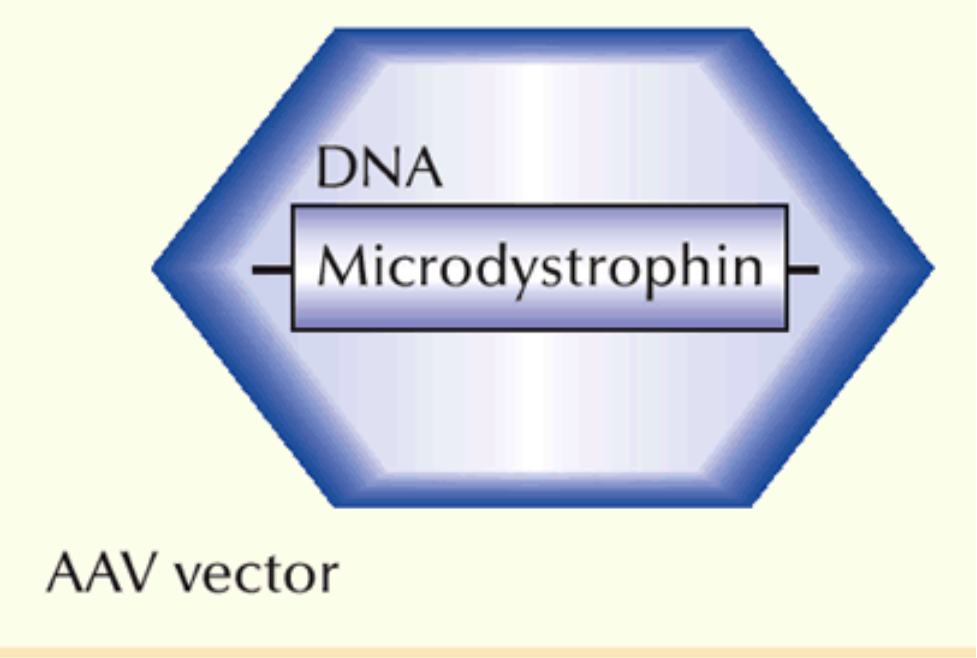


Spectrin-like central rod domain is largely dispensable

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

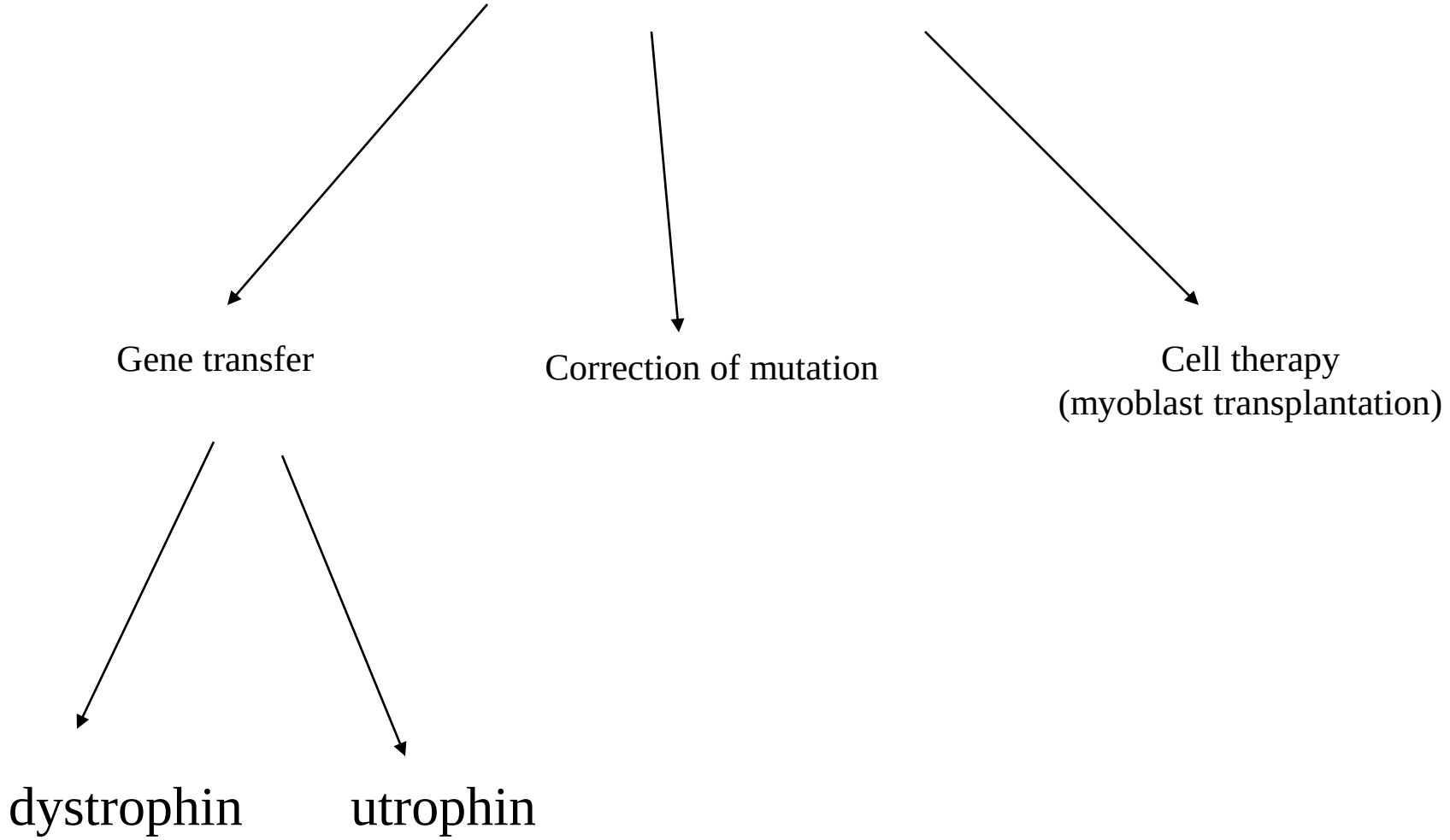
Micro-dystrophin cDNA can be delivered with AAV vector



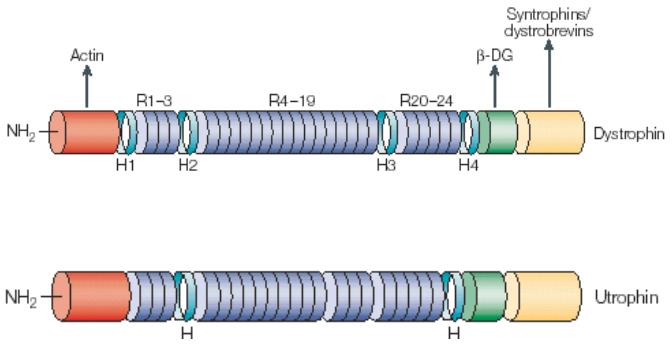
James G Tidball & Melissa J Spencer

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 utrophin transfer – gutless Ad or AAV vectors

dystrophin – guttless vector

Utrophin – guttless vector

Wild type mice

Mdx mice

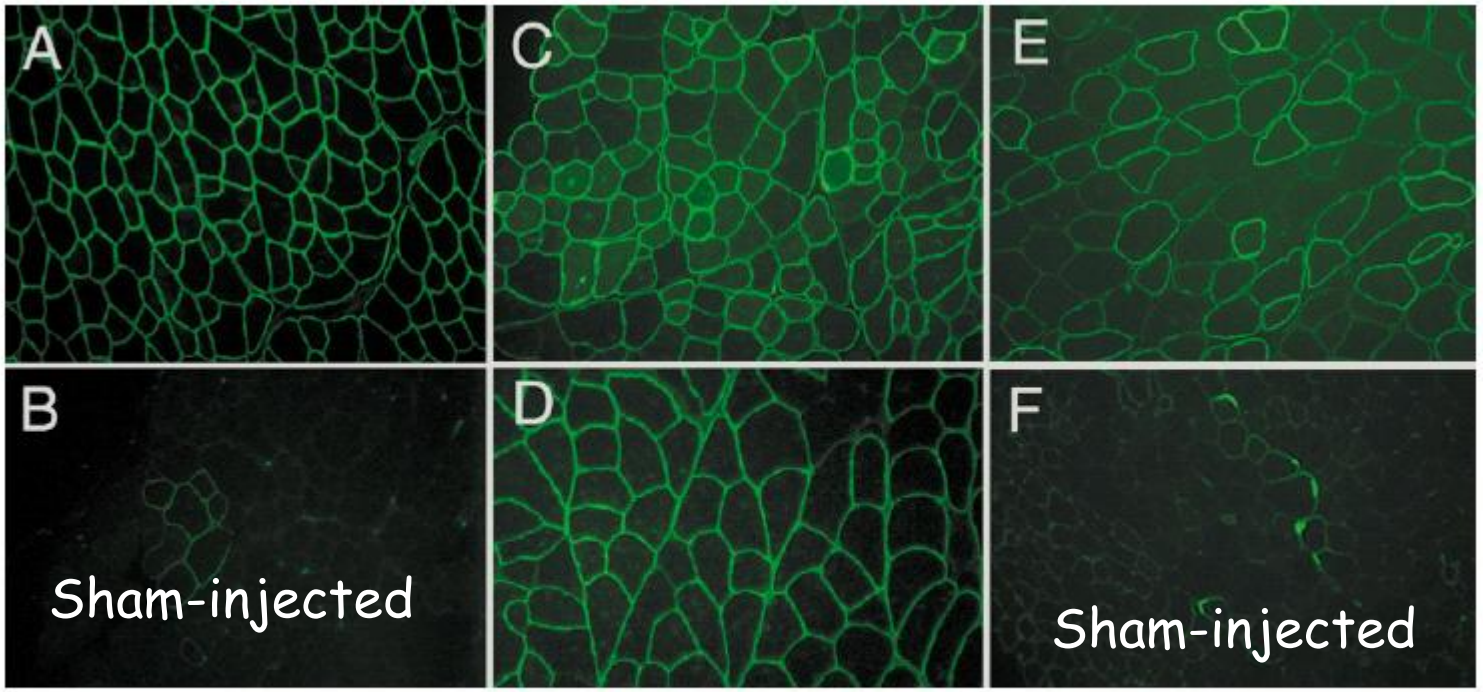


Figure 3. Transduction of adult, immunocompetent *mdx* mouse muscles by dystrophin and utrophin expression vectors. The figure shows cross-sections of C57Bl/10 (**A**) or *mdx* mouse TA muscles that had been (**B** and **F**) sham-injected (no vector), or (**C**, **D** and **E**) injected with viruses 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.

Hum Mol Genet 2002

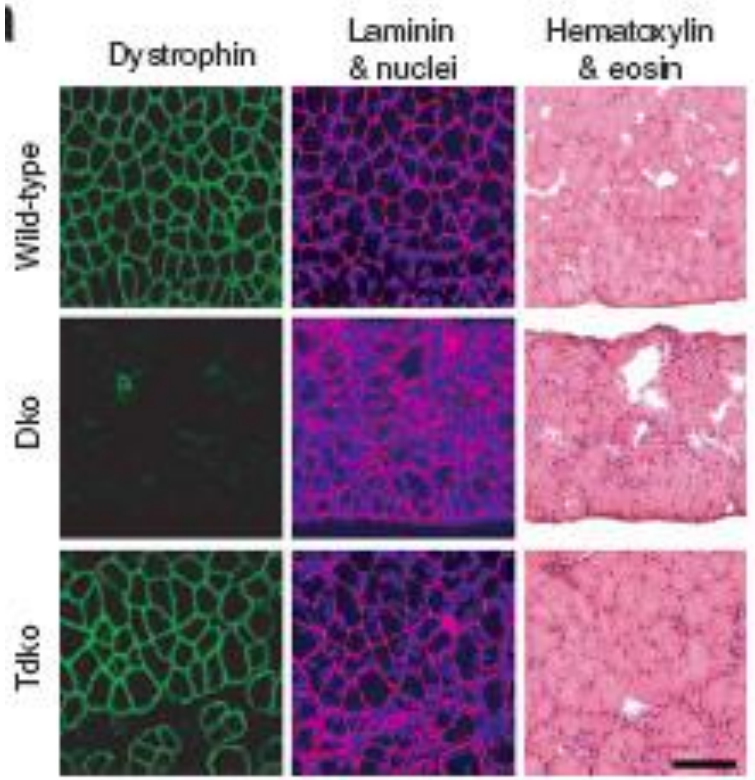
microdystrophin - AAV

Mdx mice – a model of muscular dystrophy

Nature Med., July 2006

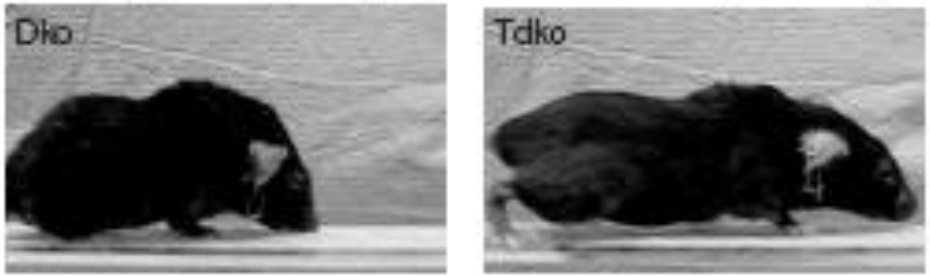
rAAV6-microdystrophin preserves muscle function and extends lifespan in severely dystrophic mice

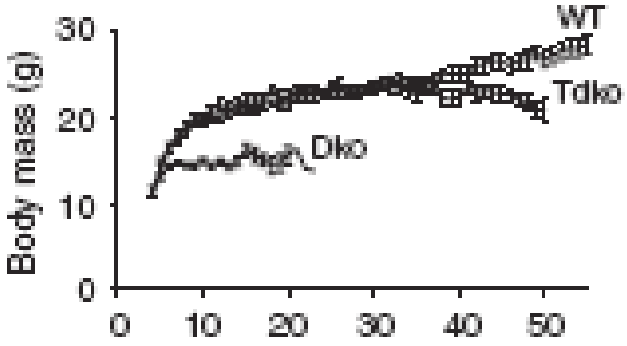
Paul Gregorevic[1], James M Allen[1,2], Elina Minami[3], Michael J Blankinship[1], Miki Haraguchi[1], Leonard Meuse[1], Eric Finn[1], Marvin E Adams[4], Stanley C Froehner[4], Charles E Murry[3] & Jeffrey S Chamberlain[1,2,5]

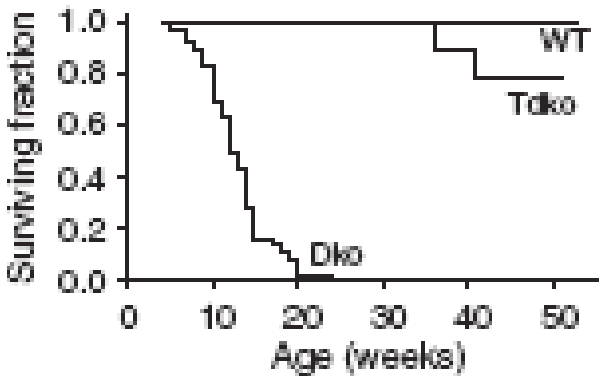


Double KO – dystrophin & utrophin

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

The generation of revertant dystrophin from mutant allele

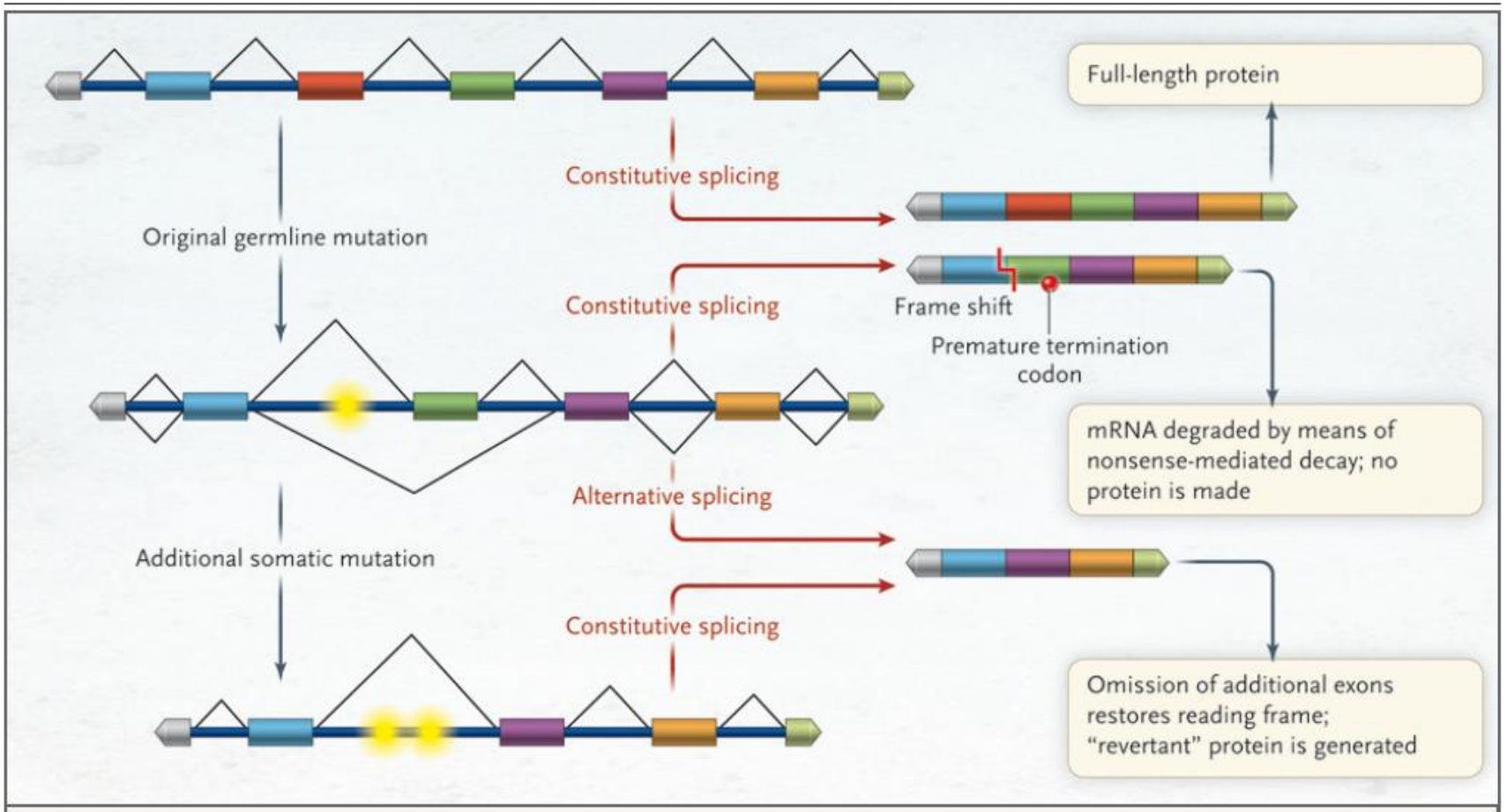


Figure 1. The Generation of Revertant Dystrophin from Mutant Alleles.

Constitutive pre–messenger RNA (mRNA) splicing of the transcript from a germline deletion or nonsense allele leads to an mRNA molecule containing a premature termination codon. Such mRNA molecules are targeted for destruction by means of nonsense-mediated decay, resulting in no production of protein. Alternative splicing of the mutant allele to exclude one or more additional exons can restore the open reading frame and generate "revertant" protein. Revertant protein can also be produced by constitutive splicing of an allele created by a secondary somatic mutation. The yellow areas denote deletions within the dystrophin gene that are known to lead to clinical muscular dystrophy.

Moore and Flotte NEJM 2010

Antisense oligonucleotides in gene therapy

1. Inhibition of gene expression

2. Exon-skipping strategies

2.1. Remove a specific mutation from the transcript (such as premature stop codon)
2.2. Restore open-reading frame to a frame-shifted transcript arising from a gene with a deletion or insertion

Duchenne muscular dystrophy, B-thalassemia

3. Displacement of protein sequestered on triplet repeats RNA:

myotonic dystrophy type 1, type 2, spincerebellar ataxia type 1 & 3, fragile X-associated ataxia

Exon-skipping strategies using antisense oligonucleotides

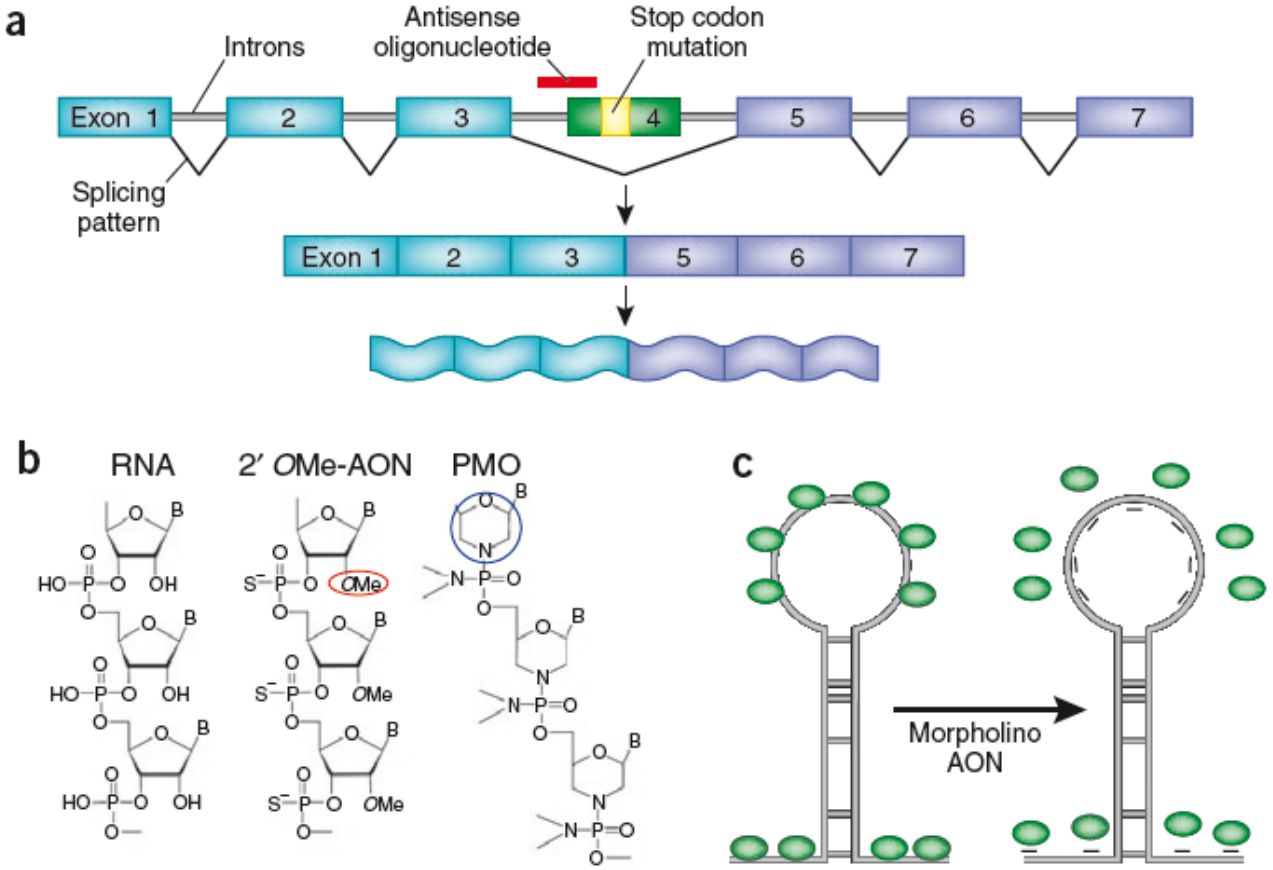
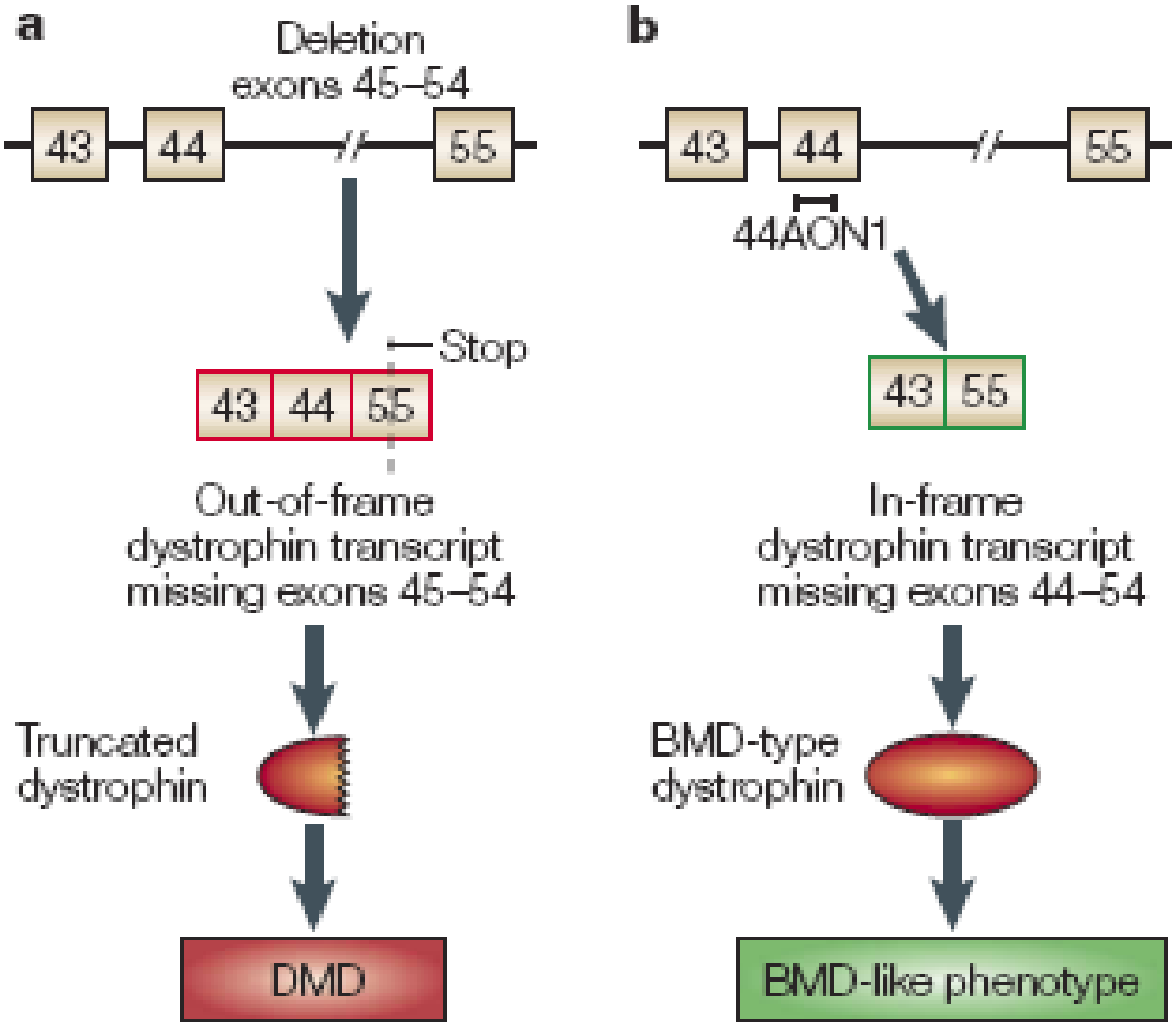


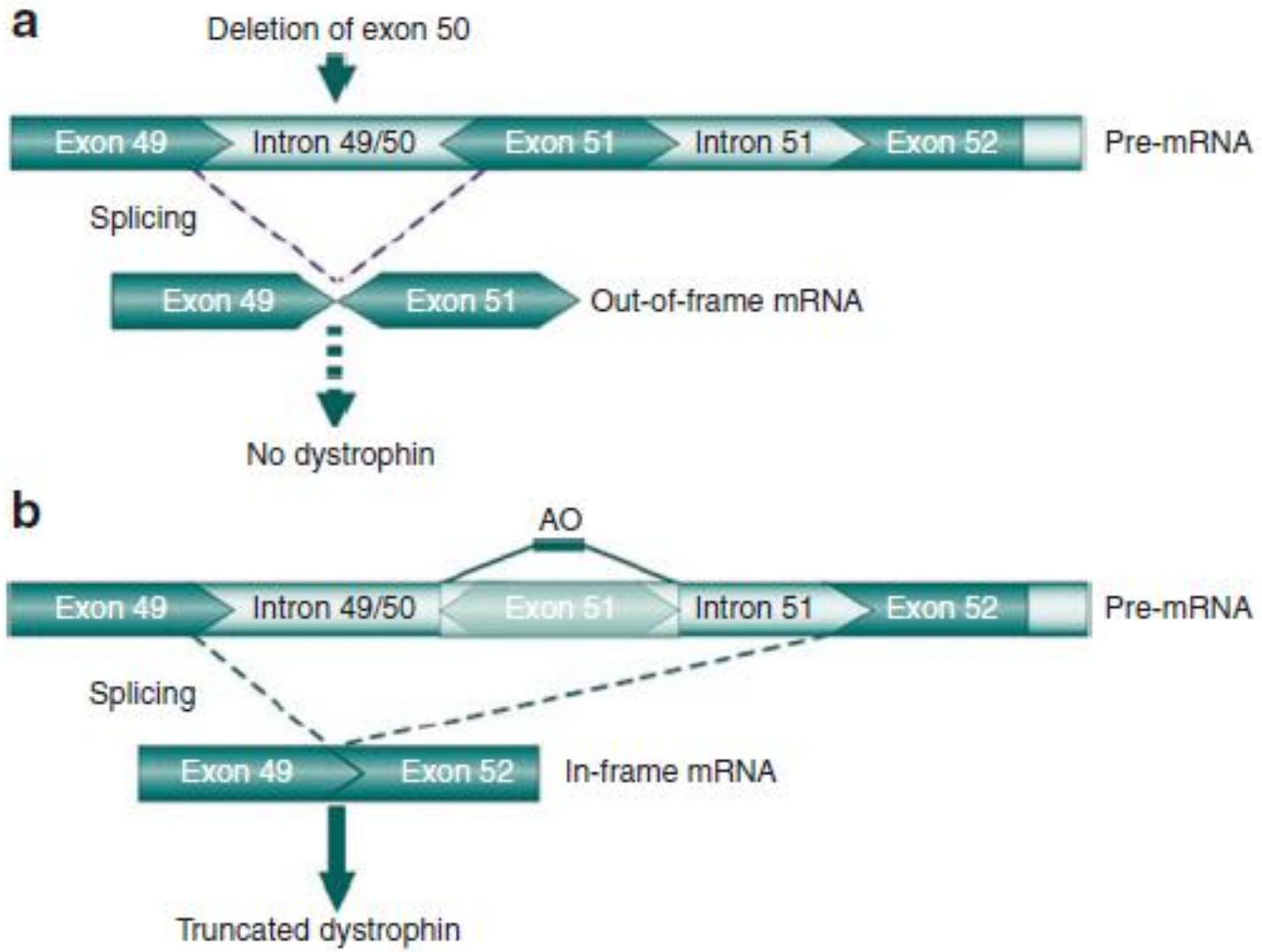
Figure 1 Exon-skipping strategies using antisense oligonucleotides. (**a**) RNA transcribed from a gene initially contains sequences corresponding to both exons and introns. In the example shown, the pre-mRNA contains a premature stop codon in exon 4. An antisense oligonucleotide (red) targeting the 3′ splice site of intron 3 is able to block splicing of exon 4, leading to exclusion of these sequences from the spliced mRNA. The truncated mRNA now contains an open reading frame able to be translated into a slightly truncated but functional protein. (**b**) Comparative structure of RNA, 2′*O*Me and phosphorodiamidate morpholino oligomer (PMO) antisense molecules. Left, ribonucleic acid structure with the characteristic ribose rings joined by phosphodiester bonds. Middle, 2′-*O*–methylated antisense oligonucleotides (AONs) use a phosphorothioate bond to join ribose rings (substitution of sulfur for oxygen). Right, PMOs incorporate morpholine rings in place of ribose rings, and the rings are joined by phosphorodiamidate bonds (substitution of nitrogen for oxygen). (**c**) Elimination of RNA toxicity using a morpholino antisense oligonucleotide. The expanded trinucleotide repeat in the mutant DM1 transcript forms extensive hairpin loops that bind and sequester MBNL1 (ref. 8). In the presence of a morphilino antisense oligonucleotide, MBNL1 is displaced, allowing it to resume normal splicing of numerous other pre-mRNAs[4].

Chamberlain & Chamberlain, Nature Medicine 2010

Exon skipping as a way to improve conditions in DMD



Exon skipping as a way to improve conditions in DMD



Pichavant et al., Mol Ther 2011

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.

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 $\alpha 2$.

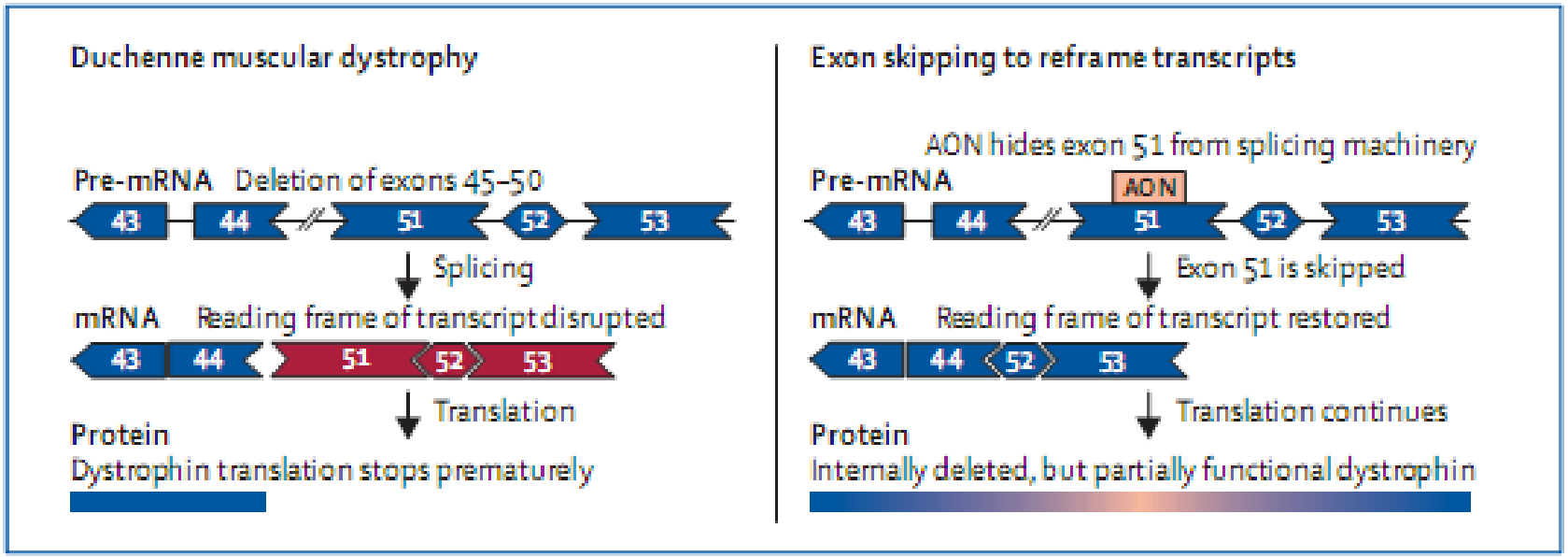
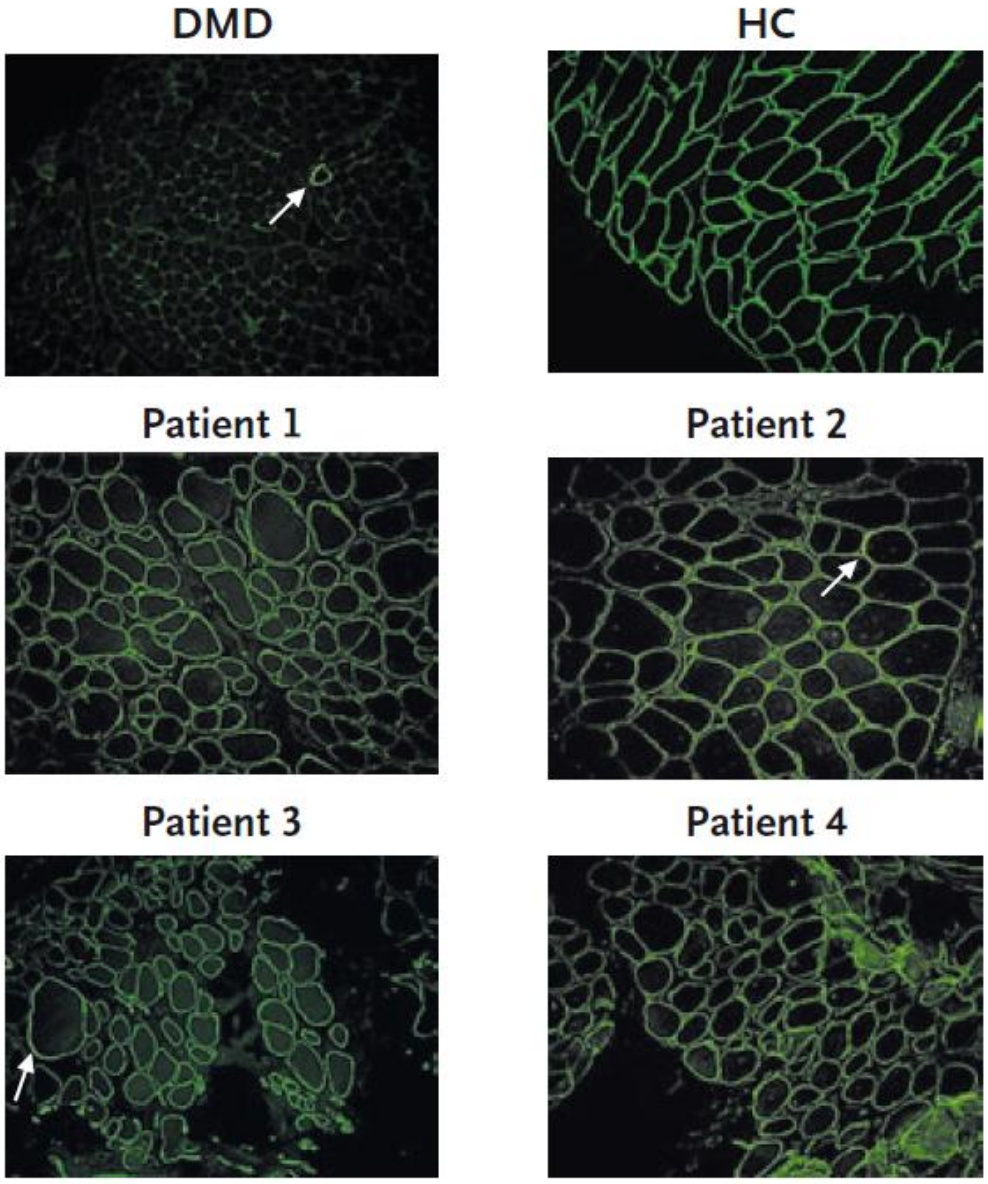
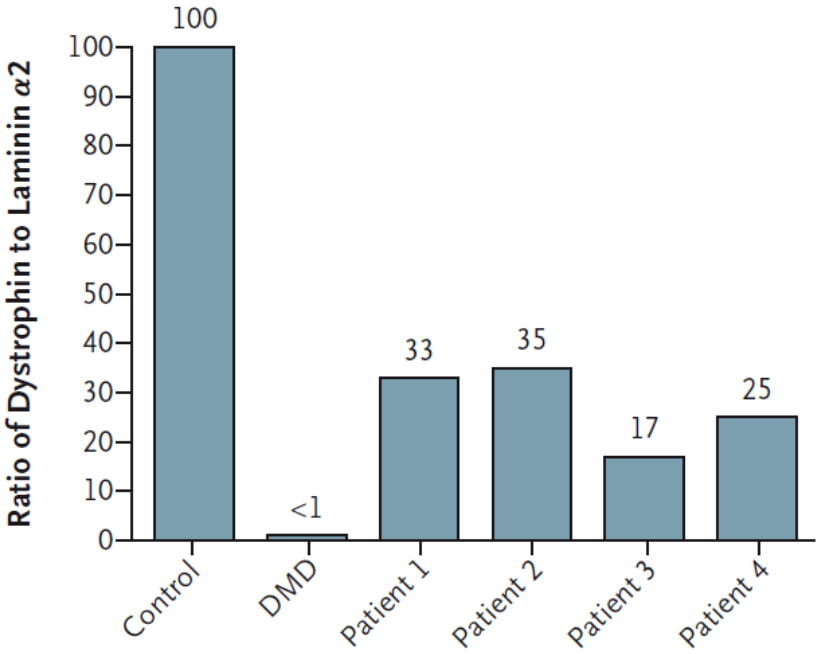


Figure 1: **Antisense-mediated exon skipping to reframe *DMD* transcripts**

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.





Van Deutekom et al, NEJM 2009

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.

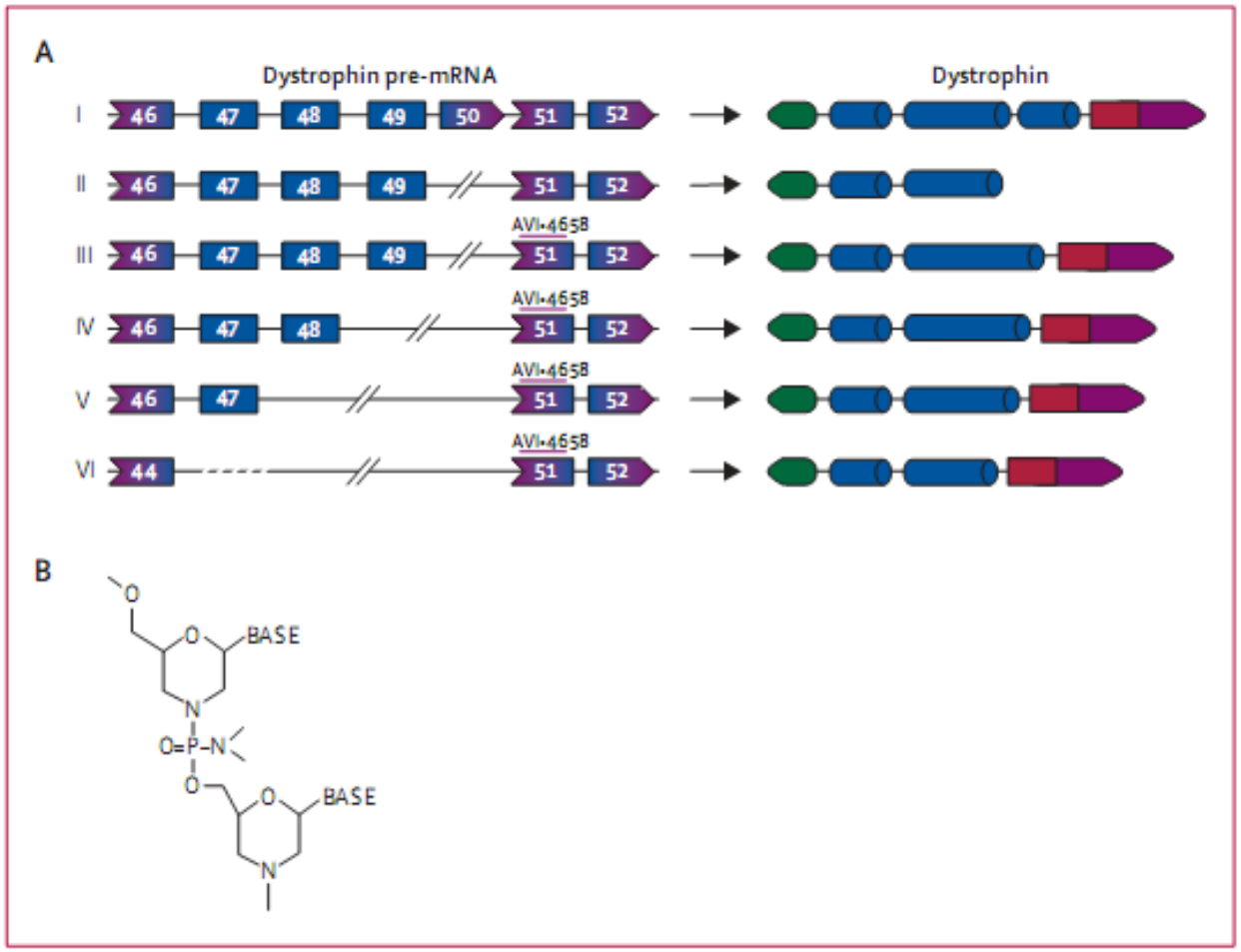
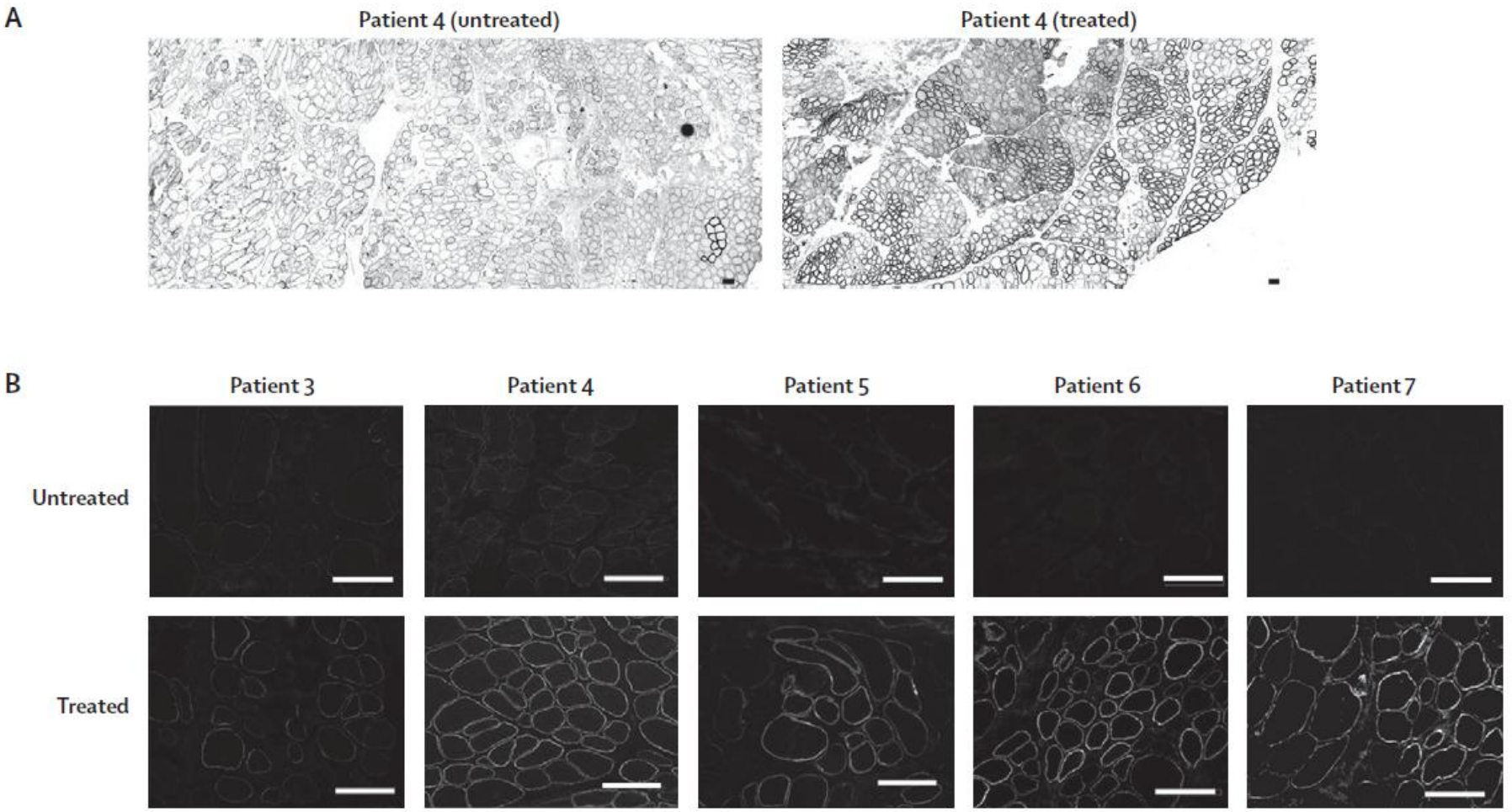


***Figure 1*: Deletions and predicted results of exon skipping in the patients who were studied**
(A) Pre-mRNA transcripts and dystrophin protein products from full length *DMD*, in patients with Duchenne muscular dystrophy, and predicted protein sequences after exon skipping. (I) The normal dystrophin 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.

Restoration of dystrophin expression in patients treated with morpholino

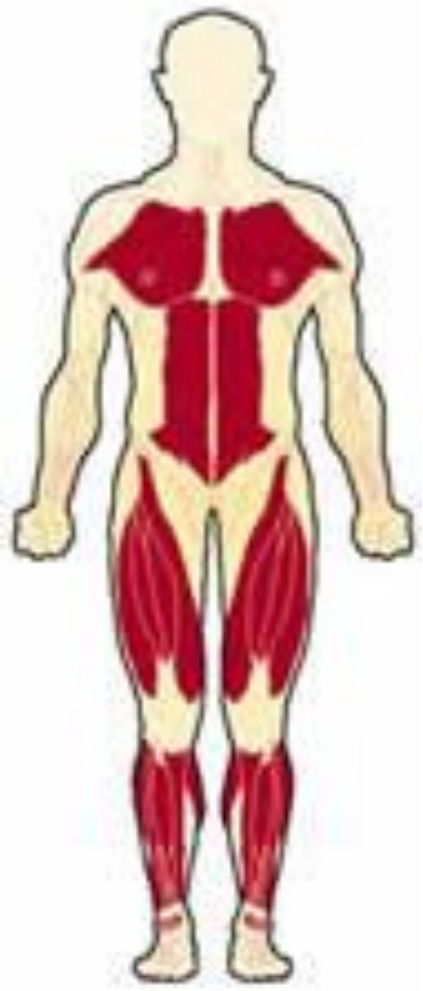


Kinali et al., Lancet Neurol 2009

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



Immunity in gene therapy of Duchenne muscular dystrophy

One recent trial (Medell JR, NEJM Oct 2010) – rAAV with dystrophin minigene

1. Some patients had immune response to epitopes represented in rAAV
2. Unexpectedly, a rapid T-lymphocyte response to dystrophin epitopes that were not expressed from the vector-expressed protein also developed in one patient
3. This patient and one other showed some level of immunoreactivity to these epitopes before receiving the vector

Reason?

-self-immunity should develop against the reverted dystrophin

- however, epitopes of revertant dystrophin are most probably not efficiently expressed in the thymus of DMD patients to induce self-tolerance

Disease targets for gene therapy

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

Merry Christmas for Patients with Hemophilia B

Katherine P. Ponder, M.D.

The first reported case of hemophilia due to FIX deficiency was in 1952 and was called "Christmas disease" after the patient, a 10-year-old boy named Stephen Christmas.[3] Queen Victoria of the United Kingdom (1819–1901) was the most famous carrier of the hemophilia B gene. Although the protein and the gene were

This article (10.1056/NEJMoa1108046) was published on December 10, 2011, at NEJM.org.

N Engl J Med 2011.

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Adenovirus-Associated Virus Vector–Mediated Gene Transfer in Hemophilia B

Amit C. Nathwani, M.B., Ch.B., Ph.D., Edward G.D. Tuddenham, M.B., B.S., M.D., Savita Rangarajan, M.B., B.S., Cecilia Rosales, Ph.D., Jenny McIntosh, Ph.D., David C. Linch, M.B., B.Chir., Pratima Chowdary, M.B., B.S., Anne Riddell, B.Sc., Arnulfo Jaquilmac Pie, B.S.N., Chris Harrington, B.S.N., James O'Beirne, M.B., B.S., M.D., Keith Smith, M.Sc., John Pasi, M.D., Bertil Glader, M.D., Ph.D., Pradip Rustagi, M.D., Catherine Y.C. Ng, M.S., Mark A. Kay, M.D., Ph.D., Junfang Zhou, M.D., Yunyu Spence, Ph.D., Christopher L. Morton, B.S., James Allay, Ph.D., John Coleman, M.S., Susan Sleep, Ph.D., John M. Cunningham, M.D., Deokumar Srivastava, Ph.D., Etiena Basner-Tschakarjan, M.D., Federico Mingozzi, Ph.D., Katherine A. High, M.D., John T. Gray, Ph.D., Ulrike M. Reiss, M.D., Arthur W. Nienhuis, M.D., and Andrew M. Davidoff, M.D.

METHODS

We infused a single dose of a serotype-8–pseudotyped, self-complementary adeno-virus-associated virus (AAV) vector expressing a codon-optimized human factor IX (FIX) transgene (scAAV2/8-LP1-hFIXco) in a peripheral vein in six patients with severe hemophilia B (FIX activity, <1% of normal values). Study participants were enrolled sequentially in one of three cohorts (given a high, intermediate, or low dose of vector), with two participants in each group. Vector was administered without immunosuppressive therapy, and participants were followed for 6 to 16 months.

RESULTS

AAV-mediated expression of FIX at 2 to 11% of normal levels was observed in all participants. Four of the six discontinued FIX prophylaxis and remained free of spontaneous hemorrhage; in the other two, the interval between prophylactic injections was increased. Of the two participants who received the high dose of vector, one had a transient, asymptomatic elevation of serum aminotransferase levels, which was associated with the detection of AAV8-capsid–specific T cells in the peripheral blood; the other had a slight increase in liver-enzyme levels, the cause of which was less clear. Each of these two participants received a short course of glucocorticoid therapy, which rapidly normalized aminotransferase levels and maintained FIX levels in the range of 3 to 11% of normal values.

1. Self-complementary AAV vector
2. AAV8-pseudotyped
3. Targeting to liver
4. Limitations of immune response (lower prevalence of anti-AAV8 antibodies)

Table 1. Characteristics of the Six Study Participants at Baseline and after Gene Transfer, According to Dose of Vector.*

Characteristic	Vector Dose, 2×10^{11} vg/kg		Vector Dose, 6×10^{11} vg/kg		Vector Dose, 2×10^{12} vg/kg	
	Participant 1	Participant 2	Participant 3	Participant 4	Participant 5	Participant 6
At baseline						
Age (yr)	31	64	43	29	32	27
Mutation in FIX gene	31280G→A E387K	2bp deletion, frame shift	30097G→T W215C	31290G→A A309T	20518C→T R180W	–52 del C
FIX prophylaxis	Twice weekly	Twice weekly	Twice weekly	Targeted (average, weekly)	Twice weekly	Thrice weekly
Hepatitis C status	Negative	Positive, spontaneous clearance	Positive, clearance with interferon plus antiviral therapy	Positive, spontaneous clearance	Positive, clearance with interferon plus antiviral therapy	Negative
Antibody titer (relative units)†						
AAV2 IgG	5	20	77	12	10	22
AAV8 IgG	1	12	37	1	5	8
After gene transfer						
Maximum FIX level (IU/dl)‡	2	2	3	4	8	12
Duration of FIX expression (mo)	>15	>11	>9	>8	>6	>5
FIX expression on in vivo transduction-inhibition assay (% of control value)§	138	116	42	109	92	93
Peak alanine aminotransferase value (IU/liter)¶	36	20	39	34	202	36

* FIX denotes factor IX, and vg vector genomes.
† The lower limit of detection for serotype-2 adenovirus-associated virus (AAV2) IgG antibodies was 3 relative units; the lower limit of detection for serotype-8 adenovirus-associated virus (AAV8) IgG antibodies was 1 relative unit.
‡ The lower limit of detection was 1%.
§ The lower limit of detection was 0.7%.
¶ The upper limit of the normal range is 41 IU per liter.

Is gene therapy for hemophilia necessary?

With the success of protein therapy, why would gene therapy be needed? In the United States and other developed countries, annual costs for a single adult patient of clotting factors for hemophilia are approximately $150,000 for on-demand therapy and $300,000 for prophylaxis,[6] which could incur a lifetime cost of over $20 million. In developing countries, prophylactic and frequent on-demand therapy is not affordable, and patients still have chronic joint disease and die young.[7]

achieved about 7% of normal activity. Expression has been seen for over 6 months in all patients, and prophylactic use of factor concentrate has either been eliminated or reduced. Since the vector is estimated to cost $30,000 per patient, dramatic cost savings have already been achieved.

Gene therapy of Leber’s congenital amaurosis

Leber's congenital amaurosis

1. Most common cause of congenital blindness in children

2. LCA2 – one of the forms – caused by mutation in the retinal pigment epithelium-specific 65-kD protein gene (RPE65)

3. RPE65 is required to keep light-sensing photoreceptor cells – the rodes and cones of the retina – in operating order

4. The *RPE65* gene encodes for the isomerohydrolase that isomerizes bleached all-trans-retinal into photosensitive 11-cis-retinal (Jin et al., 2005; Moiseyev et al., 2005). If no 11-cis-retinal is produced due to loss of or impaired RPE65 function, the *chromophore rhodopsin cannot be assembled,* and the photoreceptors remain insensitive to light stimuli

5. LCA2 is a rare diseases – in USA only 2000 people – but is untreatable and causes blindness early in life

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

Albert M Maguire, Katherine A High*, Alberto Auricchio, J Fraser Wright, Eric A Pierce, Francesco Testa, Federico Mingozzi, Jeannette L Bennicelli, Gui-shuang Ying, Settimio Rossi, Ann Fulton, Kathleen A Marshall, Sandro Banfi, Daniel C Chung, Jessica I W Morgan, Bernd Hauck, Olga Zelenaia, Xiaosong Zhu, Leslie Raffini, Frauke Coppieters, Elfride De Baere, Kenneth S Shindler, Nicholas J Volpe, Enrico M Surace, Carmela Acerra, Arkady Lyubarsky, T Michael Redmond, Edwin Stone, Junwei Sun, Jennifer Wellman McDonnell, Bart P Leroy, Francesca Simonelli, Jean Bennett*

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.

Lancet, October 2009

Successful gene therapies

Disorder	Disease type	Patients benefiting	First publication
X-SCID	Immunodeficiency	17/20	2000
ADA-SCID	Immunodeficiency	26/37	2002
Adrenoleukodystrophy	Neurologic	2/4*	2009
Leber's congenital amaurosis	Blindness	28/30	2008
Wiskott-Aldrich syndrome	Immunodeficiency	8/10	2010
β-thalassemia	Hemoglobinopathy	1/1	2010
Hemophilia	Coagulation	6/6	2011?

*Includes a patient treated too recently to see benefit

Science, 7th October 2011

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Science, 7th October 2011

Treatment of beta-thalassemia

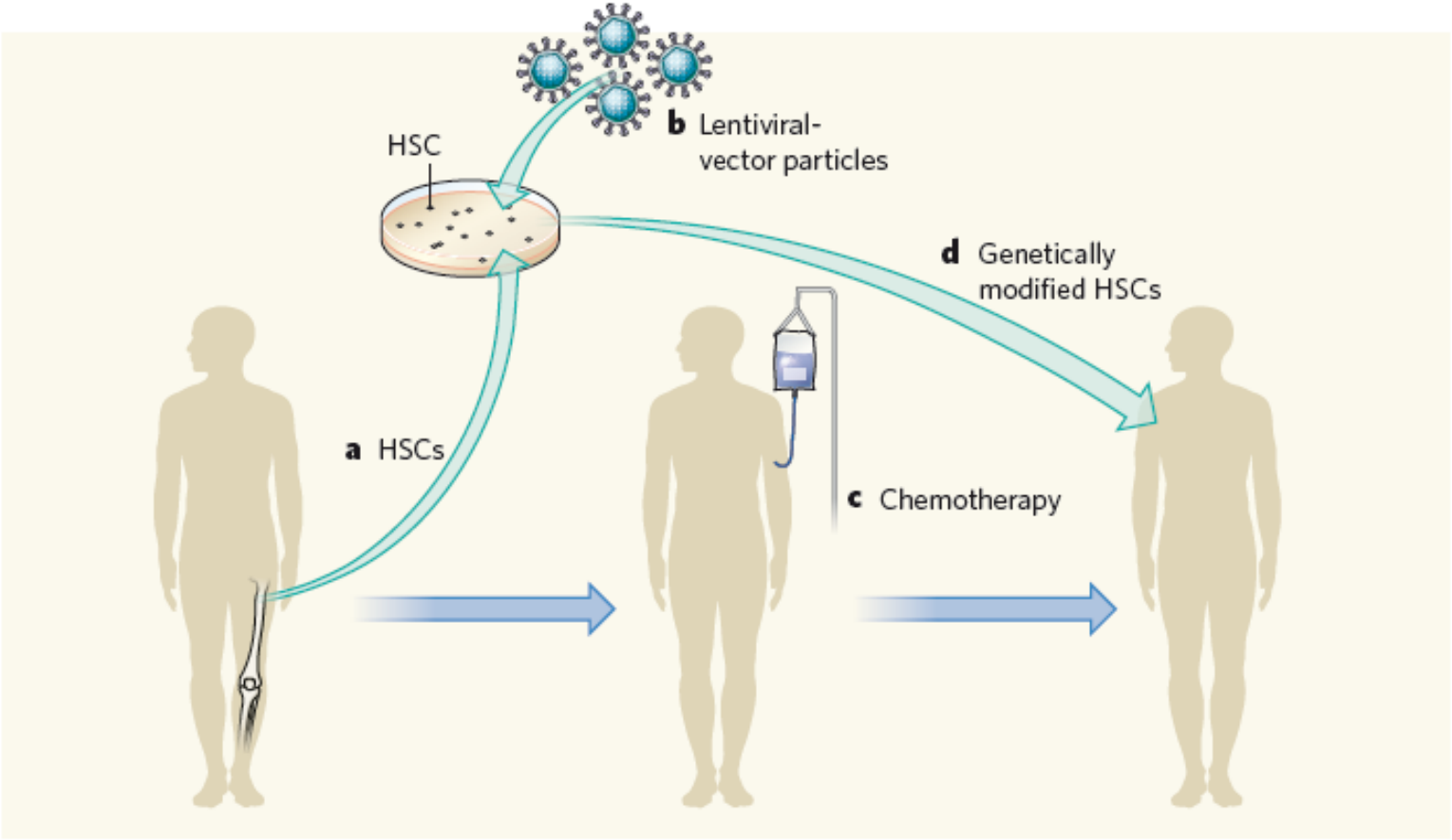

Figure 1 | Gene-therapy procedure. **a**, Cavazzana-Calvo *et al.*[2] collected haematopoietic stem cells (HSCs) from the bone marrow of a patient with β-thalassaemia and maintained them in culture. **b**, The authors then introduced lentiviral-vector particles containing a functional β-globin gene into the cells and allowed them to expand further in culture. **c**, To eradicate the patient's remaining HSCs and make room for the genetically modified cells, the patient underwent chemotherapy. **d**, The genetically modified HSCs were then transplanted into the patient.

-self-inactivating lentiviral vector

Persons, Nature September 2010

Transfusion independence and *HMGA2* activation after gene therapy of human β-thalassaemia

Marina Cavazzana-Calvo[1,2]*, Emmanuel Payen[3,4,5]*, Olivier Negre[3,4,5,6], Gary Wang[7], Kathleen Hehir[8], Floriane Fusil[3,4,5], Julian Down[8], Maria Denaro[8], Troy Brady[7], Karen Westerman[8,9], Resy Cavallesco[9], Beatrix Gillet-Legrand[6], Laure Caccavelli[1,2], Riccardo Sgarra[10], Leila Maouche-Chrétien[3,4], Françoise Bernaudin[11], Robert Girot[12], Ronald Dorazio[8], Geert-Jan Mulder[8], Axel Polack[8], Arthur Bank[13], Jean Soulier[5], Jérôme Larghero[5], Nabil Kabbara[5], Bruno Dalle[5], Bernard Gourmel[5], Gérard Socie[5], Stany Chrétien[3,4,9], Nathalie Cartier[14], Patrick Aubourg[14], Alain Fischer[1,2], Kenneth Cornetta[15], Frédéric Galacteros[16], Yves Beuzard[3,4,5], Eliane Gluckman[5], Frederick Bushman[7], Salima Hacein-Bey-Abina[1,2]* & Philippe Leboulch[3,4,9]*

The β-haemoglobinopathies are the most prevalent inherited disorders worldwide. Gene therapy of β-thalassaemia is particularly challenging given the requirement for massive haemoglobin production in a lineage-specific manner and the lack of selective advantage for corrected haematopoietic stem cells. Compound β^E/β^0-thalassaemia is the most common form of severe thalassaemia in southeast Asian countries and their diasporas[1,2]. The β^E-globin allele bears a point mutation that causes alternative splicing. The abnormally spliced form is non-coding, whereas the correctly spliced messenger RNA expresses a mutated β^E-globin with partial instability[1,2]. When this is compounded with a non-functional β^0 allele, a profound decrease in β-globin synthesis results, and approximately half of β^E/β^0-thalassaemia patients are transfusion-dependent[1,2]. The only available curative therapy is allogeneic haematopoietic stem cell transplantation, although most patients do not have a human-leukocyte-antigen-matched, geno-identical donor, and those who do still risk rejection or graft-versus-host disease. Here we show that, 33 months after lentiviral β-globin gene transfer, an adult patient with severe β^E/β^0-thalassaemia dependent on monthly transfusions since early childhood has become transfusion independent for the past 21 months. Blood haemoglobin is maintained between 9 and 10 g dl^{-1}, of which one-third contains vector-encoded β-globin. Most of the therapeutic benefit results from a dominant, myeloid-biased cell clone, in which the integrated vector causes transcriptional activation of *HMGA2* in erythroid cells with further increased expression of a truncated *HMGA2* mRNA insensitive to degradation by let-7 microRNAs. The clonal dominance that accompanies therapeutic efficacy may be coincidental and stochastic or result from a hitherto benign cell expansion caused by dysregulation of the *HMGA2* gene in stem/progenitor cells.

In 2007, Cavazzana-Calvo and colleagues[2] treated an 18-year-old male patient who had HbE/β-thalassaemia — a form of the disorder in which haemoglobin production is severely compromised. They treated the patient's HSCs with an HIV-derived lentiviral vector containing a functional β-globin gene (Fig. 1). In a bold move, the investigators gave the patient a high dose of chemotherapy before administering his genetically modified HSCs. Their aim was to eliminate most, if not all, of the diseased HSCs in the patient's body. This severe degree of pre-transplant 'conditioning' seems to have been crucial for the success of the treatment. Had the conditioning been less intense, the genetically corrected HSCs might have become diluted by residual host HSCs, possibly compromising the outcome.

blood cells: the levels of genetically modified cells rose from less than 2% in the first few months to 11% at 33 months post-transplant. Concomitantly, levels of the normal β-globin protein increased, with 10–20% of reconstituted HSCs containing the transferred globin gene; this resulted in the improved production and quality of red blood cells. Remarkably, a year after the treatment, the patient no longer needed blood transfusions. Although, three years on, he remains mildly anaemic and shows signs of compensatory expansion of red-blood-cell precursors in his bone marrow, absence of the need for blood transfusions means that this case can be viewed as a clinical success.

Nature, September 2010

SUMMARY

Gene therapy is effective in a number of monogenic diseases

1. Immunodeficiencies

- X-SCID immunodeficiency: *retroviral vectors & hematopoietic stem cells*
- ADA- immunodeficiency - *retroviral vectors & hematopoietic stem cells*
- chronic granulomatous diseases - *retroviral vectors & hematopietic stem cells*

2. Congential blindness:

- *Leber's congenital amaurosis – rAAV vectors*

Some beneficial effects have been observed in treatment of:

1. Adrenoleukodystrophy – lentiviral vector & hematopoietic stem cells
2. β-thalassemia – lentiviral vector & hematopoietic stem cells

Exam

Monday 30th January, 13.00-15.00 – room D107