Gene therapy of monogenic diseases

examples

Lecture 9

12 December 2011

Exam

Monday 30th January, 13.00-15.00 – room D107

Genetic diseases - incidence, mutated genes and detection rate

Disorder	Incidence	Gene	Mutation detection rate
Monogenic			
Cystic fibrosis	1:4000	CFTR	98%
Duchenne muscular dystrophy	1:3500	DMD	~90%
Fragile X syndrome	1:4000	FMR	100%
Huntington 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%

Inborn error of metabolism – lipid disorders

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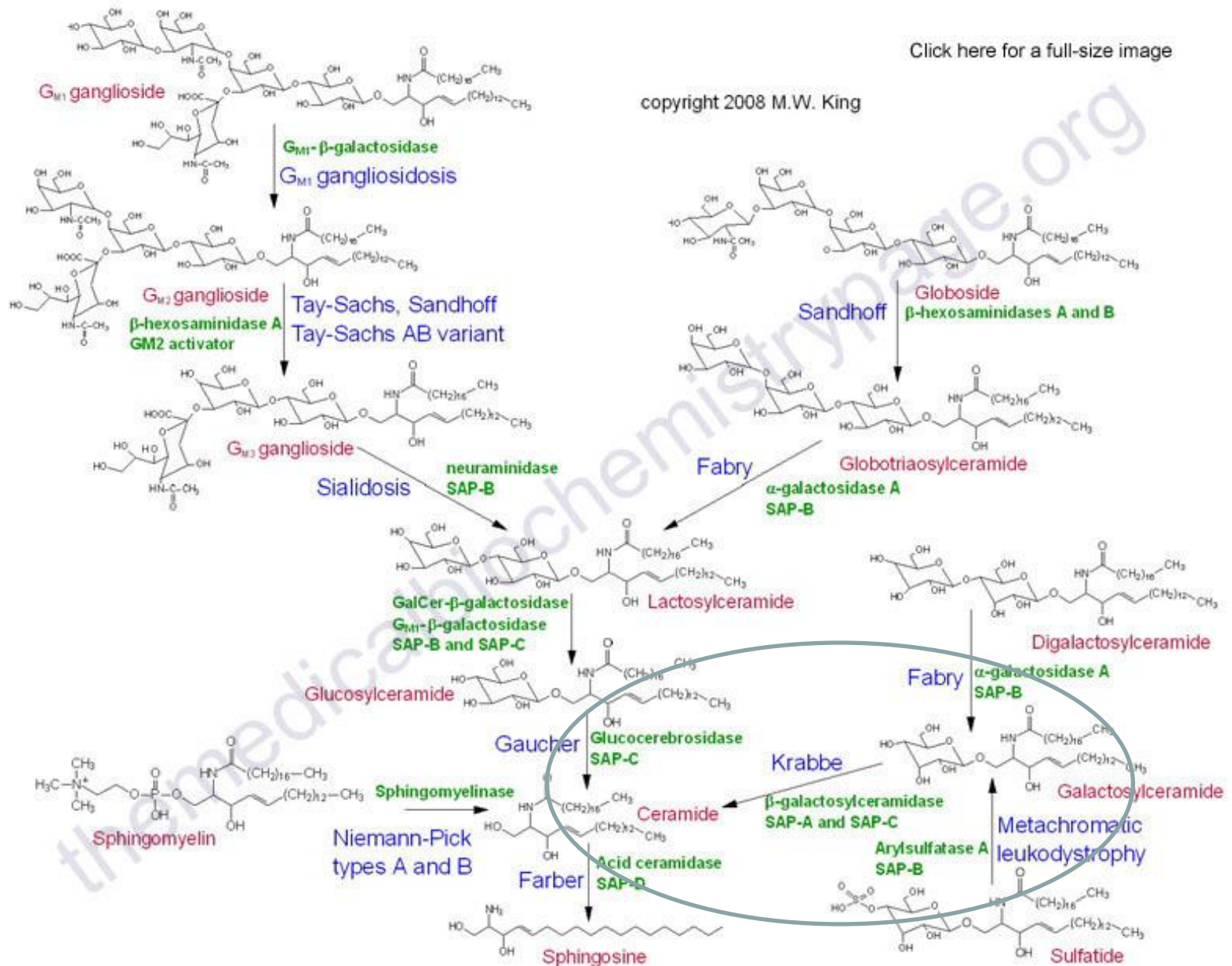
What Causes Krabbe Disease?

Krabbe Disease is an autosomal recessive disorder resulting from a deficiency in an enzyme known as **galactocerebrosidase** (GALC)

GALC is an enzyme that breaks down molecules called galactolipids, which are heavily present in the brain. There are many different galactolipids, but the buildup of one in particular, called psychosine, appears to be responsible for a good deal of the pathology of Krabbe disease.

In patients with Krabbe disease, psychosine can be at levels 100 times that of healthy individuals, and this buildup is thought to lead to the demyelination observed in Krabbe Disease.

Another name for Krabbe Disease is Globoid Cell Leukodystrophy. This name comes from a characteristic pathology of Krabbe Disease, where macrophages accumulate high levels of undegraded galactolipids as a result of the lack of GALC activity. These cells look different from healthy cells, and are termed globoid cells.

Psychosine is responsible for demyelination in Krabbe's disease

S. Giri et al., J Lipid Res 2006
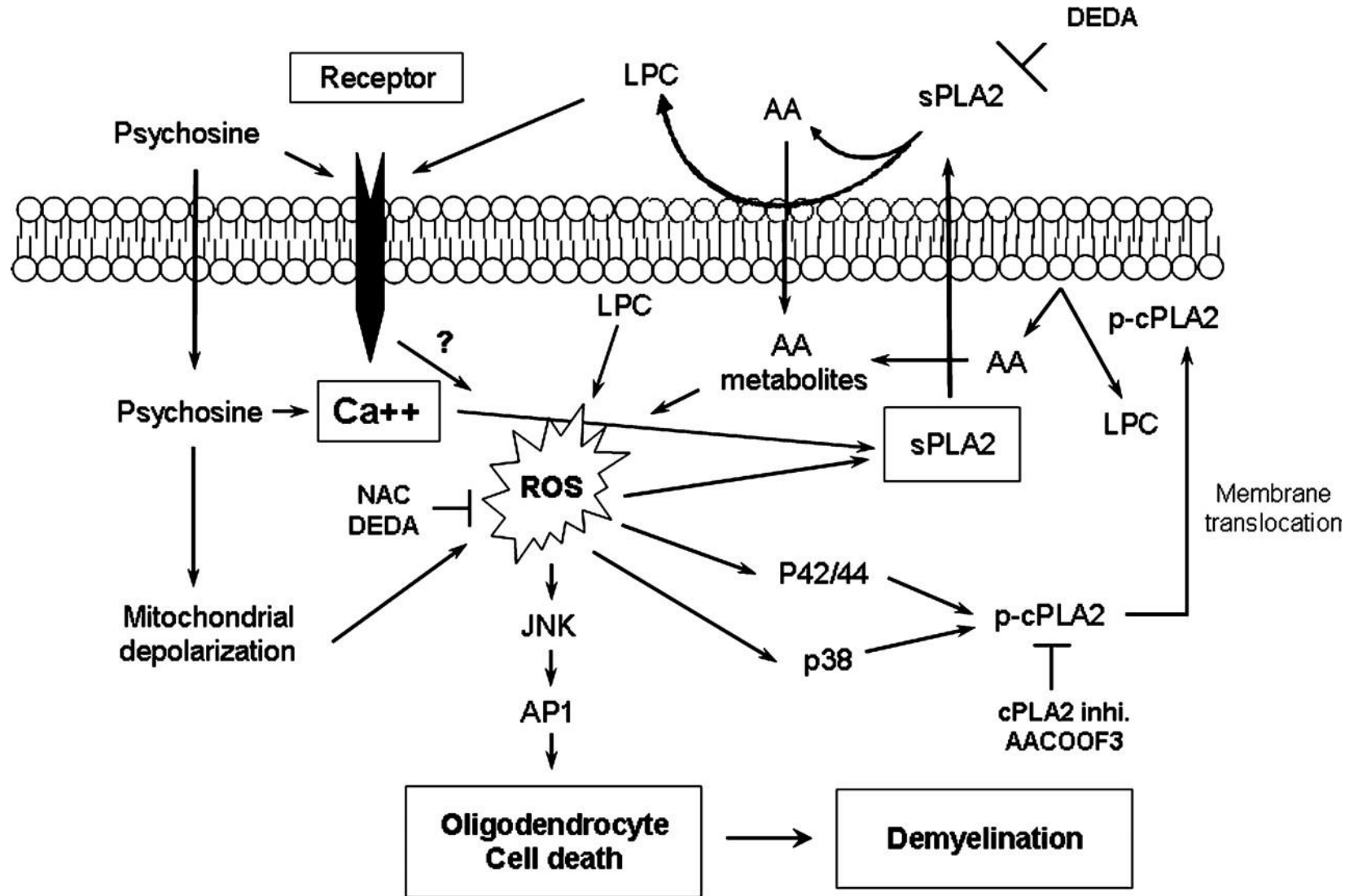
Methods of treatment of globoid-cell leukodystrophy

1. Hematopoietic stem cell transplantation - based on the concept of metabolic cross-correction in which the lysosomal enzyme of one cell is taken by the enzyme--deficient adjacent cell

   - associated with substantial morbidity and mortality

2. Enzyme replacement therapy (ERT) – thus far ineffective in achieving adequate delivery of the enzyme to the brain

3. Gene therapy

What are the symptoms of Krabbe Disease?

<u>The majority of cases of Krabbe Disease appear within the first year of life</u>. **The patients rapidly regress to a condition with little to no brain function, and generally die by age 2, though some have lived longer.** Death generally occurs as a result of a respiratory infection or brain fever.

Symptoms that might be encountered in **the infantile form of Krabbe Disease** include:
Developmental delay
Seizures
Limb stiffness
Optic atrophy: wasting of a muscle of the eye, resulting in vision diffculties
Neurosensoral deafness
Extreme irritability
Spasticity: presence of spasms
Ataxia: loss of the ability to control muscular movement
Progressive psychomotor decline: progressive decline in the coordination of movement

Although <u>the majority of Krabbe Disease patients show symptoms within the first year of life</u>, **there have been cases diagnosed at all ages, through late adulthood**. In general, the earlier the diagnosis, the more rapid the progression of the disease. Those who first show symptoms at ages 2-14 will regress and become severely incapacitated, and generally die 2-7 years following diagnosis. Some patients who have been diagnosed in the adolescent and adult years have symptoms that remain confined to weakness without any intellectual deterioration, while others may become bedridden and deteriorate both mentally and physically.

Krabbe disease

Frequency

United States

Calculated incidence of Krabbe disease is 1 case per 100,000 population.

International

Overall calculated European incidence is 1 case per 100,000 population, with a higher reported incidence in Sweden of 1.9 cases per 100,000 population. An unusually high incidence, 6 cases per 1000 live births, is reported in the Druze community in Israel.

Mortality/Morbidity

Morbidity in patients with all subtypes arises from the primary progressive neurodegeneration of the central and peripheral nervous systems and secondary effects of the disease (ie, weakness, seizure, loss of protective reflexes, immobility). The sequelae, including infection and respiratory failure, cause most deaths.

Race

Krabbe disease is panethnic, although most reported cases have been among people of European ancestry. Late-onset Krabbe disease may be more common in southern Europe.

Sex

Krabbe disease is inherited as an autosomal recessive trait and equally affects both sexes.

Age

Typical age of onset is 3-6 months for the infantile form of Krabbe disease (type 1), 6 months to 3 years for the late infantile form (type 2), 3-8 years for the juvenile form (type 3), and older than 8 years for the adult form (type 4).

Identification of Hematopoietic Stem Cell–Specific miRNAs Enables Gene Therapy of Globoid Cell Leukodystrophy

Bernhard Gentner,[1,2]* Ilaria Visigalli,[1,2]* Hidefumi Hiramatsu,[3]* Eric Lechman,[3]* Silvia Ungari,[1,2] Alice Giustacchini,[1,2] Giulia Schira,[1] Mario Amendola,[1] Angelo Quattrini,[4] Sabata Martino,[5] Aldo Orlacchio,[5] John E. Dick,[3] Alessandra Biffi,[1†‡] Luigi Naldini[1,2†‡]

Globoid cell leukodystrophy (GLD; also known as Krabbe disease) is an invariably fatal lysosomal storage disorder caused by mutations in the galactocerebrosidase (GALC) gene. Hematopoietic stem cell (HSC)–based gene therapy is being explored for GLD; however, we found that forced GALC expression was toxic to HSCs and early progenitors, highlighting the need for improved regulation of vector expression. We used a genetic reporter strategy based on lentiviral vectors to detect microRNA activity in hematopoietic cells at single-cell resolution. We report that miR-126 and miR-130a were expressed in HSCs and early progenitors from both mice and humans, but not in differentiated progeny. Moreover, repopulating HSCs could be purified solely on the basis of miRNA expression, providing a new method relevant for human HSC isolation. By incorporating miR-126 target sequences into a GALC-expressing vector, we suppressed GALC expression in HSCs while maintaining robust expression in mature hematopoietic cells. This approach protected HSCs from GALC toxicity and allowed successful treatment of a mouse GLD model, providing a rationale to explore HSC-based gene therapy for GLD.

Science Translational Medicine Nov 2010

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microRNA regulated vectors to drive expression in a specific cell


Naldini i wsp. 2009
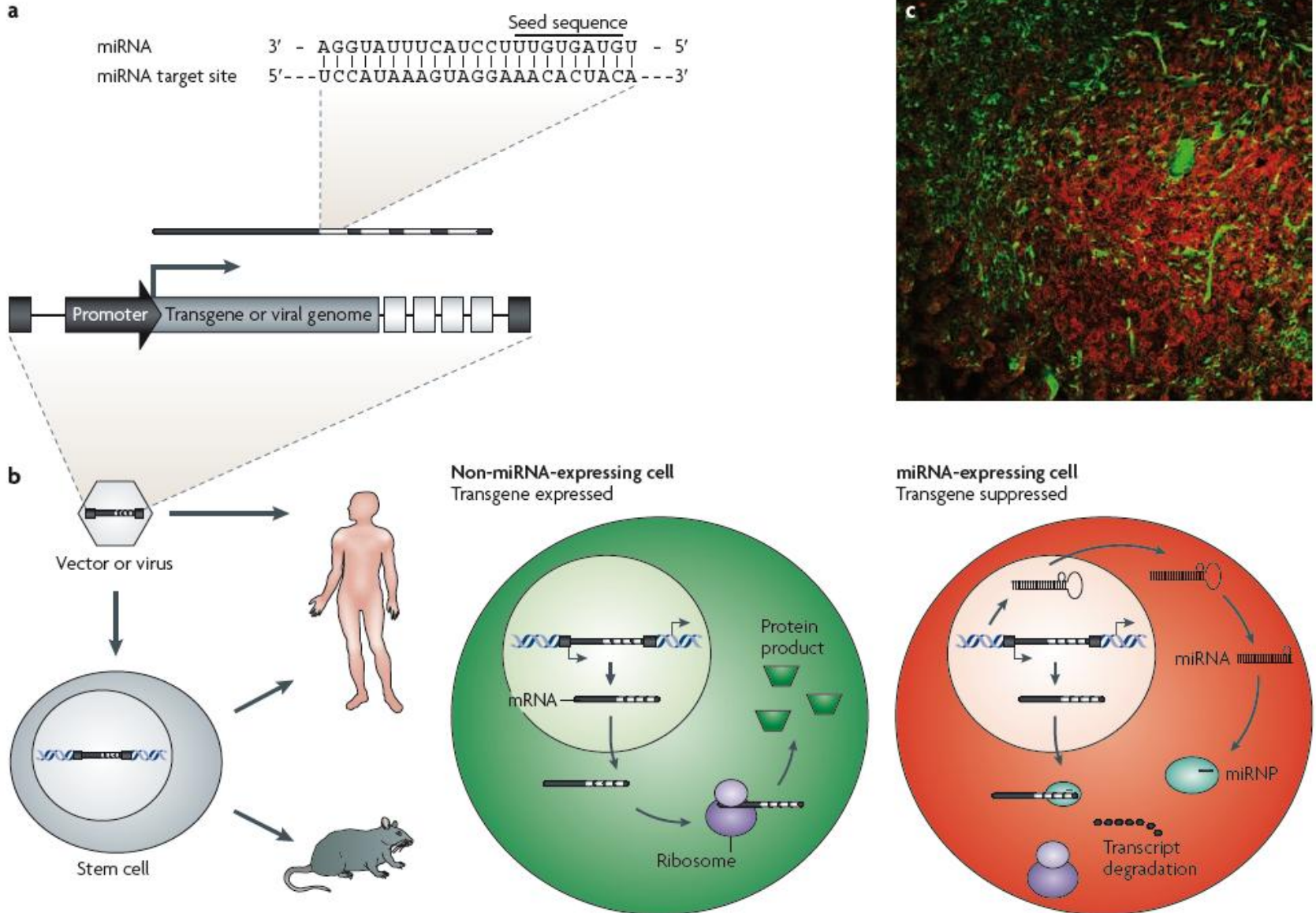
microRNA based strategy for treatment of Krabbe disease

1. HSC transplantation

2. Gene transfer of GALC into HSC

3. GALC has been found to be be toxic for early HSC

4. Identification of microRNA specific for HSC:
   mir-126 and mir-130a

5. Design and construction of microRNA regulated vector:
   mir-126/mir-130 target sites in 3' region

6. GALC is not expressed in early HSC transduced with mirRNA-regulated vector, but when HSC differentiate, expression of mir-126 and mir-130a goes down and GALC is expressed

Gene therapy for Krabbe's disease

Orchard & Wagner, NEJM 2011

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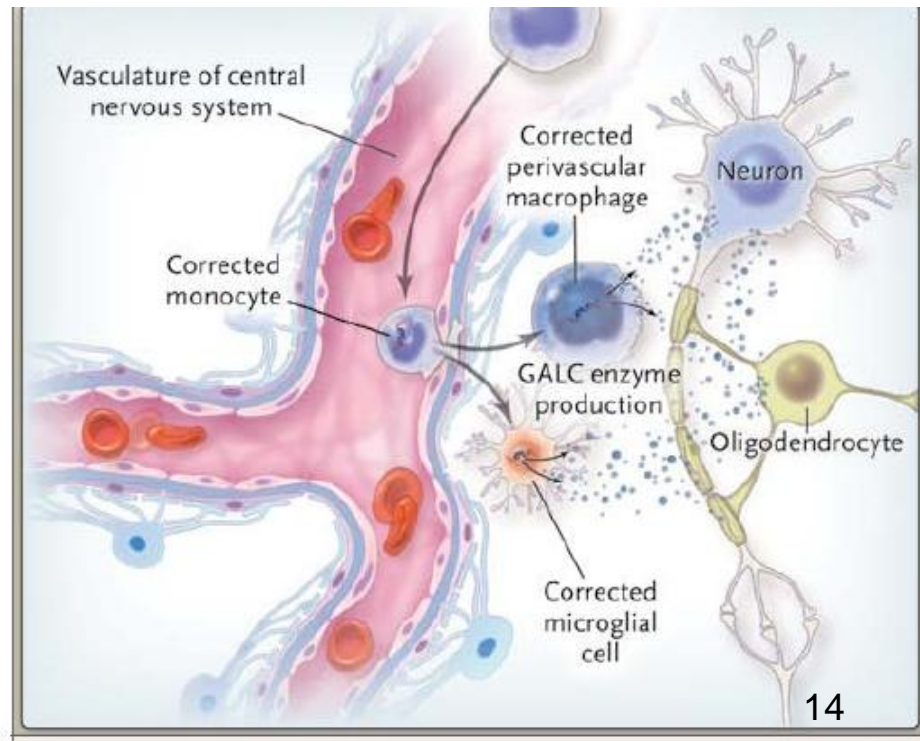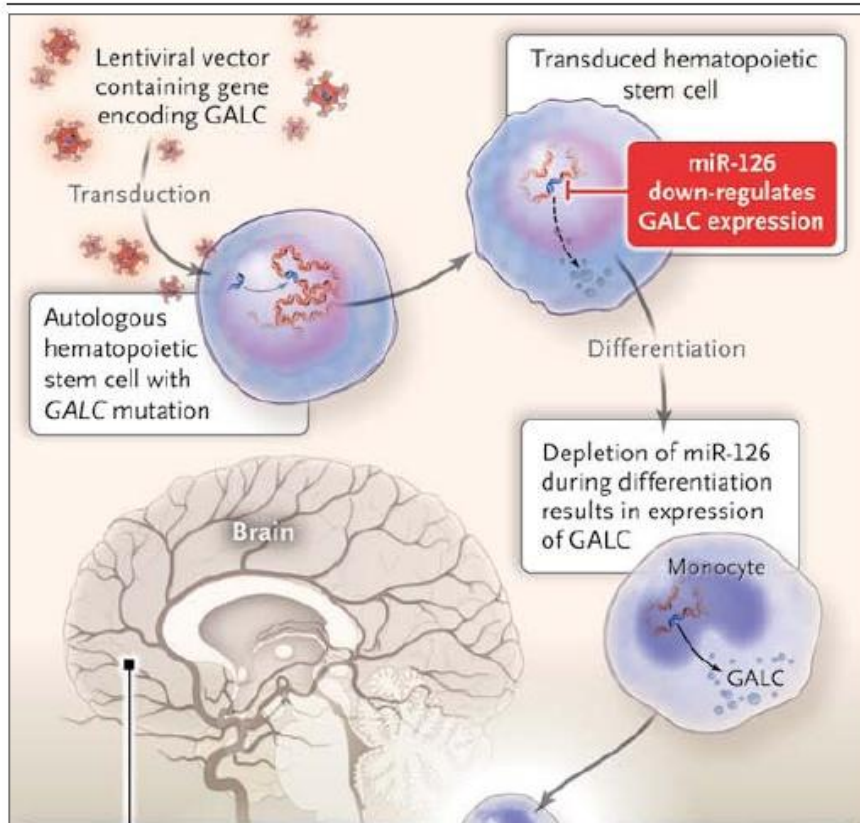
Therapeutic efficacy of HSC gene therapy in GLC mice

Gertner/Naldini, Science Transl Med. 2010
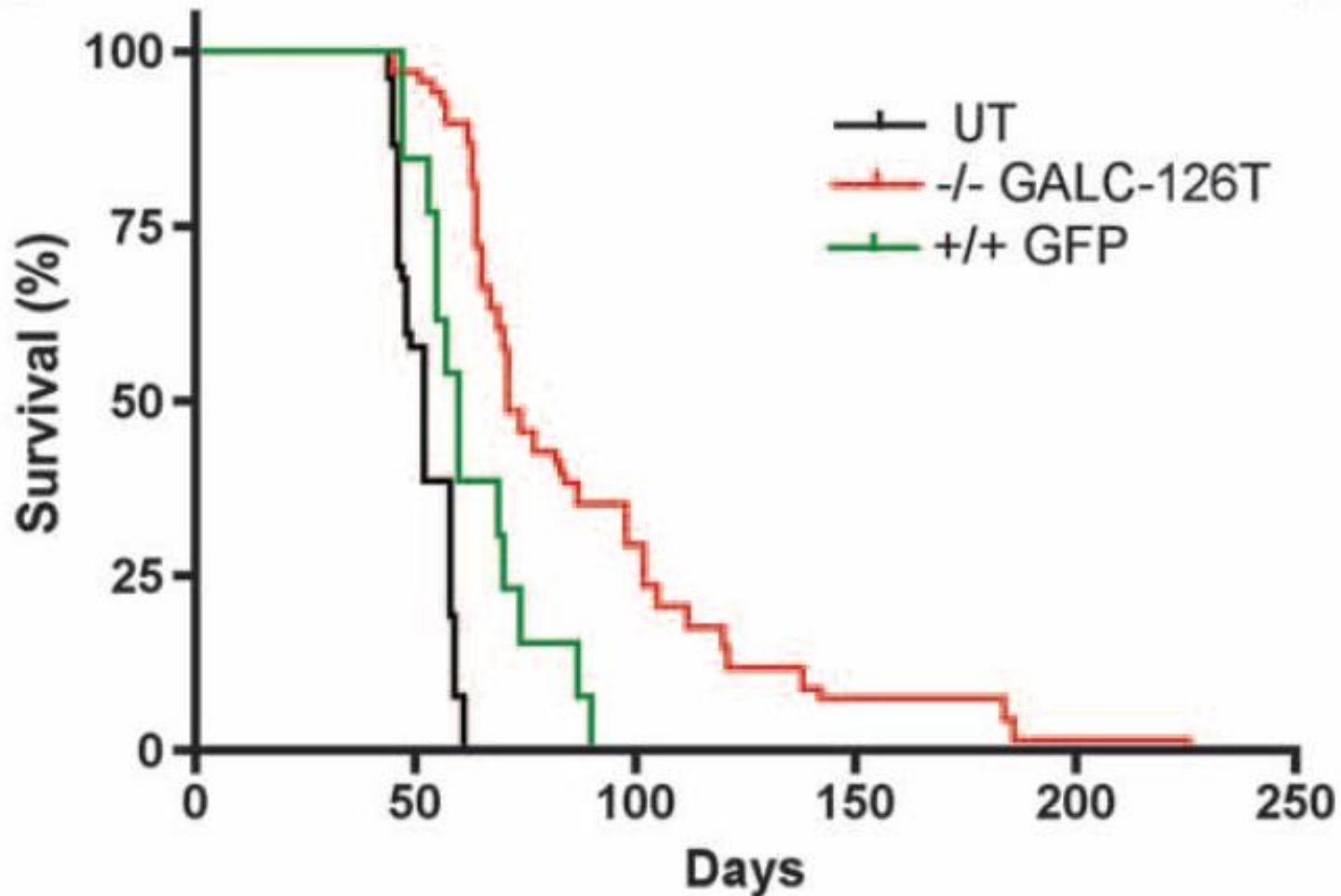
Clinical gene therapy of monogenic diseases

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

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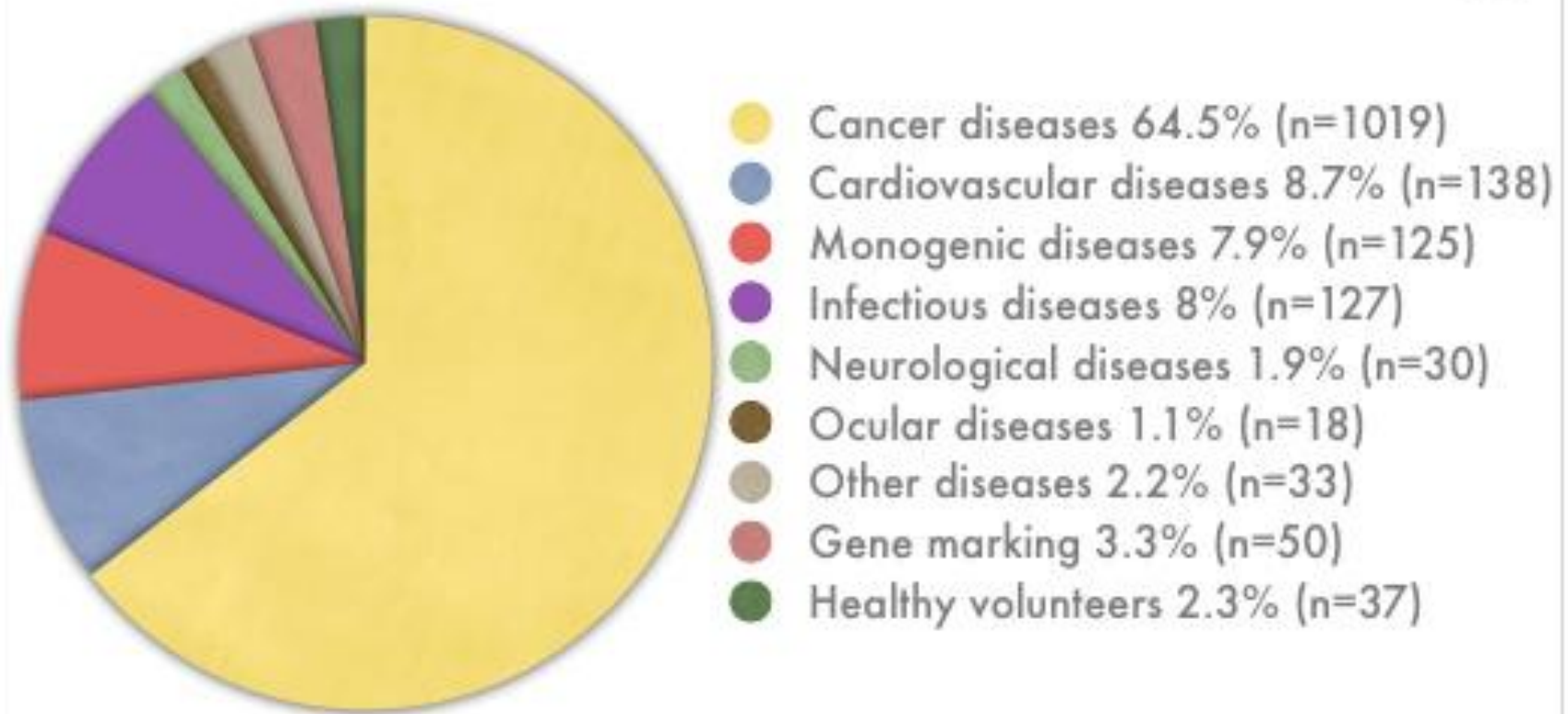
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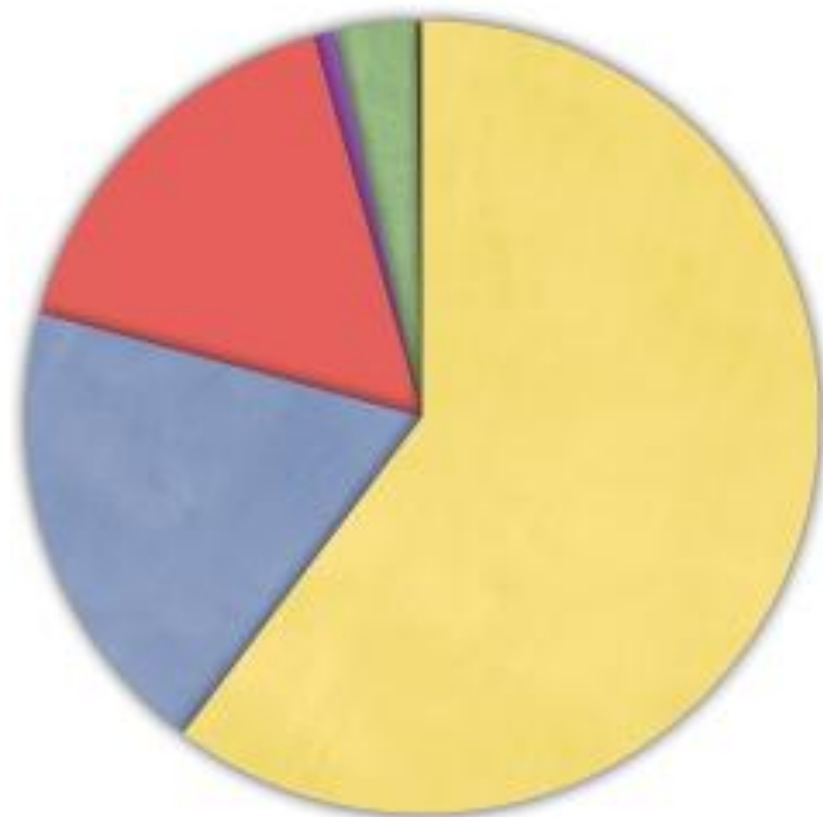# Indications Addressed by Gene Therapy Clinical Trials
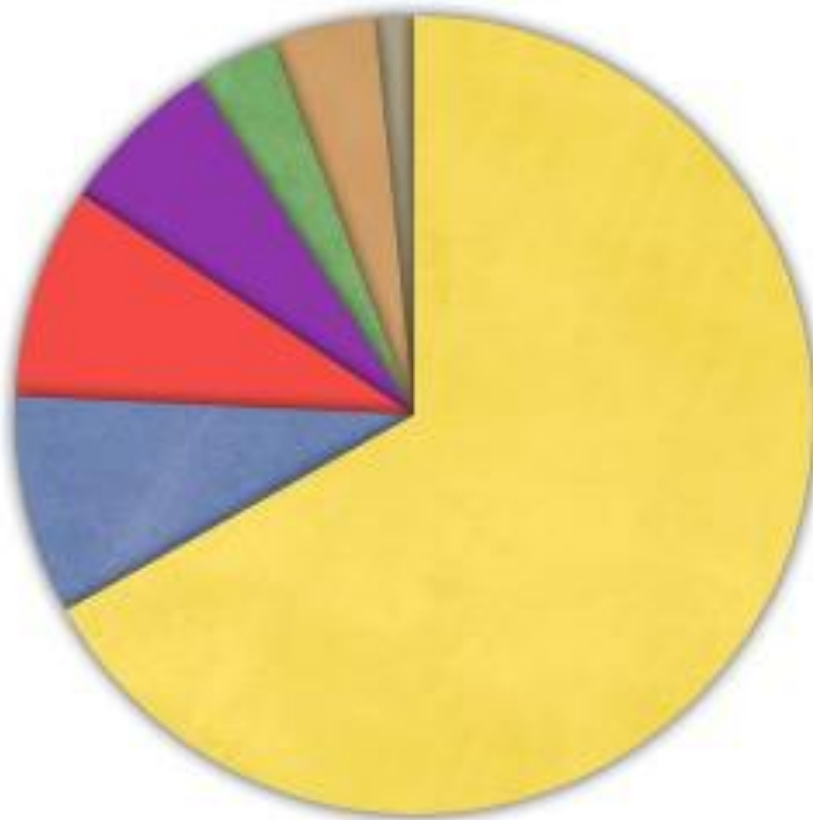
- Cancer diseases 67% (n=797)
- Vascular diseases 8.9% (n=106)
- Monogeneic diseases 8.6% (n=102)
- Infectious diseases 6.5% (n=78)
- Other diseases 3.4% (n=40)
- Gene marking 4.2% (n=50)
- Healthy volunteers 1.6% (n=19)

The Journal of Gene Medicine, © 2006 John Wiley and Sons Ltd   www.wiley.co.uk/genmed/clinical

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2009

Indications Addressed by Gene Therapy Clinical Trials

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

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Phases of Gene Therapy Clinical Trials
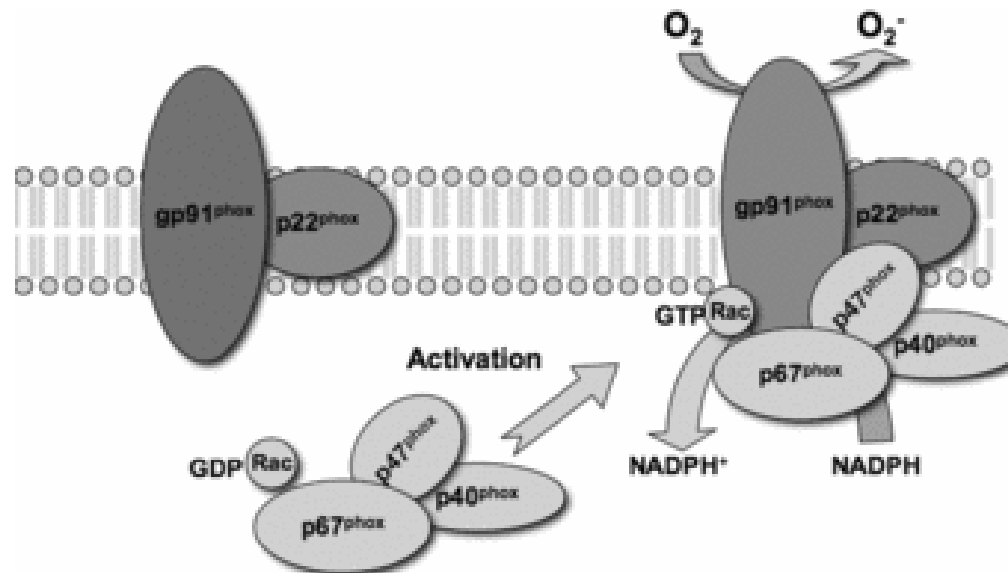

Legend:
- Phase I 60.3% (n=952)
- Phase I/II 18.9% (n=299)
- Phase II 16.3% (n=258)
- Phase II/III 0.8% (n=13)
- Phase III 3.4% (n=53)
- Phase IV 0.1% (n=2)
- Single subject 0.1% (n=2)

www.wiley.co.uk/genmed/clinical
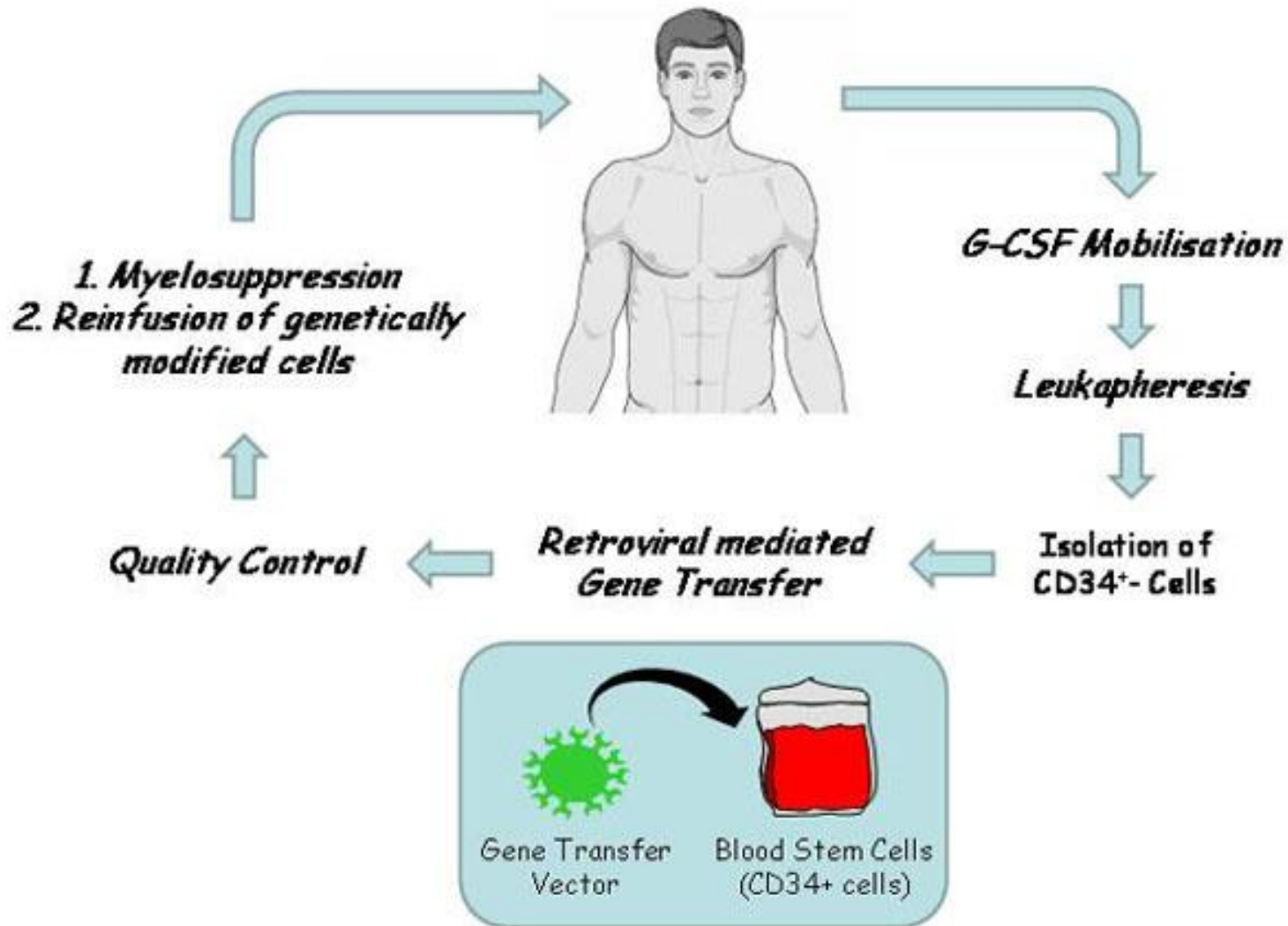
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VERY IMPORTANT INFORMATION

1. The effectiveness of any treatment (drugs, gene therapy) must be confirmed
     in controlled clinical trials.

2. In case of diseases, which development and progression is multifactorial,
     and the course of disease can vary (significantly) between individual patients,
     and the number of patients is large (eg. cardiovascular diseases, cancer)
     it is necessary to peform clinical trials at all steps/stages, before any definitive
     conclusions on the efficacy can be stated!

3. The composition of the study group is very important! Eg. it cannot be conclusively
     stated that treatment is effective in a given type of cancer, if the classification
     of the stage and patient recrutiment was not proper

4. It is always necessary to record and control the other type of treatment when the given
     therapy is tested: the patients may benefit from the other drugs used at the same
     time

5. In case of rare diseases, in which the other treatment is not effective, the
     improvement after well-performed gene therapy trial can be a strong indication
     for the effectiveness of therapy even when observed in single patients.

Chronic granulomatous disease

Chronic granulomatous disease (CGD) is a rare inherited immunodeficiency characterized by recurrent, often life threatening bacterial and fungal infections due to a functional defect in the microbial-killing activity of phagocytic neutrophils. It occurs as a result of mutations in genes encoding a multicomponent enzyme complex, the NADPH oxidase, that catalyses the respiratory burst. The majority of patients have an X-linked form of the disease which is associated with **mutations in a membrane-bound component gp91phox**. HLA-matched allogeneic hematopoietic stem cell (HSC) transplantation can be curative, but for patients without suitable donors, genetic modification of autologous hematopoietic stem cells is an attractive alternative.

Gene Therapy for X-CGD

1. Myelosuppression
2. Reinfusion of genetically modified cells

G-CSF Mobilisation

Leukapheresis

Quality Control ← Retroviral mediated Gene Transfer ← Isolation of CD34⁺- Cells

Gene Transfer Vector   Blood Stem Cells (CD34+ cells)

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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 gp91phox. We detected substantial gene transfer in both individuals' neutrophils that lead to a large number of functionally corrected phagocytes and notable clinical improvement. Large-scale retroviral integration site–distribution analysis showed activating insertions in *MDS1-EVI1*, *PRDM16* or *SETBP1* that had influenced regulation of long-term hematopoiesis by expanding gene-corrected myelopoiesis three- to four-fold in both individuals. Although insertional influences have probably reinforced the therapeutic efficacy in this trial, our results suggest that gene therapy in combination with bone marrow conditioning can be successfully used to treat inherited diseases affecting the myeloid compartment such as CGD.

Nature Medicine April 2006

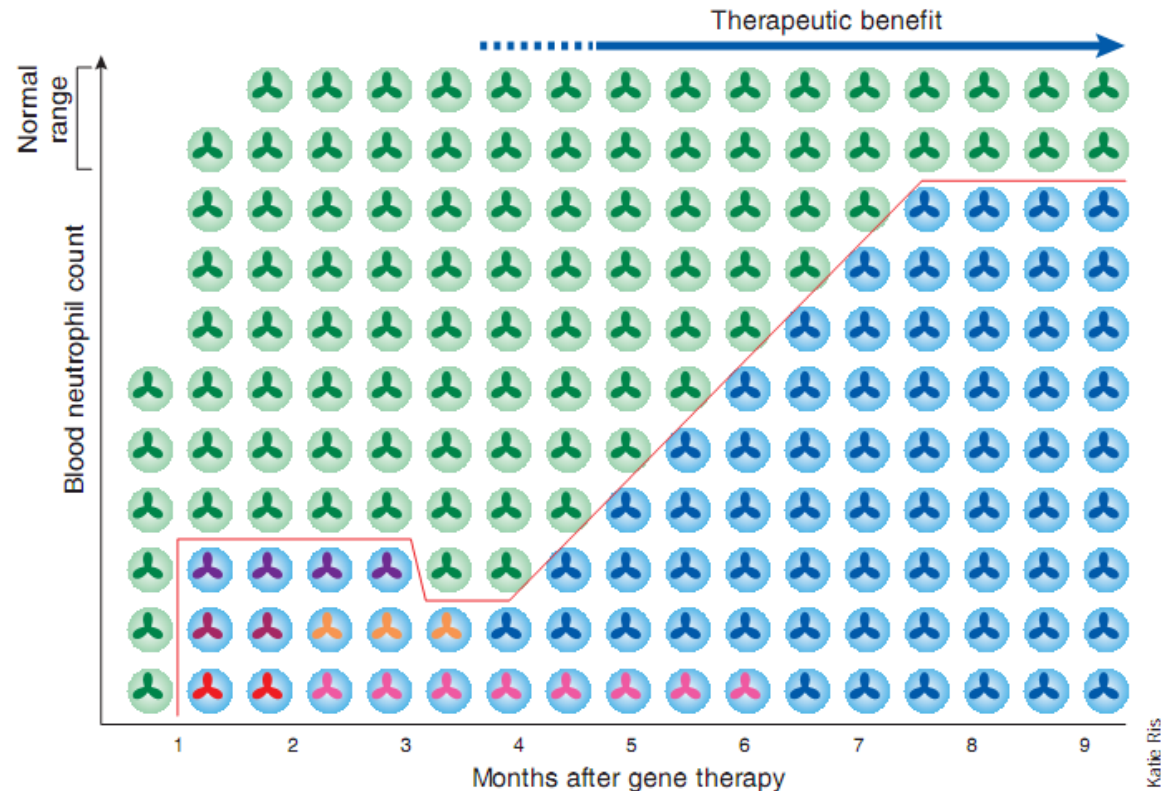# Correction of neutrophil bacteriocidal function by overexpression of gp91phox subunit of NADPH oxidase

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

Retrovirus gene therapy for X-linked chronic granulomatous disease can achieve stable long-term correction of oxidase activity in peripheral blood neutrophils.

Kang EM, Choi U, Theobald N, Linton G, Long Priel DA, Kuhns D, Malech HL.
Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA. ekang@niaid.nih.gov

Abstract

Chronic granulomatous disease (CGD) is associated with significant morbidity and mortality from infection. The first CGD gene therapy trial resulted in only short-term marking of 0.01% to 0.1% of neutrophils. A recent study, using busulfan conditioning and an SFFV retrovirus vector, achieved more than 20% marking in 2 patients with X-linked CGD. However, oxidase correction per marked neutrophil was less than normal and not sustained. Despite this, patients clearly benefited in that severe infections resolved. As such, we initiated a gene therapy trial for X-CGD to treat severe infections unresponsive to conventional therapy. We treated 3 adult patients using busulfan conditioning and an MFGS retroviral vector encoding gp91(phox), achieving early marking of 26%, 5%, and 4% of neutrophils, respectively, with sustained long-term marking of 1.1% and 0.03% of neutrophils in 2 of the patients. Gene-marked neutrophils have sustained full correction of oxidase activity for 34 and 11 months, respectively, with full or partial resolution of infection in those 2 patients. Gene marking is polyclonal with no clonal dominance. We conclude that busulfan conditioning together with an MFGS vector is capable of achieving long-term correction of neutrophil oxidase function sufficient to provide benefit in management of severe infection. This study was registered at www.clinicaltrials.gov as #NCT00394316.

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 is succesful in treatment of disease

Some Gene Therapy Successes

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

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Science, 7th October 2011

Other gene therapy trials

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

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

Skin biopsy to isolate fibroblasts

Transfection with factor VIII plasmid

Selection and cloning of cells expressing factor VIII

Expansion and characterization of clone

Implantation of cells in omentum

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
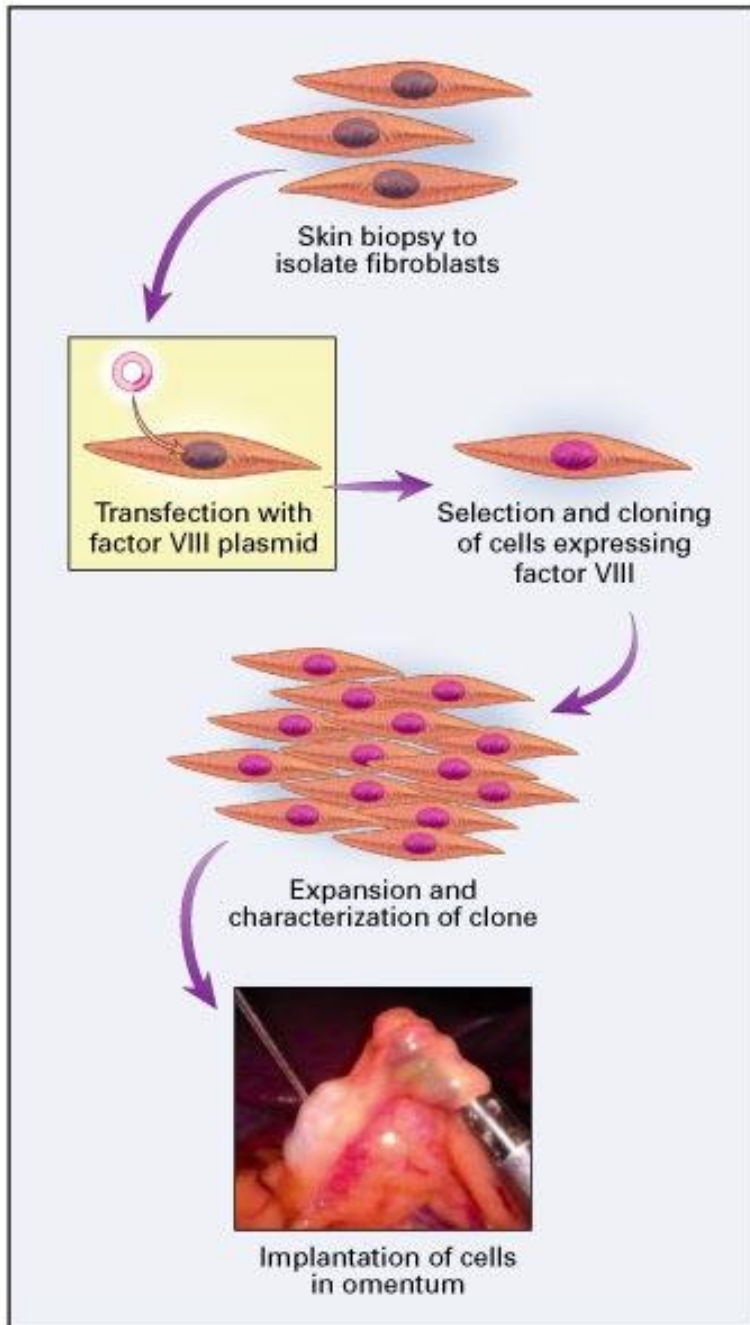
Clinical gene therapy for haemophilia A

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	6
<0.8% of normal (no. of patients)	
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.

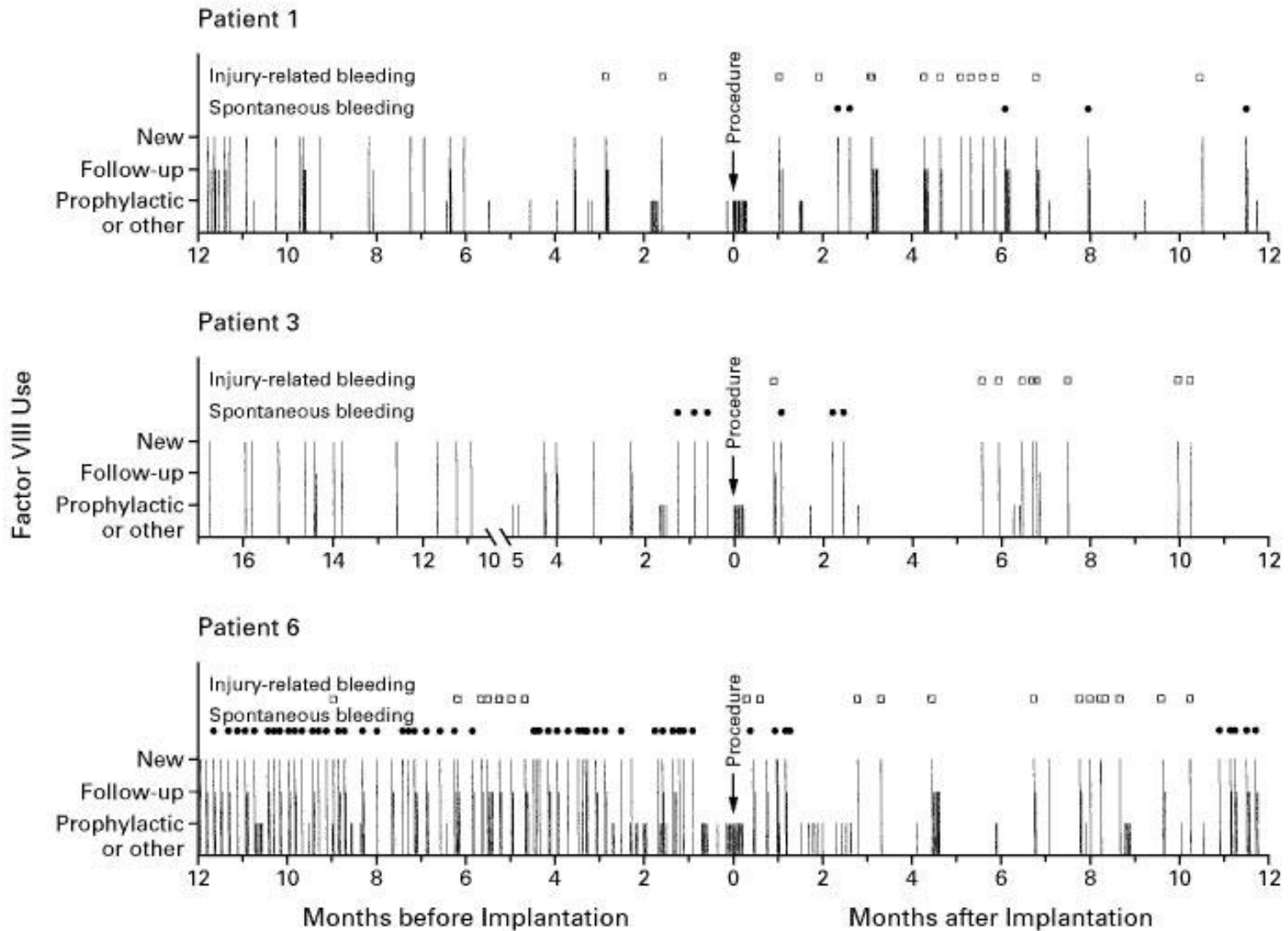**TABLE 2.** TOTAL FACTOR VIII PRODUCTION BY IMPLANTED AUTOLOGOUS FIBROBLASTS.*

PATIENT NO.	FACTOR VIII PRODUCTION BY HARVESTED CELLS†	NO. OF CELLS IMPLANTED	TOTAL FACTOR VIII PRODUCTION BY IMPLANTED CELLS‡
	IU/10^8 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.

Bleeding Events and Use of Exogenous Factor VIII in Three of the Six Patients.

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

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
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β-thalassemia	Hemoglobinopathy	1/1	2010
Hemophilia	Coagulation	6/6	2011?

*Includes a patient treated too recently to see benefit

Science, 7th October 2011

Table 1 | Summary of clinical trials using AAV gene transfer

Disease	Transgene product	Serotype	Route of administration	Clinical trial
AAV clinical trials for inherited diseases				
α1 antitrypsin deficiency	α1 antitrypsin	AAV2	Intramuscular	Phase I/II
		AAV1		
Batten's disease	CLN2	AAV2	Direct intracranial administration	Phase I
		AAVrh10		
Canavan's disease	Aspartoacylase	AAV2	Direct intracranial administration	Phase I
Cystic fibrosis	CFTR	AAV2	Direct instillation to maxillary sinus, bronchoscopy to right lower lobe, aerosol to whole lung	Phase I/II
Haemophilia B	Factor IX	AAV2	Intramuscular	Phase I/II
			Hepatic	
		AAV8	Intravenous	
LPL deficiency	LPL	AAV1	Intramuscular	Phase I/II
Pompe's disease	GAA	AAV1	Series of intradiaphragmatic injections	Phase I/II
Muscular dystrophy: Duchenne	Microdystrophin	AAV1–AAV2 hybrid	Intramuscular	Phase I
Muscular dystrophy: limb girdle	α-sarcoglycan	AAV1	Two to six separate injections into the selected muscle	Phase I

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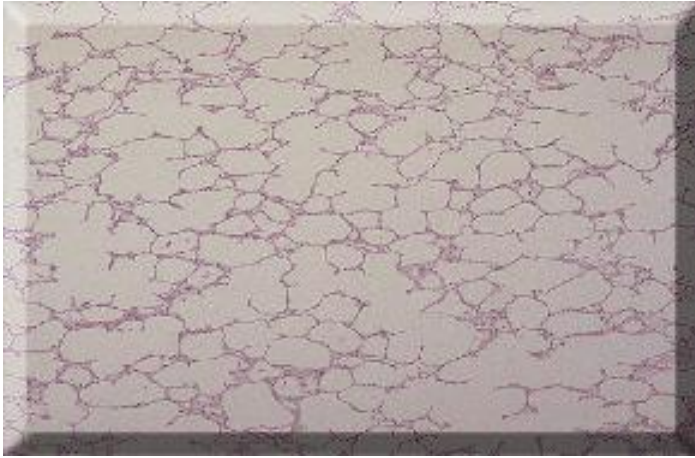
Cystic fibrosis
Mukowiscydoza
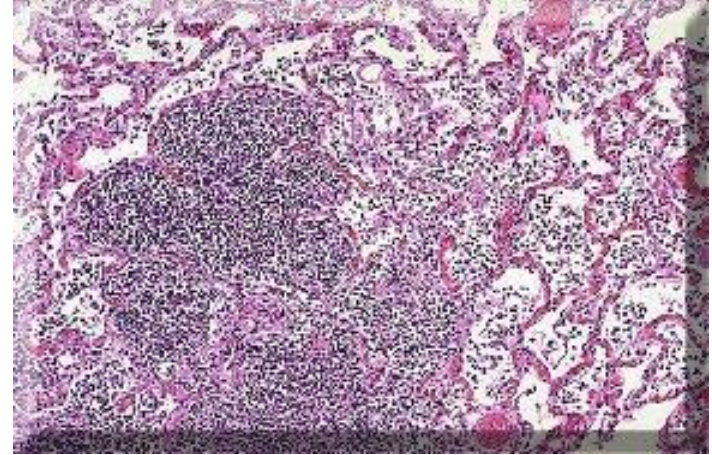
Cystic fibrosis
Mukowiscydoza

- 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
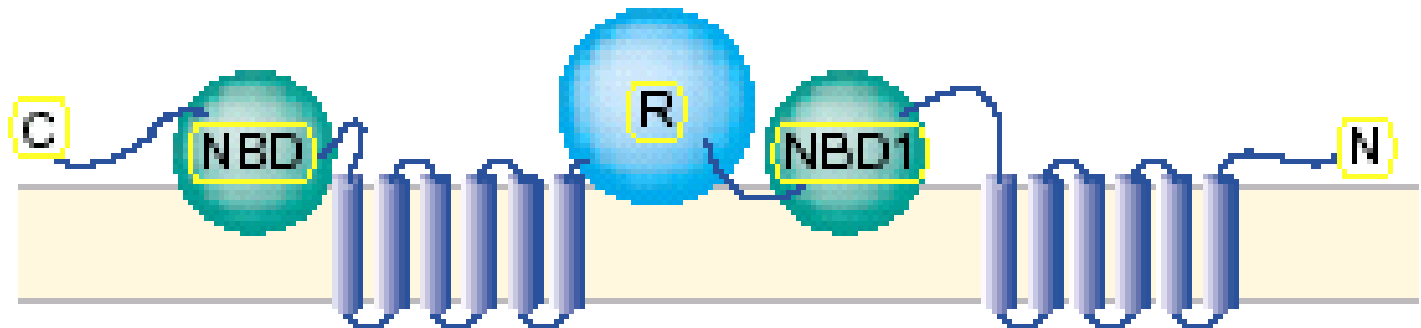
Median survival remains about 35 years

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)

History of research on cystic fibrosis

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.

CF Mouse

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.

45
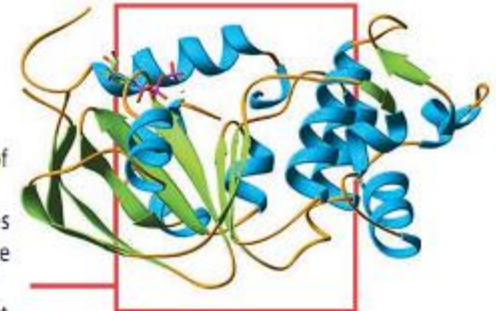
Cystic fibrosis key dates

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.

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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

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

51
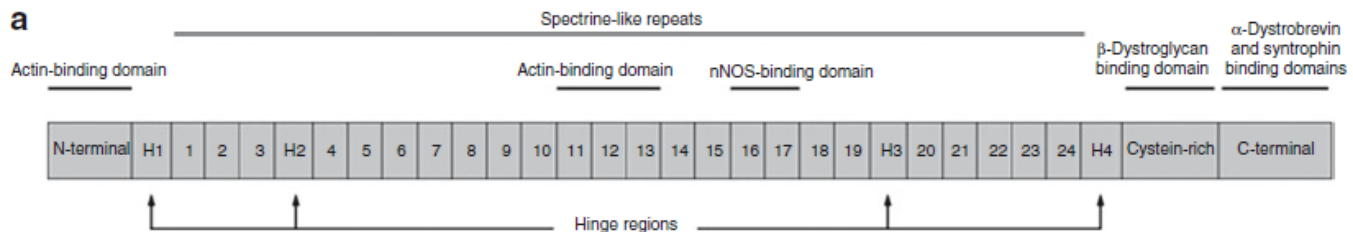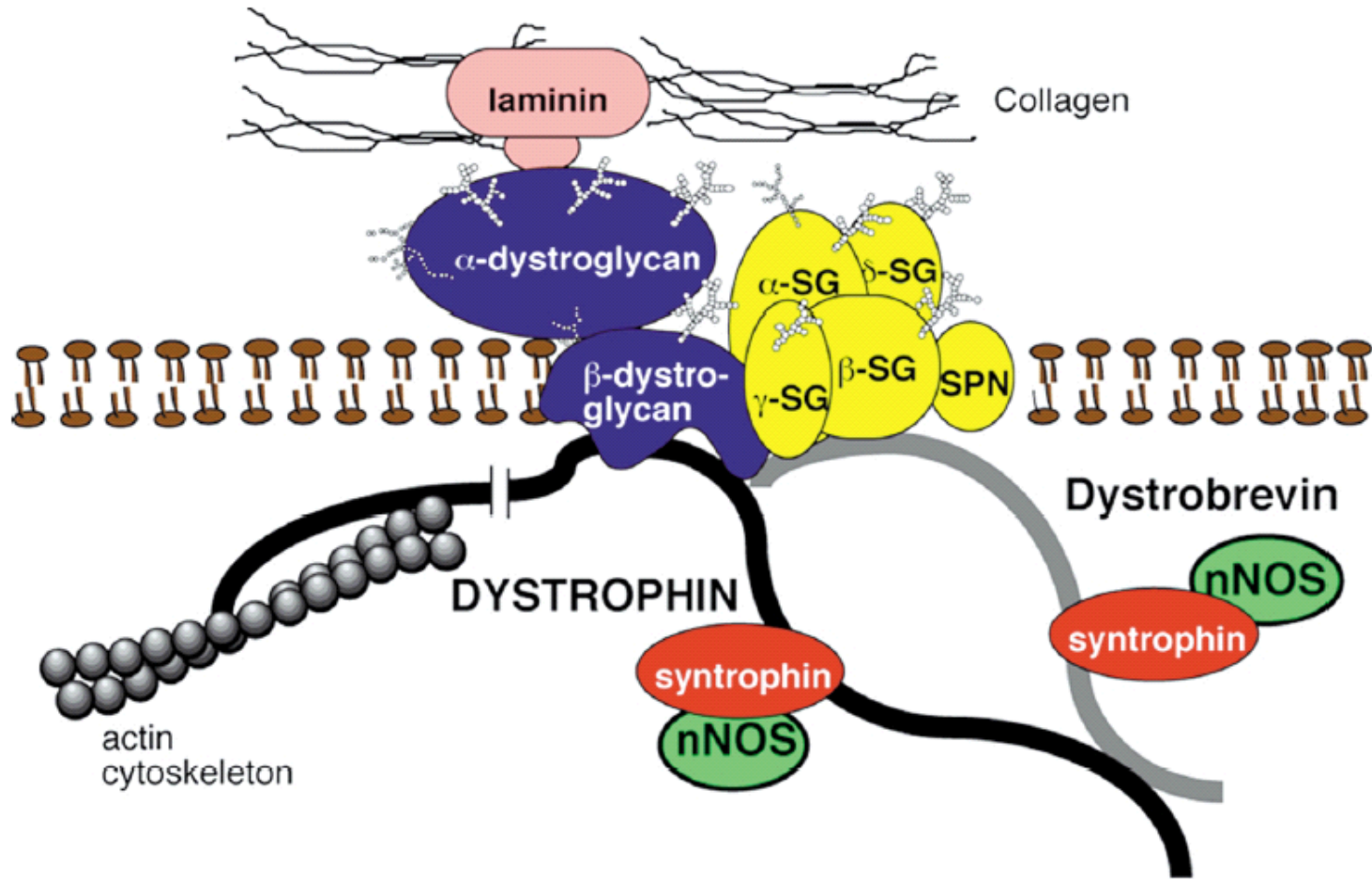
Approaches to treat Duchenne muscular dystrophy

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

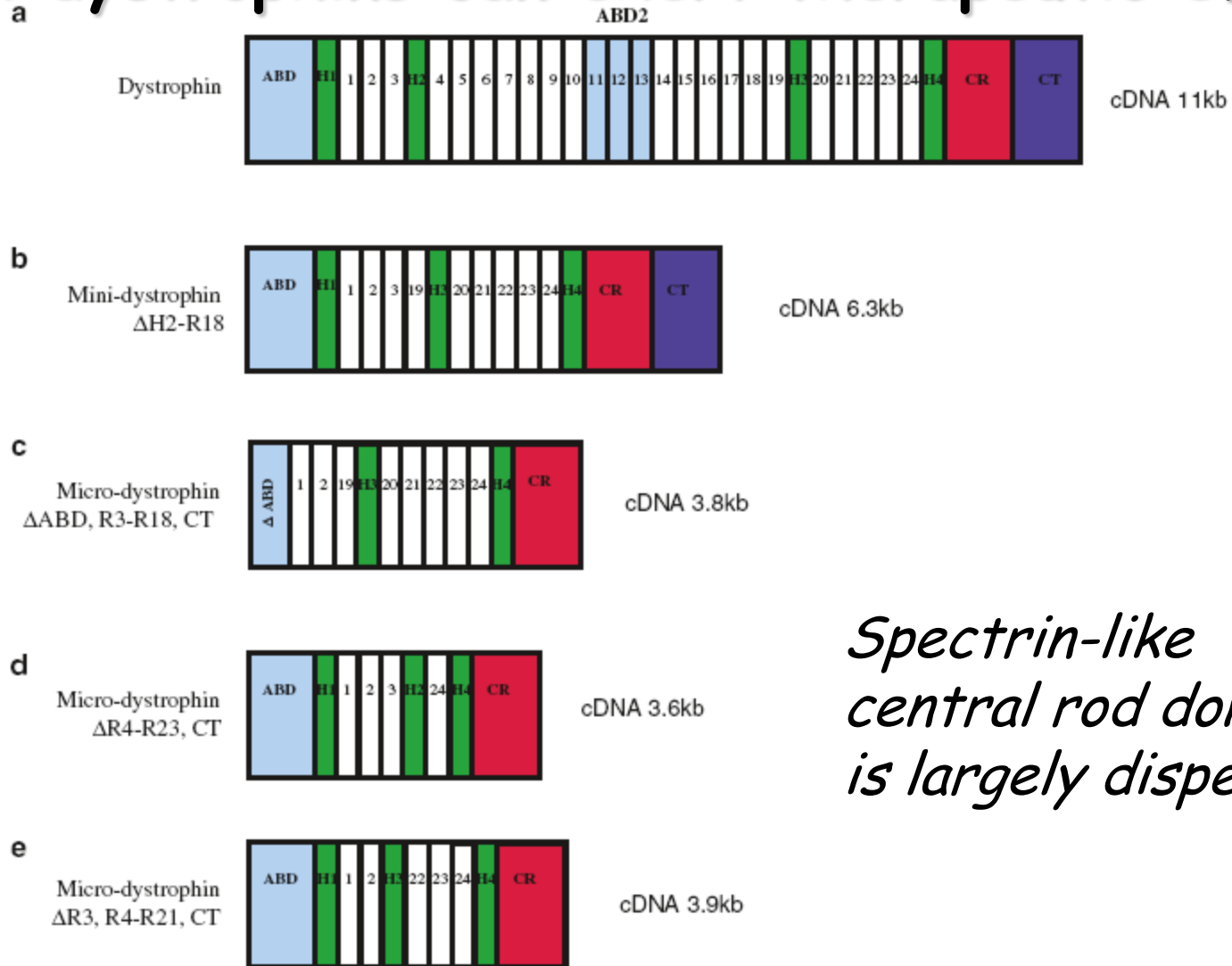# Phase I study of dystrophin plasmid-based gene therapy in Duchenne/Becker muscular dystrophy.

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.

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Partial dystrophins can exert therapeutic effect

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.

Spectrin-like central rod domain is largely dispensable

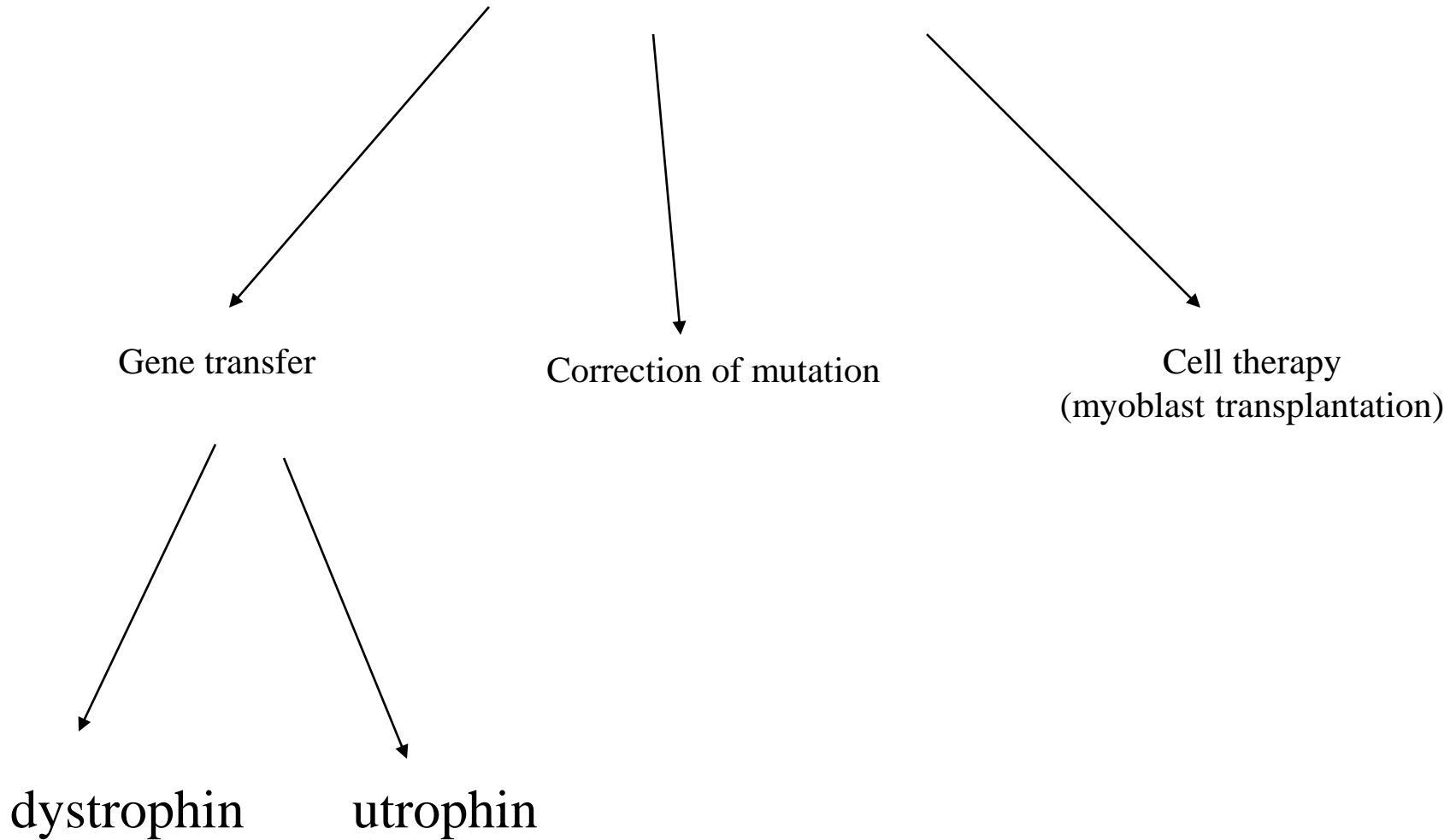*Foster et al., Gene Therapy 2006*

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Micro-dystrophin cDNA can be delivered with AAV vector
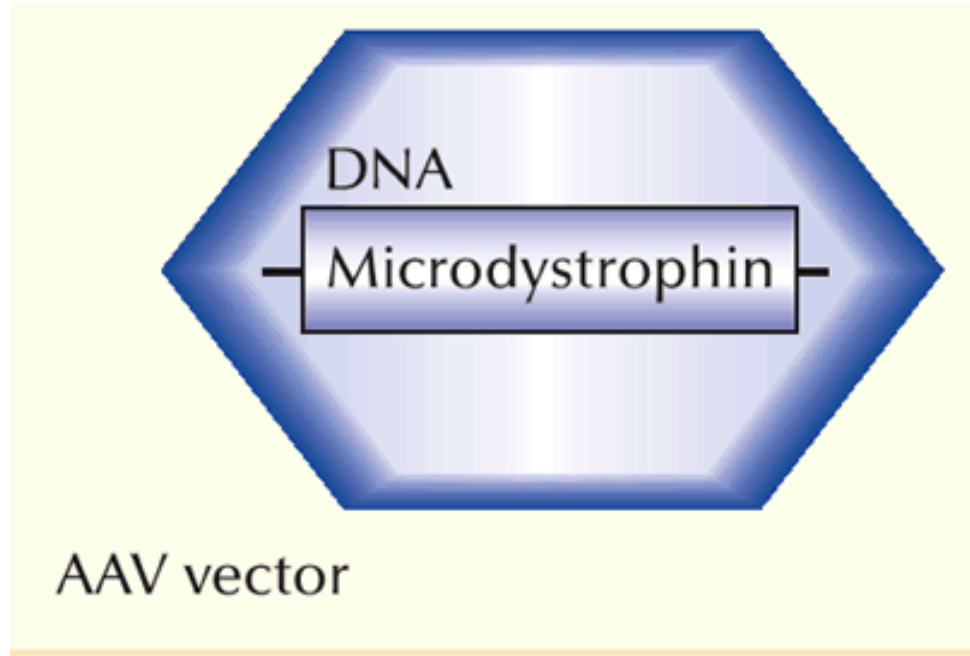
James G Tidball & Melissa J Spencer

Nature Medicine 9, 997 - 998 (2003)

Gene therapy for Duchenne muscular dystrophy

Gene transfer

Correction of mutation

Cell therapy
(myoblast transplantation)

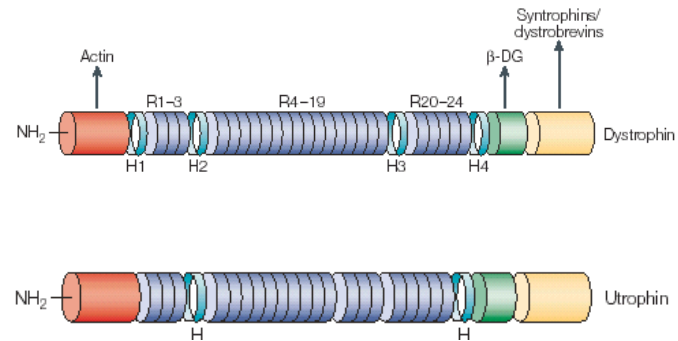dystrophin   utrophin

Utrophin

-A homologue of dystrophin

-Present in DMD patients

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

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

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

Animal models of DMD

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

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

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

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

dystrophin – guttless vector   **Utrophin – guttless vector**
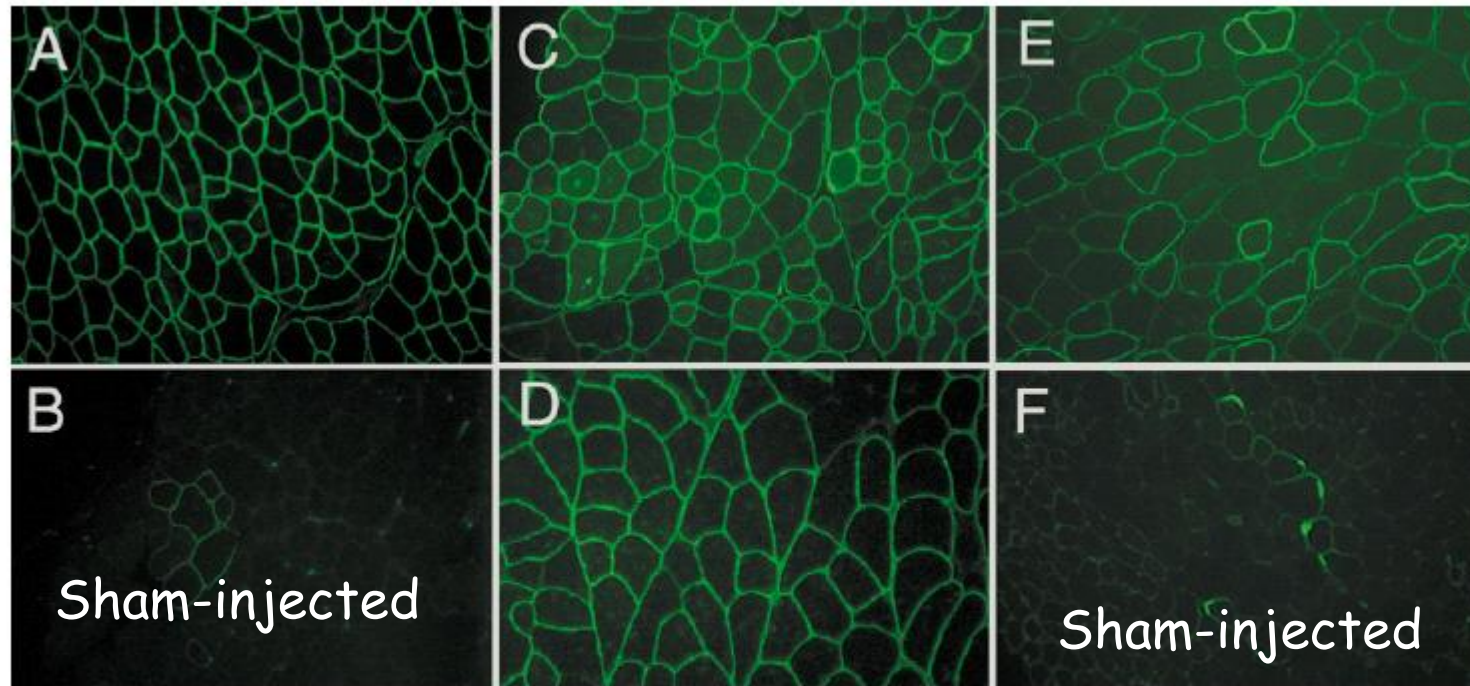
Wild type mice

Mdx mice

Sham-injected   Sham-injected

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

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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]

Double KO – dystrophin & utrophin
80% mortality at 15 weeks of age
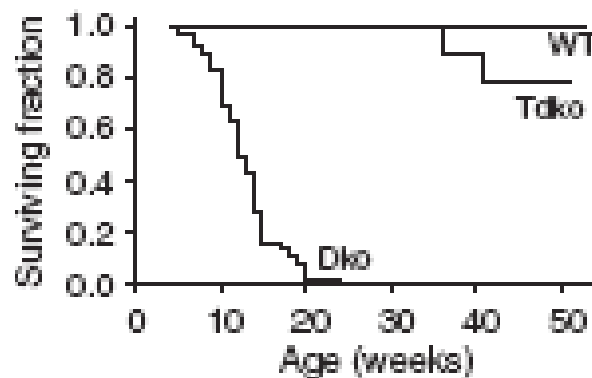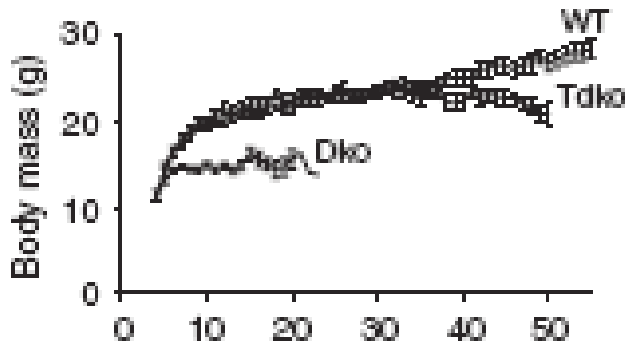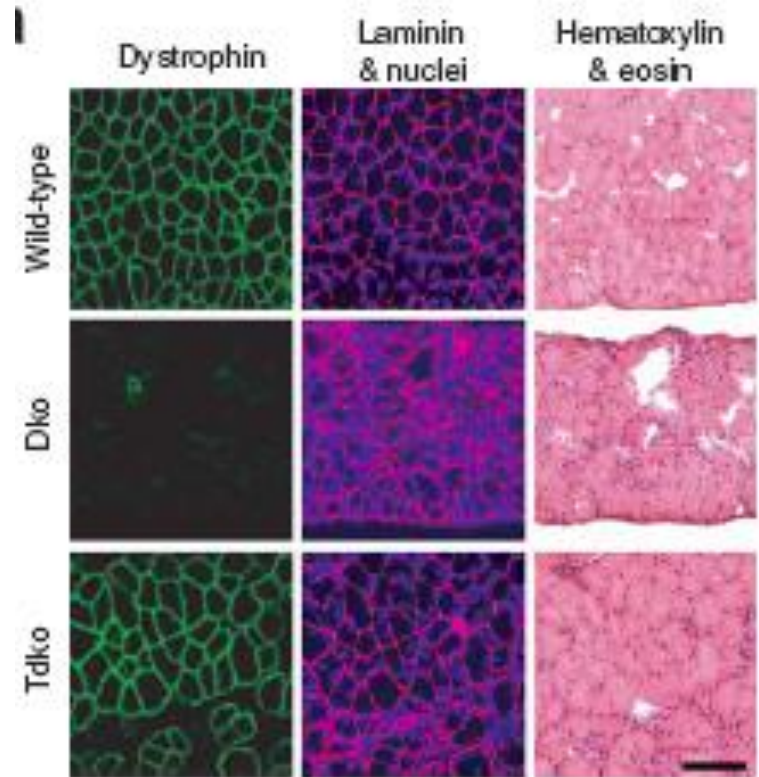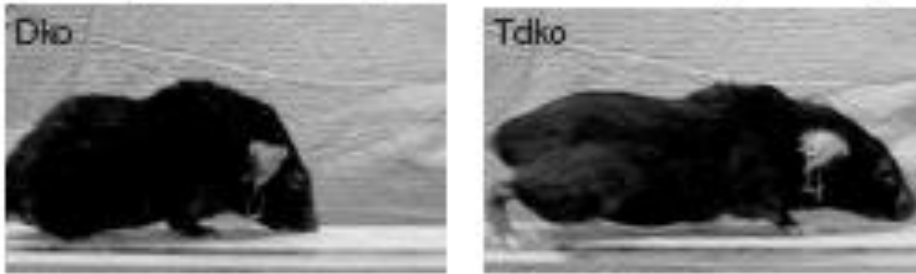
dystrophin in diaphragm after AAV6-delivery

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

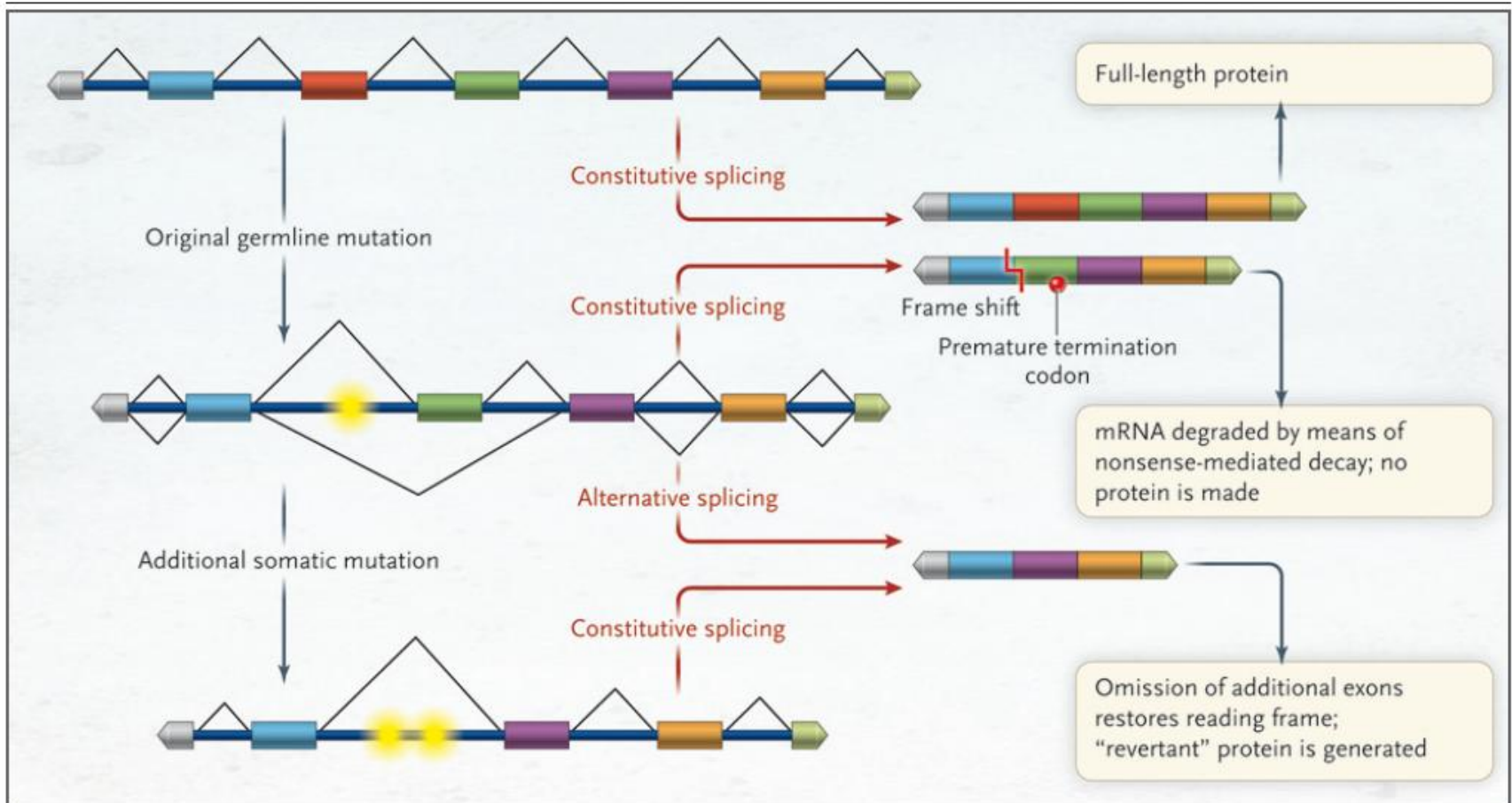# The generation of revertant dystrophin from mutant allele

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.

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Exon skipping as a way to improve conditions in DMD
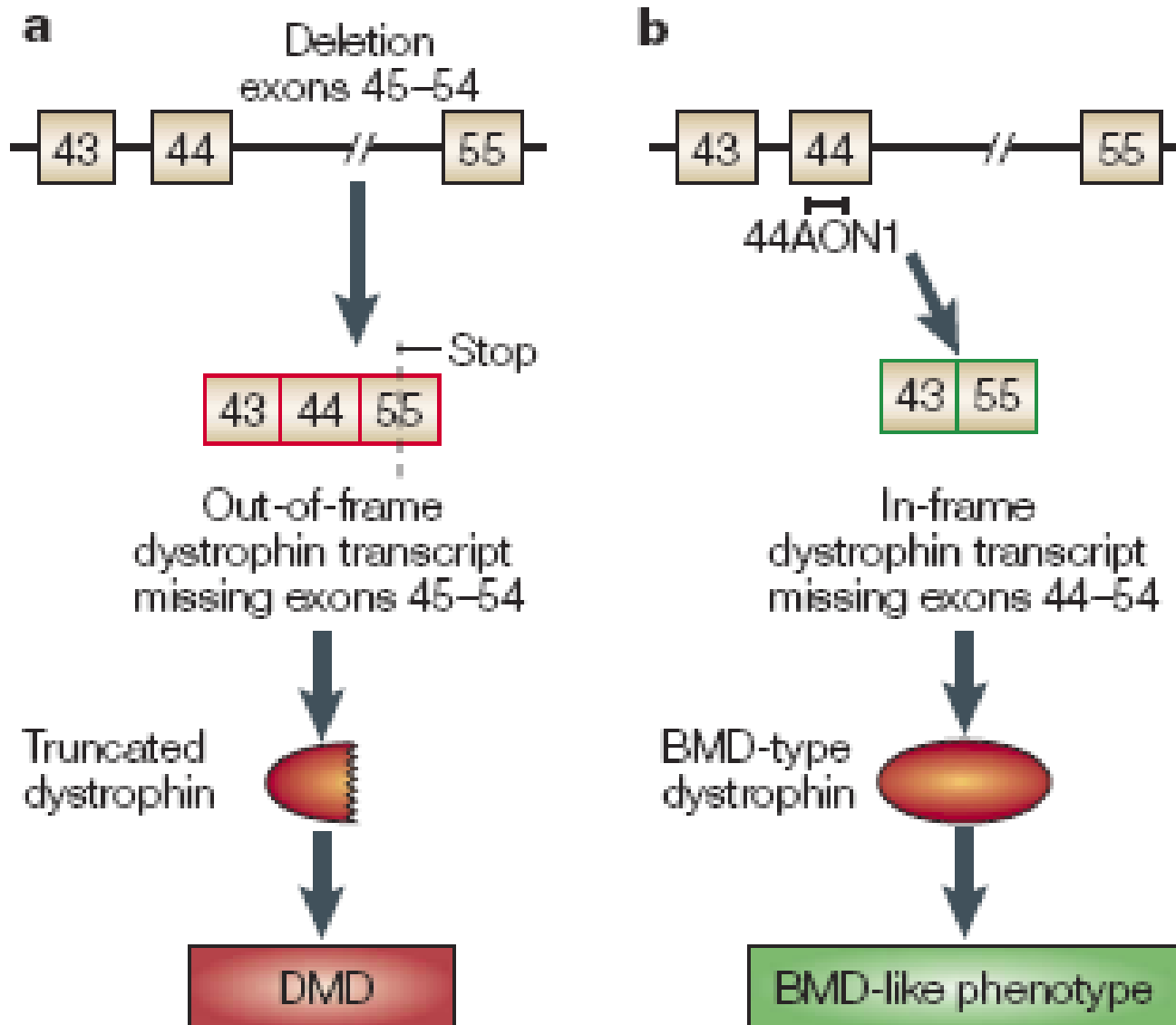
Exon skipping as a way to improve conditions in DMD
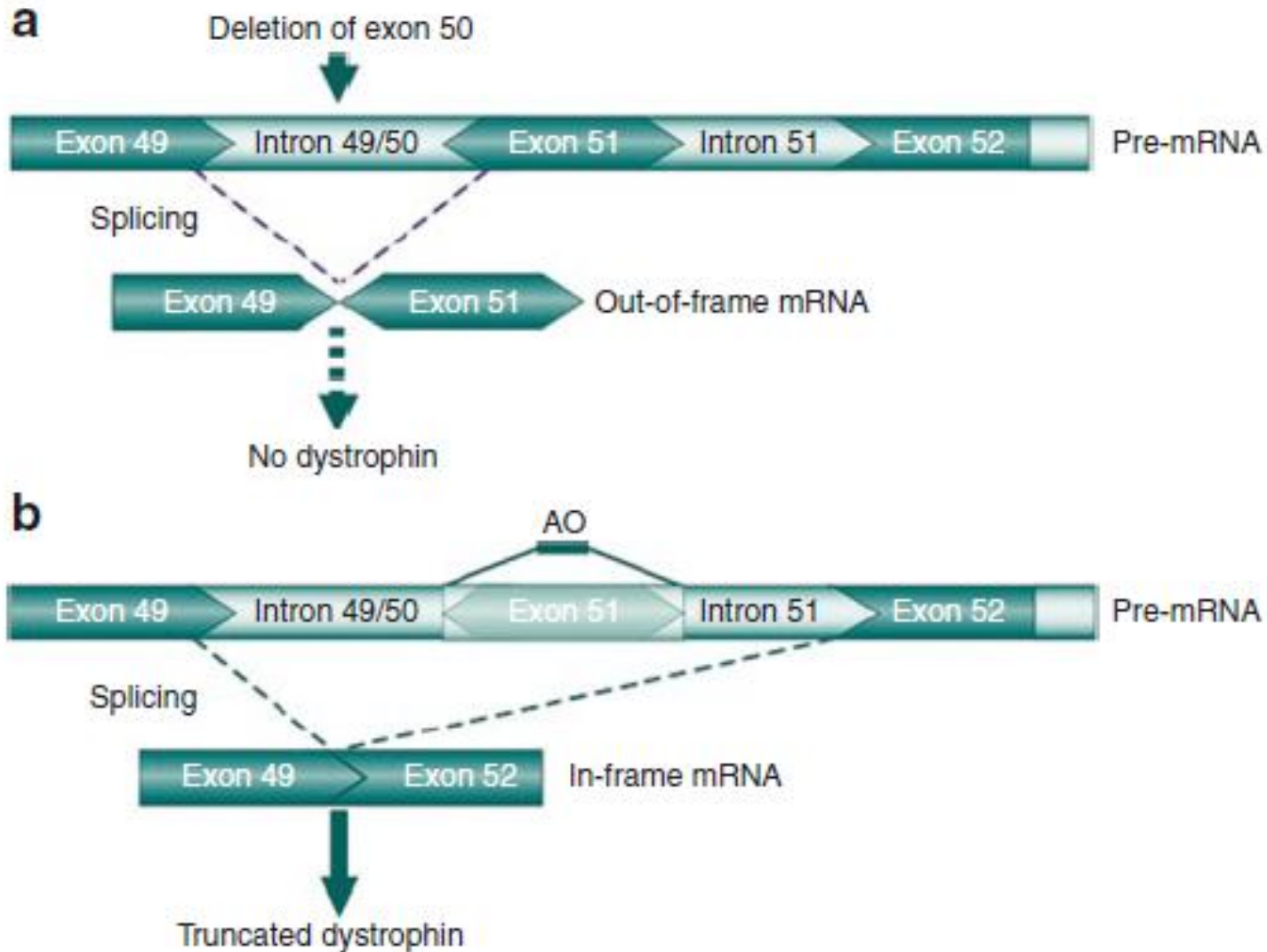
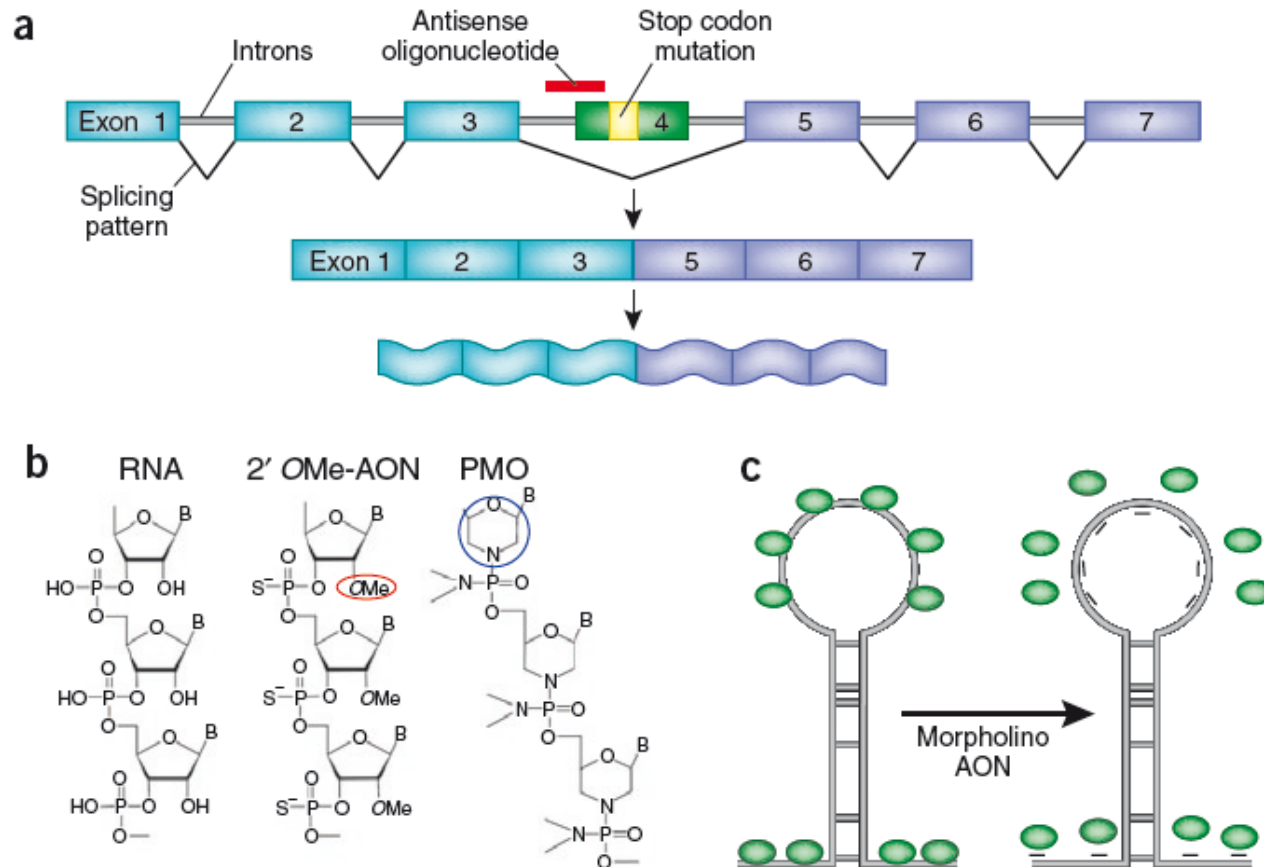# Exon-skipping strategies using antisense oligonucleotides

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′OMe 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].

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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

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.

66
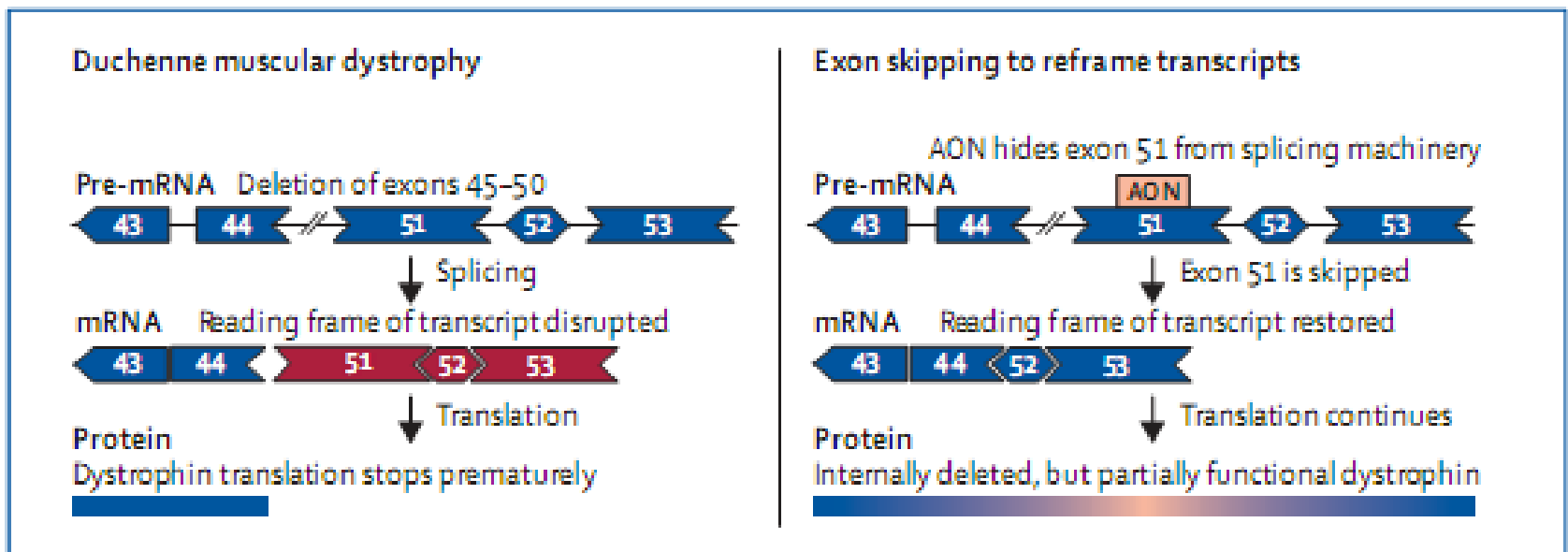
Figure 1: Antisense-mediated exon skipping to reframe DMD transcripts

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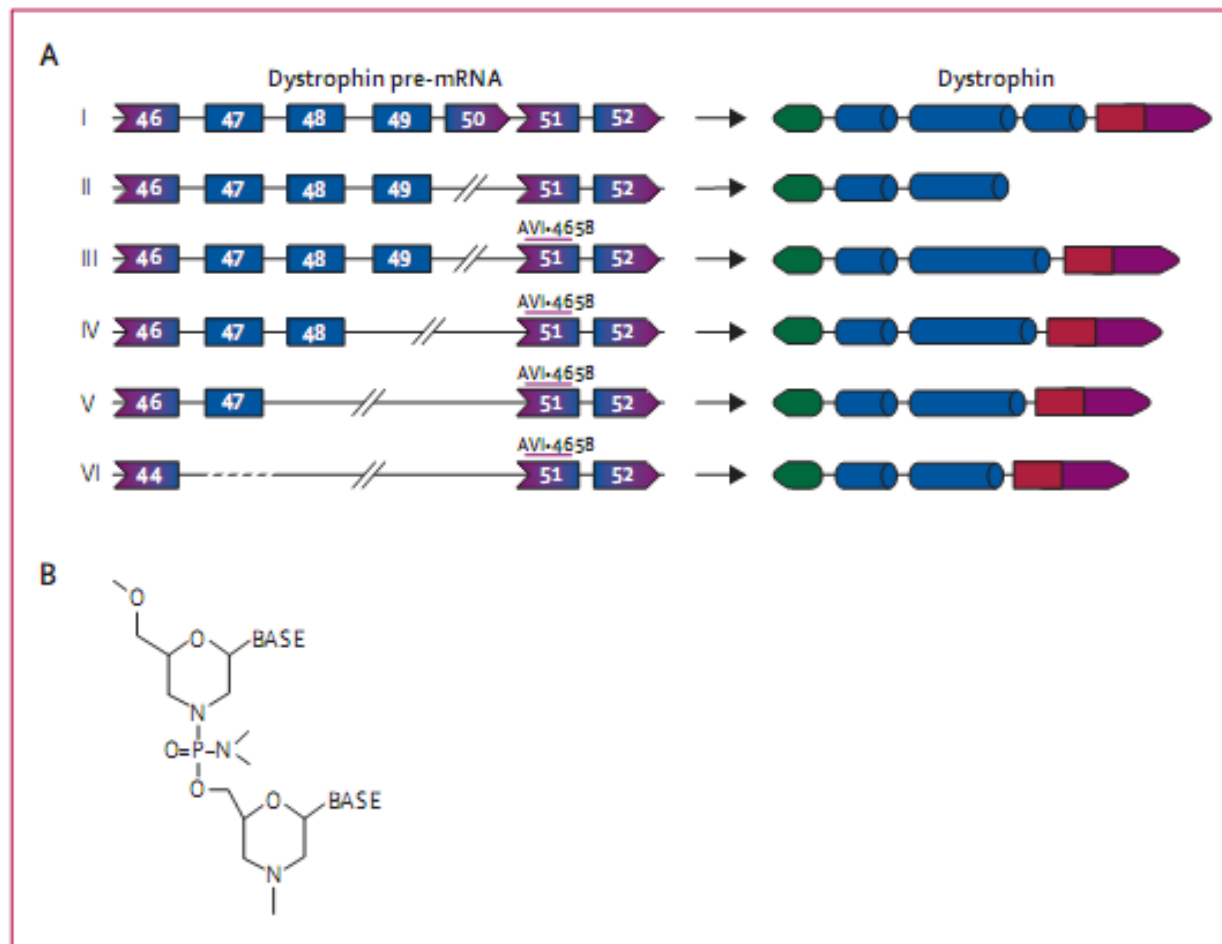
Figure 1: Deletions and predicted results of exon skipping in the patients who were studied
(A) Pre-mRNA transcripts and dystrophin protein products from full length DMD, in patients with Duchenne muscular dystrophy, and predicted protein sequences after exon skipping. (I) The normal dystrophin gene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

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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

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.

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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

Gene therapy of Leber's congenital amaurosis

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

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 ($1 \cdot 5 \times 10^{10}$ vector genomes), medium ($4 \cdot 8 \times 10^{10}$ vector genomes), or high dose ($1 \cdot 5 \times 10^{11}$ vector genomes) for up to 2 years.

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

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

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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
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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

Treatment of beta-thalassemia

HSC

b Lentiviral-vector particles

d Genetically modified HSCs

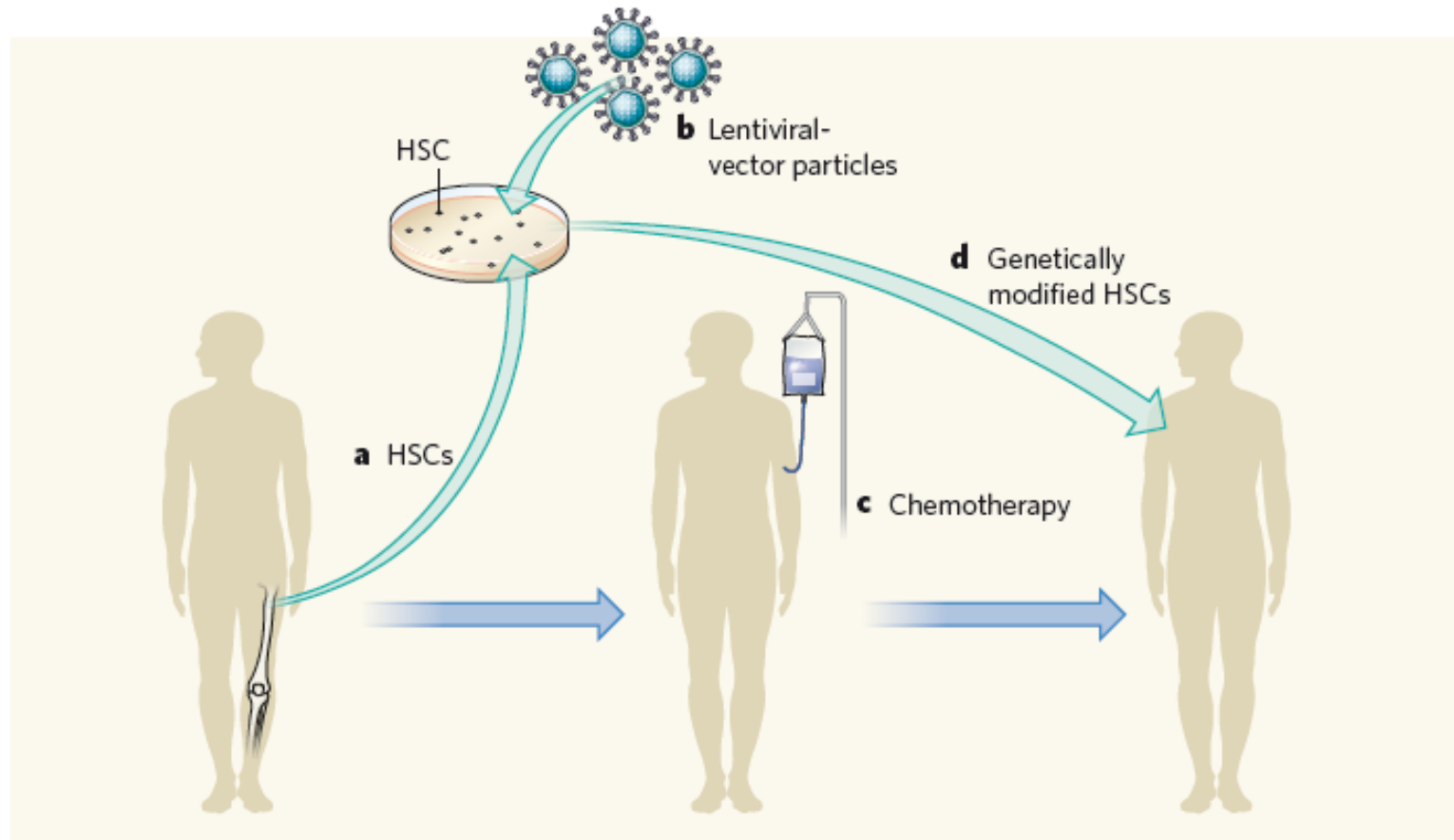**a** HSCs

c Chemotherapy

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 pretransplant '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.

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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

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]