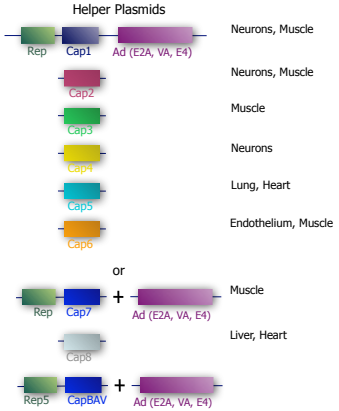
## AAV Vector Unit (AVU) facility at ICGEB Trieste: production of AAV vectors with different serotypes

www.icgeb.org/RESEARCH/TS/COREFACILITIES/AVUTRC.htm

The AVU produces, purifies, titers and characterizes research-grade recombinant AAV vectors

Both rAAV2 or hybrid vectors pseudotyped with capsid of serotypes from 1 to 9 are reproducibility obtained at a sterility and purity grade suitable for in vitro and animal experimentation




**Activities performed at the AVU**

Plasmid amplification and purification

Vector packaging and purification from cellular lysates

Vector titration by real-time PCR

**Characteristics of final vector preparation**

Yield: $1x10^3$ vg / cell

Physical Titer: $1x10^{12}$ to $1x10^{12}$ vg / ml

Final Volume of a standard vector batch: 3 ml in PBS

# AAV gene therapy clinical trials

- Melanoma
- Prostate cancer
- Cystic fibrosis
- Hemophilia B
- Muscular distrophies
- Amiotrophic lateral sclerosis
- Canavan disease
- Alpha 1-antitrypsin deficiency
- Healthy subjects (aerosol)
- Rheumatoid arthritis
- Parkinson disease
- Retinal degeneration

# Gene therapy of muscle

For inherited disorders of muscle

For the release of therapeutic proteins in the blood

(To improve muscle function in adults)
(Gene doping?)

Gene Therapy (2008) 15, 329–337

www.nature.com/gt

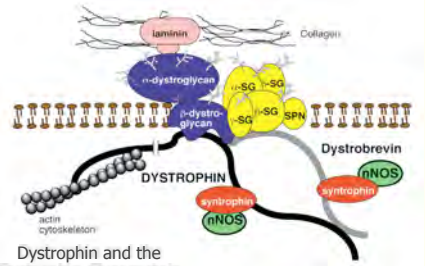
REVIEW

*Progress and prospects: gene therapy for performance and appearance enhancement*

M Kiuru and RG Crystal
*Department of Genetic Medicine, Weill Medical College of Cornell University, New York, NY, USA*

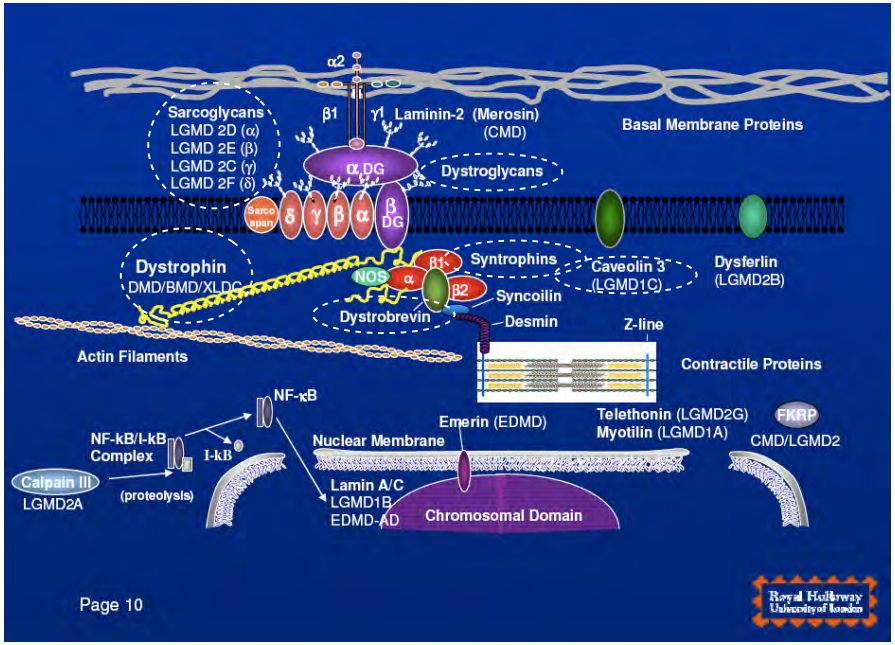
# Distrofie muscolari

| Malattia | Gene mutato |
|---|---|
| Distrofia muscolare di Duchenne (DMD) | Distrofina; incidenza di circa 1/3500 bambini maschi |
| Distrofia muscolare di Becker (BMD) | Distrofina; incidenza di circa 1/20.000 bambini maschi |
| Distrofia muscolare di Emery-Dreifuss | Emerina, lamina A o lamina C |
| Distrofia dei cingoli (LGMD) | Piu' di 15 geni diversi<br><br>autosomiche dominanti<br>LGMD 1A: miotilina<br>LGMD 1B: lamina A/C<br>LGMD 1C: caveolina 3<br>ed altre<br><br>autosomiche recessive<br>LGMD 2A: calpaina-3<br>LGMD 2B: disferlina<br>LGMD 2C: γ-sarcoglicano<br>LGMD 2D: α-sarcoglicano<br>LGMD 2E: β-sarcoglicano<br>LGMD 2F: δ-sarcoglicano<br>ed altre |
| Distrofia facio-scapolo-omerale o di Lnadouzy-Dejerine (FSHD) | Non noto |
| Distrofia miotonica o malattia di Steinert (MMD) | DMPK (DM1) e ZNF9 (DM2) |
| Distrofia oculo-faringea (OPMD) | Poly(A)-binding protein nuclear 1 (PABPN1) |
| Distrofia muscolare distale (DD) | Almeno 8 geni diversi (disferlina, titina, desmina ed altri) |
| Distrofia muscolare congenita (CMD) | Geni diversi (Laminina α2 – merosina, fukutina, collagene di tipo VI, integrina a7, ed altri) |

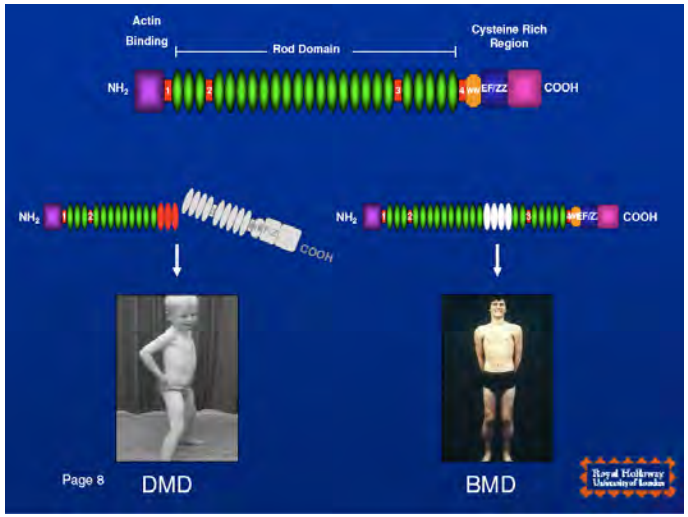



Dystrophin and the Dystrophin-Glycoprotein Complex (DCG)

# Molecular defects in muscular dystrophies




# Molecular defects in dystrophin lead to Duchenne and Becker muscular dystrophy




427 kDa, gene 2.4 Mbp with 70 exons, mRNA 14 kb (coding region 11 kb) - transcription lasts 16 hrs
4 structural domains (N-terminal, rod, cystein-rich and C-terminal)
N-terminal: binds actin, Cystein rich: binds b-dystroglycan

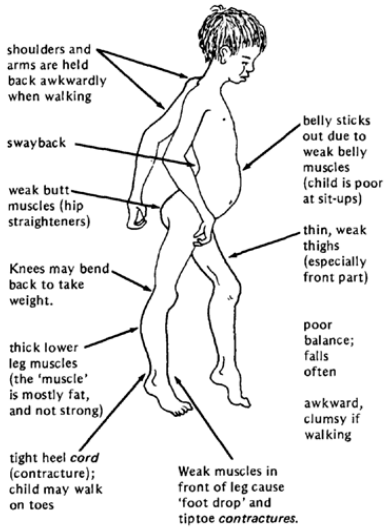
# DMD: il problema clinico

La DMD ha un decorso ingravescente e devastante (esaurimento delle cellule satelliti).

Alla nascita, i bambini maschi affetti sembrano normali, ed i primi sintomi insorgono tra i 3 ed i 5 anni di vita sotto forma di blanda debolezza muscolare, che si manifesta con la difficolta' nel salire le scale, alzarsi nella posizione seduta o con l'incespicare di frequente. Con il passare del tempo, la muscolatura si indebolisce progressivamente.
Solitamente entro i 10 anni di vita gli individui affetti sono costretti sulla sedia a rotelle, e molti decedono entro il 20° anno di eta'.

Non esistono attualmente terapie per la malattia, se non quelle di supporto.

Oltre al muscolo scheletrico, i pazienti con DMD mostrano un interessamento piu' o meno marcato del cuore che spesso evolve in una forma franca di cardiomiopatia dilatativa.




# DMD: current treatment options

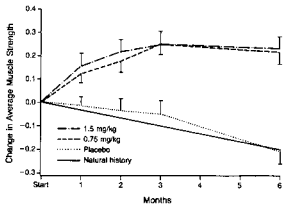
Only glucocorticoids have consistently demonstrated efficacy in DMD

1592 THE NEW ENGLAND JOURNAL OF MEDICINE June 15, 1989

**RANDOMIZED, DOUBLE-BLIND SIX-MONTH TRIAL OF PREDNISONE IN DUCHENNE'S MUSCULAR DYSTROPHY**

J.R. Mendell, R.T. Moxley, R.C. Griggs, M.H. Brooke, G.M. Fenichel, J.P. Miller, W. King, L. Signore, S. Pandya, J. Florence, J. Schierbecker, J. Robison, K. Kaiser, S. Mandel, C. Arpken, and B. Gilder

At 6 months: improvement in muscle strength, pulmonary function, time to rise from supine to standing, to walk 9m, to climb 4 stairs.




Randomized, blinded trial of weekend vs daily prednisone in Duchenne muscular dystrophy

*Neurology*© 2011;77:444-452

**Conclusions:** Weekend dosing of prednisone is equally beneficial to the standard daily dosing of prednisone. Analysis of side effect profiles demonstrated overall tolerability of both dosing regimens.

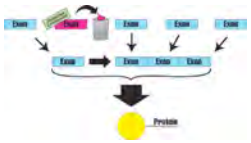
Side effects:
- weight gain with cushingoid appearance, high risk for hypertension, cataract, loss of bone density, vertebral compression fractures and long bone fractures
- long term administration limited by steroid-induced behavioral problems

# DMD: emerging drugs or small molecule therapies

- Exon skipping
- Mutation suppression
- Gene therapy
- Muscle building strategy

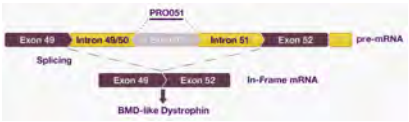
## Exon skipping

Exon skipping is targeted at the pre-mRNA level, allowing one or more exons to be omitted to restore the dystrophin reading frame. This is accomplished with splice-switching oligomers (20-30 nt), complementary to sequences of the pre-mRNA transcript.




## 2 proof-of-principle clinical trials targeting exon51

PRO051/GSK2402968, **Prosensa** - oligo antisenso




The NEW ENGLAND JOURNAL of MEDICINE

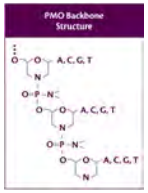
ORIGINAL ARTICLE

Systemic Administration of PRO051 in Duchenne's Muscular Dystrophy

Nathalie M. Goemans, M.D., Mar Tulinius, M.D., Ph.D., Johanna T. van den Akker, Ph.D., Brigitte E. Burm, Ph.D., Peter F. Ekhart, M.Sc., Niki Heuvelmans, Tjadine Holling, Ph.D., Anneke A. Janson, Gerard J. Platenburg, M.Sc., Jessica A. Sipkens, M.Sc., J.M. Ad Sitsen, M.D., Ph.D., Annemieke Aartsma-Rus, Ph.D., Gert-Jan B. van Ommen, Ph.D., Gunnar Buyse, M.D., Ph.D., Niklas Darin, M.D., Ph.D., Jan J. Verschuuren, M.D., Ph.D., Giles V. Campion, M.D., Sjef J. de Kimpe, Ph.D., and Judith C. van Deutekom, Ph.D.

N ENGL J MED 364;16 NEJM.ORG APRIL 21, 2011

AVI-4658/**Eteplirsen** - Avi Biopharma - morpholino oligomer

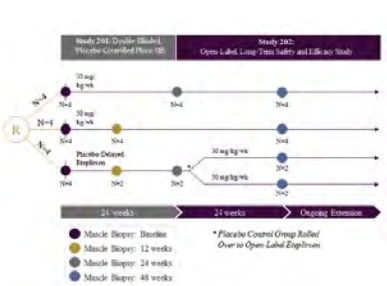


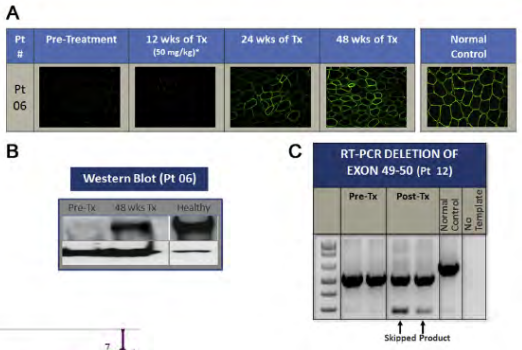

**Exon skipping and dystrophin restoration in patients with Duchenne muscular dystrophy after systemic phosphorodiamidate morpholino oligomer treatment: an open-label, phase 2, dose-escalation study**

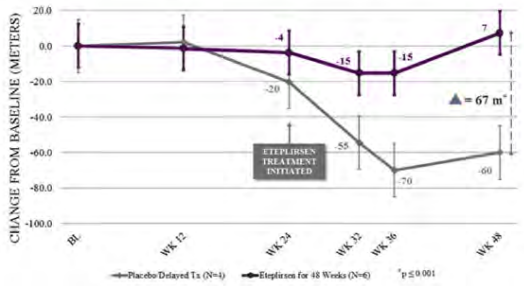
*Sebahattin Cirak*, Virginia Arechavala-Gomeza*, Michela Guglieri, Lucy Feng, Silvia Torelli, Karen Anthony, Stephan Abbs, Maria Elena Garralda, John Bourke, Dominic J Wells, George Dickson, Matthew J A Wood, Steve D Wilton, Volker Straub, Ryszard Kole, Stephen B Shrewsbury, Caroline Sewry, Jennifer E Morgan, Kate Bushby, Francesco Muntoni*

www.thelancet.com Vol 378 August 13, 2011

## Molecular and functional efficacy Eteplirsen




No side effects observed at 1.5 years follow-up

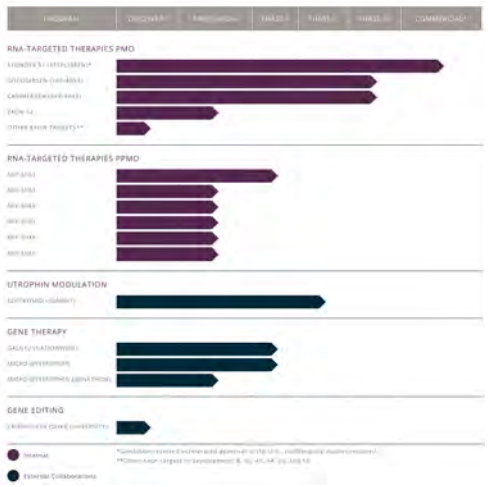
## Accelerated FDA approval of the exon-skipping drug eteplirsen

Approval to market eteplirsen was given in September 2016 to pharmaceutical company Sarepta Therapeutics. Eteplirsen will be the first disease-modifying drug on the market in the United States to treat DMD, and approximately 13 percent of DMD patients potentially may be eligible for treatment. Under the terms of the FDA's accelerated approval, Sarepta must conduct a clinical trial of eteplirsen to confirm clinical benefit. The approval is provisional, pending results of the ongoing phase III clinical trial,

SAREPTA THERAPEUTICS

"Our distinctive PMO-based platform chemistries allow us to address specific genetic diseases by altering the RNA transcription process and thereby modifying protein structure."

Gunnar J. Hanson, Ph.D., Senior Director of Research Chemistry



# EMA has not approved Eteplirsen as Duchenne MD Therapy in Europe

December 2016

Sarepta Therapeutics, Inc. (NASDAQ:SRPT), a commercial-stage developer of innovative RNA-targeted therapeutics, today announced that the European Medicines Agency(EMA) validated the previously submitted Marketing Authorization application (MAA) for eteplirsen to treat Duchenne muscular dystrophy amenable to exon 51 skipping. Sarepta is seeking conditional approval of eteplirsen in the EU through the centralized procedure. Validation of the MAA confirms that the submission is accepted and starts the formal review process by the EMA's Committee for Human Medicinal Products (CHMP). The standard review period is 210 days (plus additional time for applicant to respond to questions from the agency).

Under the brand name Exondys 51, eteplirsen was approval by the U.S. Food and Drug and Administration (FDA) in September 2016, making it the first FDA-approved therapy specifically indicated to treat Duchenne MD. But Exondys 51's development and approval process was a prolonged one. The FDA decision also followed a campaign by muscular dystrophy advocacy groups urging access to a therapy offering some level of clinical improvement for a disease with no therapeutic options.

September 2018

## Duchenne secondo "no" europeo per eteplirsen

## DMD: emerging drugs or small molecule therapies

- Exon skipping
- Mutation suppression
- Gene therapy
- Muscle building strategy

## Challenges to gene therapy for DMD

Gene therapy requires delivery of a new gene to the vast majority of muscles in the body and to the heart - a daunting challenge, since muscle tissues makes up **>40% of body mass**

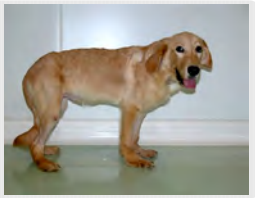
The **dystrophyn mRNA** is **14 kb** in size. A delivery vector must be identify that can carry this expression cassette; alternatively, truncated version of the gene can be used

Muscle transduction must not trigger toxic or immunological reactions that are harmful to the patient or that lead to further muscle damage

## Duchenne muscular dystrophy - natural animal models

mdx mice (point mutation leading to a premature truncation)


xdm golden retriever dog (exon 7 skipping)

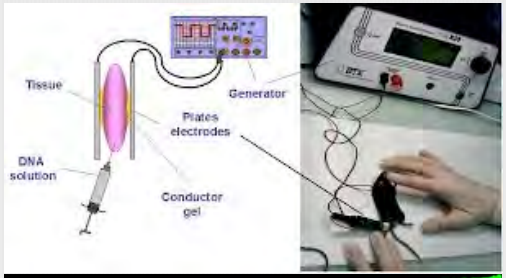


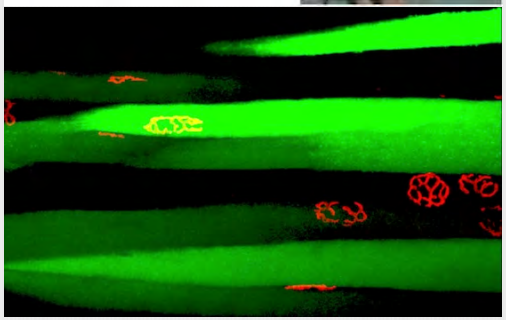
# Vectors for muscle gene therapy

• ***Plasmid DNA:*** displays a remarkable ability to transfer genes to muscle, specially if coupled with high pressure injection and/or electroporation

The first clinical trial, closed in 2006, entailed the injection of a plasmid containing the whole dystrophin cDNA under the control of the CMV promoter into the radialis muscle of 9 DMD/BMD patients. However, dystrophin expression resulted too low and not homogenous.

Different strategies can be used to increased transduction efficiency, including polymeres, ultrasounds (with microbubbles), and electroporation



# Vectors for muscle gene therapy

## Adeno-associated virus serotype 8 efficiently delivers genes to muscle and heart

Zhong Wang[1,3], Tong Zhu[1,3], Chunping Qiao[1], Liqiao Zhou[1], Bing Wang[1], Jian Zhang[1], Chunlian Chen[1], Juan Li[1] & Xiao Xiao[1,2]

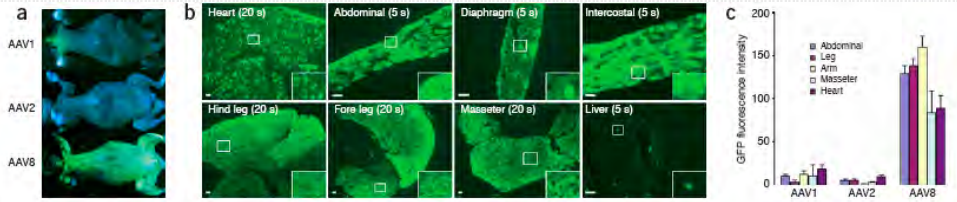



**Figure 2** Systemic gene delivery to muscle of neonatal mice by different AAV serotypes via i.v. injection. (**a**) Whole-body fluorescent photography taken one month after i.v. (temporal vein) injection of $2 \times 10^{11}$ v.g. of various dsAAV-CB-GFP at 3 d of age. (**b**) GFP expression seen in cryosections of heart, muscle and nonmuscle tissues one month after neonatal i.v. injection with $2 \times 10^{11}$ v.g. of dsAAV8-CB-GFP. Numbers in parentheses denote microscopy exposure time in seconds (s). Scale bar, 100 μm. Note the strong GFP expression in heart and muscles, and the minimal to undetectable GFP expression in nonmuscle tissues. (**c**) Quantitative analysis of average fluorescence intensity of cryosections from various muscles of AAV1, 2 and 8 treated mice (as shown in **a**).

# Distrofina e DGC



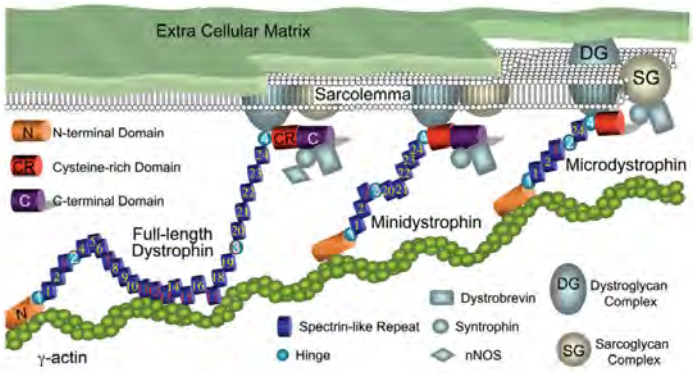



**Figure 1.** Schematic outline of full-length dystrophin, minidystrophin and microdystrophin and their interaction with other cellular proteins. Spectrin-like repeats are numbered from 1 to 24 (positively charged repeats are in red color, other repeats are in yellow color). Proline-rich hinges are numbered from 1 to 4. Hinge 3 is in pink color to indicate that it can be cleaved by viral protease. Hinge 2 to repeat 19 are deleted in minidystrophin. Repeat 4–23 and the C-terminal domain are deleted in microdystrophin. Not drawn to scale.

**minidistrofine (~6-7 kb) e microdistrofine (~4 kb)**

Queste versioni ridotte della distrofina presentano delezioni comuni della regione centrale a bastoncello e nel dominio C-terminale della proteina parentale, lasciando intatti i domini funzionali essenziali della proteina, in particolare quello ricco in cisteine (CR)

## Safety Study of Mini-Dystrophin Gene to Treat Duchenne Muscular Dystrophy

Study Type: Interventional
Study Design: Treatment, Randomized, Double-Blind, Placebo Control, Single Group Assignment, Safety Study
Official Title: Phase 1 Clinical Trial of rAAV2.5-CMV-Mini-Dystrophin Gene Vector in Duchenne Muscular Dystrophy

Primary Outcome Measures:
- Safety

Secondary Outcome Measures:
- mini-dystrophin gene expression at the site of gene transfer
- muscle strength evaluated by Maximal Volume Isometric Contraction Testing

This phase I randomized double blind dose escalation study investigates the safety and efficacy of the mini-dystrophin gene transferred to the **biceps** muscle for **Duchenne muscular dystrophy** patients, **ages 5 to 12 years** of age, using a recombinant adeno-associated virus. Eligible participants must have a known dystrophin gene mutation and may be concurrently treated with corticoid steroids. The mini-dystrophin gene or a placebo agent (normal saline or empty viral capsids) are injected directly into both biceps muscles while under conscious sedation. Following the gene transfer, patients are admitted to the hospital for 48 hours of observation followed by weekly outpatient visits at the Columbus Children's Hospital Neuromuscular Clinic. A **bilateral muscle biopsy** is preformed following 6 weeks with long term follow up will consisting of bi-annual visits for the next 2 years.

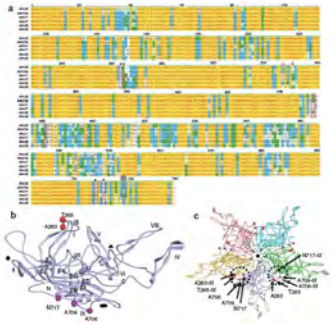
Principal Investigator:
Jerry R. Mendell, MD
Nationwide Children's Hospital

NCT00428935

# Phase 1 Gene Therapy for Duchenne Muscular Dystrophy Using a Translational Optimized AAV Vector

Dawn E Bowles[1], Scott WJ McPhee[2], Chengwen Li[3], Steven J Gray[1], Jade J Samulski[3], Angelique S Camp[4], Juan Li[3], Bing Wang[3], Paul E Monahan[4], Joseph E Rabinowitz[4], Joshua C Grieger[4], Lakshmanan Govindasamy[2], Mavis Agbandje-McKenna[2], Xiao Xiao[3] and R Jude Samulski[3]

[1]Department of Surgery, Division of Surgical Sciences, Duke University Medical Center, Durham, North Carolina, USA; [2]AskBio BioPharmaceutical Inc., Chapel Hill, North Carolina, USA; [3]Gene Therapy Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; [4]Joint Vector Core, University of North Carolina, Chapel Hill, North Carolina, USA; [5]Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina, USA; [6]Center for Translational Medicine, Department of Medicine, Thomas Jefferson University, Philadelphia, Philadelphia, Pennsylvania, USA; [7]Department of Biochemistry and Molecular Biology, Center for Structural Biology, McKnight Brain Institute, University of Florida, Gainesville, Florida, USA



www.moleculartherapy.org vol. 20 no. 2 feb. 2012

Table 2 The clinical data in patients with AAV2.5/minidystrophin muscular delivery

6 boys
Good safety
Unsuccessful transgene expression

| Subject | Age | | | | | | | | | | | Revertant fibers |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 8 | | | | | | | | | | | 30–125 |
| 2 | 9 | | | | | | | | | | | 0–9 |
| 3 | 9 | | | | | | | | | | | 0–9 |
| 4 | 5 | 49–54 | 15.8 | N | <1:2 | $3.0 \times 10^{12}$ | Saline | $6.6 \times 10^{11}$ Intermediate | D43 | 2.56 | ND | 0–11 |
| 5 | 11 | 3–17 | 57.1 | Y | 1:100 | $3.0 \times 10^{12}$ | Empty capsid | $3.3 \times 10^{12}$ High | D90 | 0.08 | ND | ND |
| 6 | 9 | 46–52 | 28.7 | Y | 1:2 | $3.0 \times 10^{12}$ | Empty capsid | $6.6 \times 10^{12}$ High | D43 | 1.42 | weak | 1–25 |
| | | | | | | | | $6.6 \times 10^{12}$ | | | | |

*Abbreviations*: AAV, adeno-associated virus; ND, none detected; qPCR, quantitative PCR.
[a]AAV2.5 minidystrophin vector genome dose (vector genomes/patient). [b]Total capsid dose (minidystrophin + empty capsid in subject's 5 and 6) capsid particles/patient. [c]Vector genome copy number isolated per nucleus as determined by qPCR (skeletal muscle cells are multinucleated).

ARTICLE

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DOI: 10.1038/ncomms16105 OPEN

# Long-term microdystrophin gene therapy is effective in a canine model of Duchenne muscular dystrophy

Caroline Le Guiner[1,2], Laurent Servais[3], Marie Montus[2], Thibaut Larcher[4], Bodvaël Fraysse[1], Sophie Moullec[5], Marine Allais[1], Virginie François[1], Maeva Dutilleul[4], Alberto Malerba[6], Taeyoung Koo[6], Jean-Laurent Thibaut[7,8], Béatrice Matot[7], Marie Devaux[1], Johanne Le Duff[1], Jack-Yves Deschamps[5], Inès Barthelemy[8,9], Stéphane Blot[8,9], Isabelle Testault[10], Karim Wahbi[11], Stéphane Ederhy[12], Samia Martin[2], Philippe Veron[2], Christophe Georger[2], Takis Athanasopoulos[6,13,†], Carole Masurier[2], Federico Mingozzi[2], Pierre Carlier[7], Bernard Gjata[2], Jean-Yves Hogrel[14], Oumeya Adjali[1], Fulvio Mavilio[2], Thomas Voit[15,*], Philippe Moullier[1,16,*] & George Dickson[6,*]

nature COMMUNICATIONS

Duchenne muscular dystrophy (DMD) is an incurable X-linked muscle-wasting disease caused by mutations in the dystrophin gene. Gene therapy using highly functional microdystrophin genes and recombinant adeno-associated virus (rAAV) vectors is an attractive strategy to treat DMD. Here we show that locoregional and systemic delivery of a rAAV2/8 vector expressing a canine microdystrophin (cMD1) is effective in restoring dystrophin expression and stabilizing clinical symptoms in studies performed on a total of 12 treated golden retriever muscular dystrophy (GRMD) dogs. Locoregional delivery induces high levels of microdystrophin expression in limb musculature and significant amelioration of histological and functional parameters. Systemic intravenous administration without immunosuppression results in significant and sustained levels of microdystrophin in skeletal muscles and reduces dystrophic symptoms for over 2 years. No toxicity or adverse immune consequences of vector administration are observed. These studies indicate safety and efficacy of systemic rAAV-cMD1 delivery in a large animal model of DMD, and pave the way towards clinical trials of rAAV–microdystrophin gene therapy in DMD patients.

**Table 2 | Levels of cMD1-positives fibres found after immunostaining analysis (NCL-DYSB) within the muscles of GRMD dogs injected by the LR route.**

| Dog | Injected forelimb ($n = 13$ muscles) | | | | Noninjected forelimb ($n = 13$ muscles) | Other muscles at distance ($n = 17$ muscles) | Heart | Diaphragm |
|---|---|---|---|---|---|---|---|---|
| | Mean of cMD1+ fibres | CV | Mean of cMD1+ fibres for the group | CV for the group | Mean of cMD1+ fibres | Mean of cMD1+ fibres | Mean of cMD1+ fibres | Mean of cMD1+ fibres |
| LR1 | 51% | 54% | 50% | 47% | 3% | 10% | <0.5% | 13% |
| LR2 | 59% | 30% | | | 1% | 10% | <0.5% | <0.5% |
| LR3 | 49% | 58% | | | 3% | 11% | <0.5% | 18% |
| LR4 | 43% | 49% | | | 1% | 7% | <0.5% | 1% |
| LR C1 | <0.5% | NA | <0.5% | NA | <0.5% | <0.5% | <0.5% | <0.5% |
| LR C2 | <0.5% | NA | | | <0.5% | <0.5% | <0.5% | <0.5% |
| LR C3 | <0.5% | NA | | | <0.5% | <0.5% | <0.5% | <0.5% |

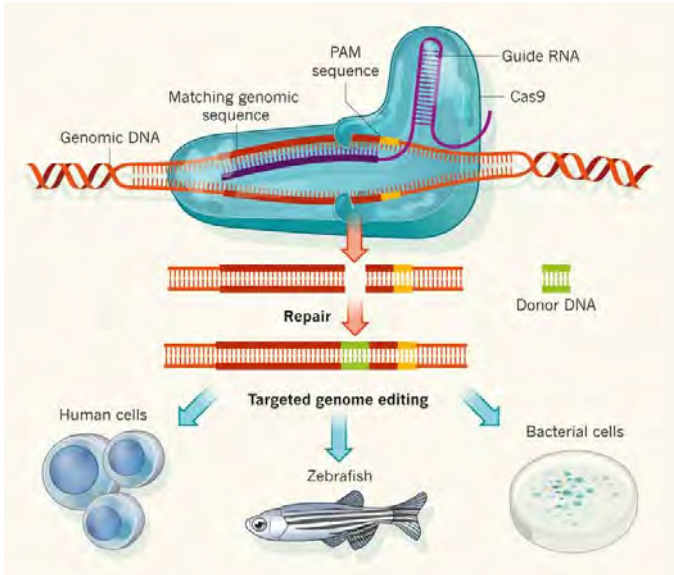
CV, coefficient of variation.
For the complete list of muscles and tissues sampled at the time of killing, see Supplementary Table 2 in ref. 33.

shedding of the vector after release of the tourniquet

# DMD: emerging drugs or small molecule therapies

- Exon skipping
- Mutation suppression
- Gene therapy
- Muscle building strategy
- Genome editing

## CRISPR/Cas9 and Targeted Genome Editing: A New Era in Molecular Biology




The functions of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and CRISPR-associated (Cas) genes are essential in adaptive immunity in select bacteria and archaea, enabling the organisms to respond to and eliminate invading genetic material.

**In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy**

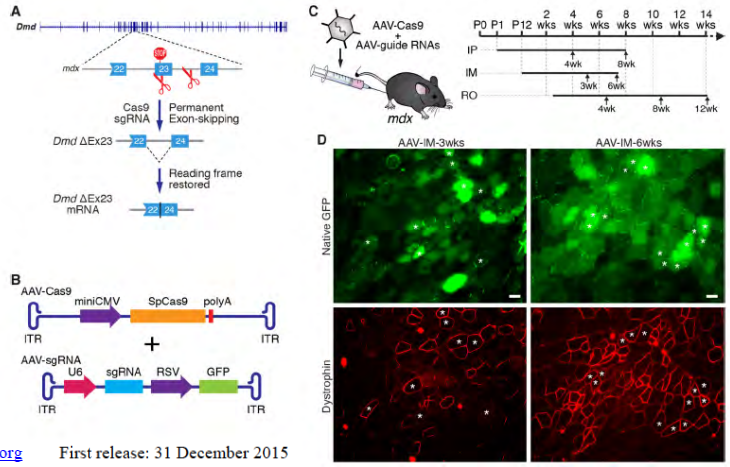
Christopher E. Nelson,[1,2] Chady H. Hakim,[3] David G. Ousterout,[1,2] Pratiksha I. Thakore,[1,2] Elrik A. Moreb,[1,2] Ruth M. Castellanos Rivera,[3] Sarina Madhavan,[1,2] Xinfang Pan,[3] F. Ann Ran,[1,4] Winston X. Yan,[1,2,5] Aravind Asokan,[6] Feng Zhang,[1,4,7,8,9] Dongsheng Duan,[3,10] Charles A. Gersbach[1,2,10]*

**Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy**

Chengzu Long,[1,2,10] Leonela Amoasii,[1,2,10] Alex A. Mireault,[1,2,3] John R. McAnally,[1,2,3] Hui Li,[1,2,3] Efrain Sanchez-Ortiz,[1,2,3] Samadrita Bhattacharyya,[1,2,3] John M. Shelton,[4] Rhonda Bassel-Duby,[1,2,3] Eric N. Olson[1,2,3]*

**In vivo gene editing in dystrophic mouse muscle and muscle stem cells**

Mohammadsharif Tabebordbar,[1,2,3] Kexian Zhu,[1,2,3] Jason K. W. Cheng,[1] Wei Leong Chew,[2,4] Jeffrey J. Widrick,[5] Winston X. Yan,[6,7] Claire Maesner,[1] Elizabeth Y. Wu,[1,2] Ru Xiao,[6] F. Ann Ran,[6,7] Le Cong,[6,7] Feng Zhang,[6,7] Luk H. Vandenberghe,[4] George M. Church,[4] Amy J. Wagers[1,2]*




www.sciencemag.org First release: 31 December 2015

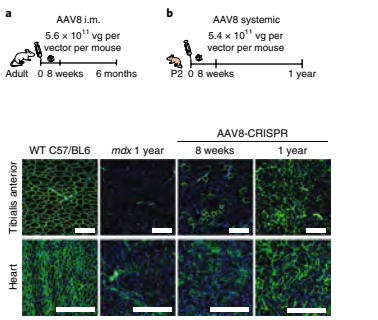
nature medicine
LETTERS
https://doi.org/10.1038/s41591-019-0344-3

## Long-term evaluation of AAV-CRISPR genome editing for Duchenne muscular dystrophy

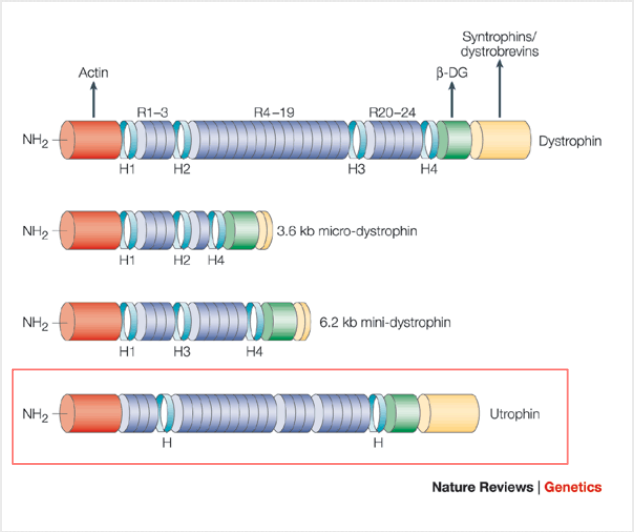
Christopher E. Nelson[1,2], Yaoying Wu[1], Matthew P. Gemberling[1,2], Matthew L. Oliver[1], Matthew A. Waller[1,2], Joel D. Bohning[1,2], Jacqueline N. Robinson-Hamm[1,2], Karen Bulaklak[1,2], Ruth M. Castellanos Rivera[3], Joel H. Collier[1], Aravind Asokan[4,5] and Charles A. Gersbach[1,2,6]*

**Duchenne muscular dystrophy (DMD) is a monogenic disorder and a candidate for therapeutic genome editing. There have been several recent reports of genome editing in preclinical models of Duchenne muscular dystrophy[1–6], however, the long-term persistence and safety of these genome editing approaches have not been addressed. Here we show that genome editing and dystrophin protein restoration is sustained in the *mdx* mouse model of Duchenne muscular dystrophy for 1 year after a single intravenous administration of an adeno-associated virus that encodes CRISPR (AAV-CRISPR). We also show that AAV-CRISPR is immunogenic when administered to adult mice[7]; however, humoral and cellular immune responses can be avoided by treating neonatal mice. Additionally, we describe unintended genome and transcript alterations induced by AAV-CRISPR that should be considered for the development of AAV-CRISPR as a therapeutic approach. This study shows the potential of AAV-CRISPR for permanent genome corrections and highlights aspects of host response and alternative genome editing outcomes that require further study.**

Genome editing is sustained for 1 year in neonatal mice treated by intravenous administration.



Structural domains of the human full-length dystrophyn, Becker mini- and micro-dystrophyns, and utrophyn.

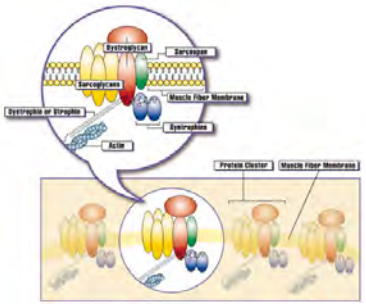


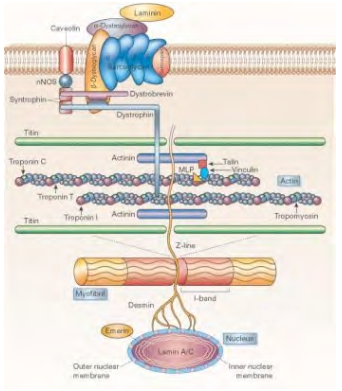

L'**utrofina** e' una proteina, codificata da un gene posto sul cromosoma 6, omologa alla distrofina sia dal punto di vista funzionale sia strutturalmente. L'utrofina e' normalmente presente alla giunzione neuromuscolare delle fibre muscolari mature, ed e' anche espressa sul sarcolemma delle fibre muscolari fetali e del muscolo rigenerante. L'utrofina e' anche espressa sul sarcolemma dei muscoli dei pazienti con DMD; tuttavia, i suoi livelli sono troppo bassi per vicariare la funzione della distrofina.

N-terminal domain (red) binds to F-actin; the cysteine-rich domain (green) binds to -Dystroglycan (-DG) and the C-terminal domain (yellow) binds to Dystrobrevins and syntrophins. The central coiled-coil rod domain (blue) contains 24 spectrin -like repeats (R1–R24) and 4 'hinge' regions (turquoise; H1–H4).

## Successful Compensation for Dystrophin Deficiency by a Helper-dependent Adenovirus Expressing Full-length Utrophin

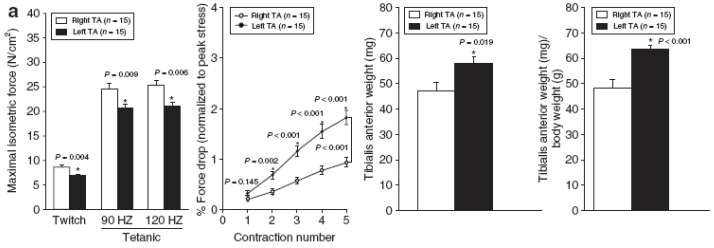
Jatinderpal R Deol[1,2], Gawiyou Danialou[3], Nancy Larochelle[1], Mylène Bourget[1], Joon-Shik Moon[1*], An-Bang Liu[1†], Rénald Gilbert[4], Basil J Petrof[2,3], Josephine Nalbantoglu[1,2,5] and George Karpati[1,5]





**First generation Ad vectors:**
- good expression levels
- transient expression (10-15 days)
- impossible re-administration

**Gutless Ad vectors:**
- tricky production
- not moved to the clinics yet




*Molecular Therapy* vol. 15 no. 10, 1767–1774 oct. 2007

## SMT C1100 (Ezutromid)

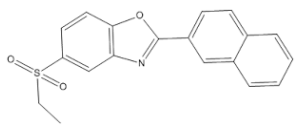
## A Small Molecule Utrophin Modulator for Duchenne

*SMT C1100 has successfully completed a **Phase 1** clinical trial in **healthy male volunteers** in **2012**.*

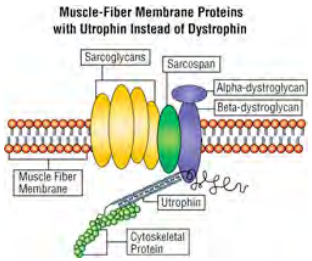
*The **Phase 1b** dose-escalating trial was conducted in **12 patients** with DMD aged between **5 and 11 years** old. These preliminary results show that SMT C1100 was **safe** and well tolerated at all doses tested in the study, and that there were no issues with patient compliance. All the boys had variable blood plasma concentrations of SMT C1100 with only two of the boys achieving concentrations similar to those of the adult volunteers in the 2012 Phase 1 study. Initial evidence suggests that the **variability in drug uptake** may be due to differences in **diet** and to other **disease-related factors**.*

*The non-placebo controlled trial also measured creatine kinase ('CK') levels, an enzyme that is associated with muscle fibre damage and elevated in boys with DMD. In the majority of patients there was a **reduction in CK** levels during dosing with SMT C1100. These data are consistent with non-clinical in vivo efficacy studies in the mdx model of DMD that showed SMT C1100 reduced CK levels after only 15 days.*

***Phase 2 trial initiated in 2016***



SMT C1100 (also known as BMN 195)
5-(ethylsulfonyl)-2-(naphthalen-2-yl)benzo[*d*]oxazole




# Summit Therapeutics Ends Development of Ezutromid Therapy for DMD After Trial Failure

48-week PhaseOut DMD trial which included 40 boys with DMD

Primary endpoint was the change from baseline in magnetic resonance parameters related to the leg muscles

Secondary endpoint consisted of biopsy measures evaluating utrophin and muscle damage, with patients having 2 biopsies, 1 at baseline and a second at either 24- or 48-weeks


Statistical decreases in developmental myosin and magnetic resonance T2 measures were seen after 24 weeks of treatment, but were not seen after 48 weeks

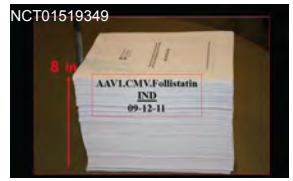
June 2018

# DMD: emerging drugs or small molecule therapies

- Exon skipping
- Mutation suppression
- Gene therapy
- Muscle building strategy

## Follistatin Gene Transfer to Patients With Becker Muscular Dystrophy and Sporadic Inclusion Body Myositis

Phase I Clinical Intramuscular Gene Transfer of rAAV1.CMV.huFollistatin (an antagonist to myostatin) Trial to Patients With Becker Muscular Dystrophy and Sporadic Inclusion Body Myositis.

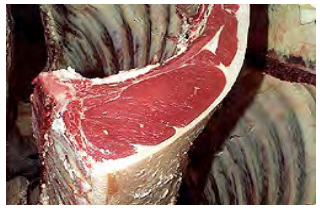



*Proc. Natl. Acad. Sci. USA*
Vol. 94, pp. 12457–12461, November 1997
Genetics

### Double muscling in cattle due to mutations in the myostatin gene

ALEXANDRA C. MCPHERRON AND SE-JIN LEE*
Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205

FIG. 2. A fullblood Belgian Blue bull showing the double muscling phenotype.



### Suppression of body fat accumulation in myostatin-deficient mice

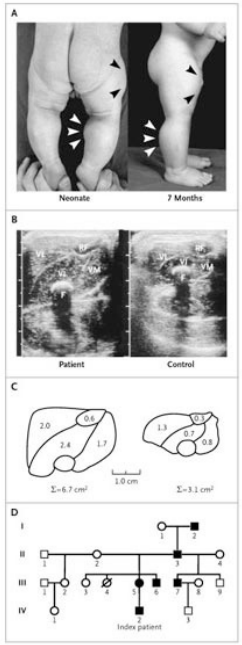
Alexandra C. McPherron and Se-Jin Lee
Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
Address correspondence to: Se-Jin Lee, Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, 725 N. Wolfe Street, Baltimore, Maryland 21205, USA. Phone: (410) 614-0198; Fax: (410) 955-0831; E-mail: sjlee@jhmi.edu.

Received for publication June 19, 2001, and accepted in revised form January 30, 2002.

## Myostatin

Myostatin belongs to the TGF-β superfamily of signal proteins, and it is normally made and secreted by skeletal muscle cells, providing negative feedback to limit muscle growth.

Small amounts of the protein can be detected in the circulation of adult humans, and it has been reported that the amount is raised in AIDS patients who show muscle wasting. Thus, myostatin may act as a negative regulator of muscle growth in adult life as well as during development. The growth of some other organs is similarly controlled by a negative-feedback action of a factor that they themselves produce.

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Review

### Doping in the recombinant era: Strategies and counterstrategies☆

Hassan M.E. Azzazy [b,*], Mai M.H. Mansour [a], Robert H. Christenson [b]

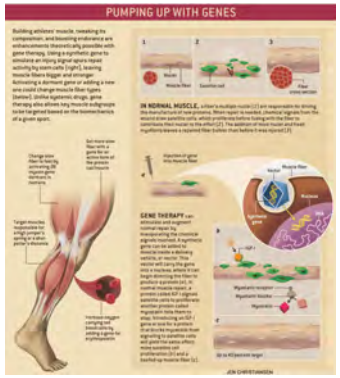



Table 1
Candidate genes for sports doping

| Gene/product | System/organ targets | Gene product properties | Physiologic response |
|---|---|---|---|
| ACE | Skeletal muscles | Peptidyl dipeptidase | ACE-D is involved in fast twitch muscles<br>ACE-I seems to correlate with endurance |
| ACTN3 | Skeletal muscle | Actin-binding proteins related to dystrophin | Involved in fast twitch muscles |
| Endorphins | Central and peripheral nervous systems | Widely active peptides | Pain modulation |
| EPO | Hematopoietic system | Glycoprotein hormone | Increases RBC mass and oxygen delivery |
| HGH | Endocrine system | 191–amino acid protein | Increases muscle size, power, and recovery |
| HIF | Hematologic and immune systems | Multisubunit protein | Regulates transcription at hypoxia response elements |
| IGF-1 | Endocrine/metabolic/skeletal muscle | 70–amino acid protein | Increases muscle size, power, and recovery by increasing regulator cells |
| Myostatin | Skeletal muscle | 2-subunit protein | Regulates skeletal muscle. Inhibition increases muscle size, power, and recovery. |
| PPAR-delta | Skeletal muscle and adipose tissue | Nuclear hormone receptor protein | Promotes fat metabolism and increases number of slow twitch fibers |
| VEGF | Vascular endothelium | Glycosylated disulfide-bonded homodimers | Induces development of new blood vessels |

## GH as a performance-enhancing drug

The use of GH and IGF-I is banned by the World Anti Doping Agency not only because of the principle of fair play in competitive sports, but also because of the adverse effects of supraphysiological doses on health. In addition, although rhGH has been available since 1985, GH extracted from the pituitary glands of human cadavers is still available in some countries. Administration from this source carries a significant risk of infection of transmissible brain diseases.

The use of GH in amateur and professional sports seems to be widespread, although the evidence is quite strong that supraphysiological GH administration does not potentiate the effects of exercise on muscle mass and strength in healthy individuals. IGF-I use is probably more limited as it is less readily available than GH.

Attraction of GH abuse:

- GH is lipolytic
- GH has known effects on collagen and bone turnover
- GH has anectodal side effects such as improving skin tone, eyesight and recovery time from injury
- combination of testosterone and GH leads to improved body composition and $VO_2$ max in elderly men

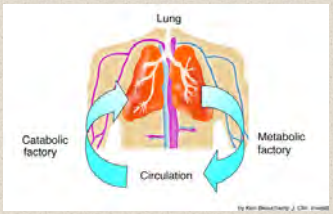
**GENE DOPING?**

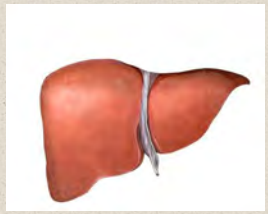
## Genetically engineered metabolic factory to treat genetic diseases

Gene transfer by

viral vectors

Lung | Liver | Muscle



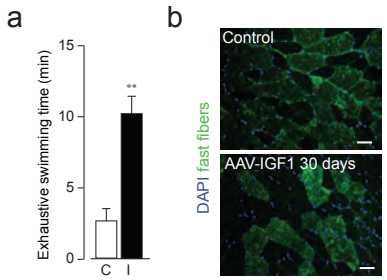



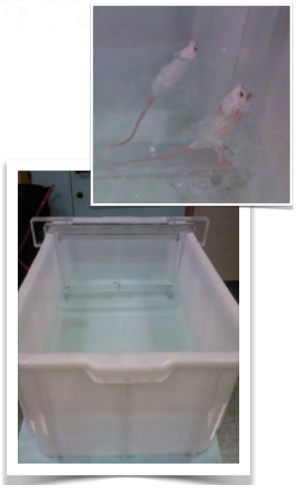
Ectopic production of genetically engineered protein products (Factor IX, Lysosomal enzymes, AAT-1, Epo, ecc)

Delivery into the circulation

## Enhanced Athletic Performance on Multisite AAV-IGF1 Gene Transfer Coincides with Massive Modification of the Muscle Proteome

Antero Macedo,[1,*] Manuela Moriggi,[2,*] Michele Vasso,[2,3] Sara De Palma,[2] Mauro Sturnega,[1] Giorgio Friso,[4] Cecilia Gelfi,[2,3] Mauro Giacca,[1] and Serena Zacchigna[1]



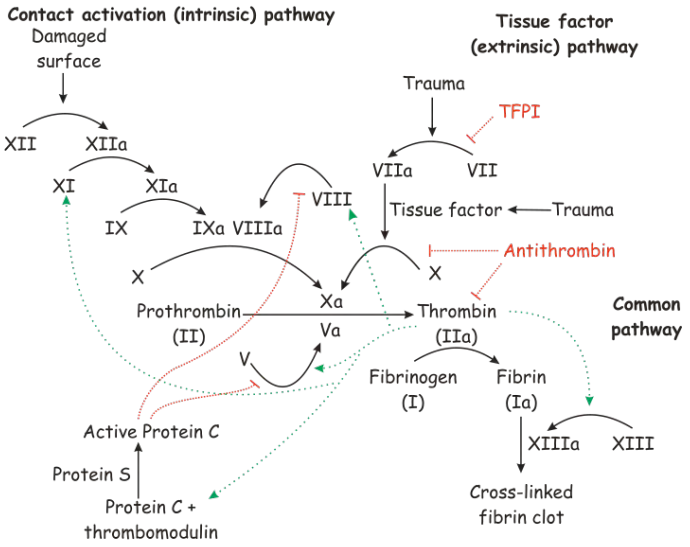
HUMAN GENE THERAPY 23:146–157 (February 2012)

DOI: 10.1089/hum.2011.157

## Hemophilia

- Affects 1:5,000 males
  - 80% hemophilia A due to Factor VIII deficiency
  - 20% hemophilia B due to Factor IX deficiency
- Results in spontaneous bleeding, which can be fatal
- Treated with prophylactic or therapeutic infusion of the deficient factor
- Correction to 1% of normal activity would reduce spontaneous bleeding
- Correction to 10% of normal activity would eliminate most spontaneous bleeding

# Blood coagulation




# Historic Overview on Hemophilia Therapy

- **Replacement therapy: blood transfusion since ‘70s**
- **Recombinant FVIII/FIX infusion since ‘90s**

## Gene Therapy Approaches for Hemophilia:

- Existence of small (KO mice) and large (dog) animal models
- Easy assessment of efficacy (coagulation tests)
- Small correction should be sufficient (1%)
- Liver-directed, Muscle-directed
- Expensive therapy available

# Haemophilia gene transfer trials

1, 2, 5 - FVIII (>8 kb)
3, 4 - FIX (1.4 kb)

| Sponsor | Trial No. | *n* | Vector/Route | Factor Level† | Side Effects |
|---|---|---|---|---|---|
| Chiron | 1—Phase I | 13 | Retrovirus/IV | 0–1% | None |
| TKT | 2—Phase I | 6 | Plasmid/omentum | 0–4% | None |
| Avigen | 3—Phase I | 9 | AAV2/IM | 0–1% | None |
| Avigen | 4—Phase I | 6 | AAV2/intrahepatic | 3–12% | Elevated transaminase‡ |
| Genstar | 5—Phase I | 3 | Adenovirus/IV | 0–1% | Elevated transaminase,‡ thrombocytopenia |

working only after hepatectomy (in animals so far)

Gutless vectors?

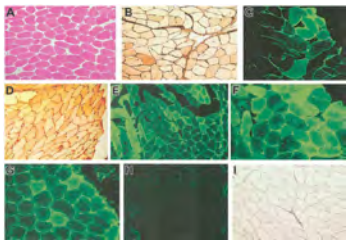
One patient so far.
At 7 days: persistent elevation of transaminases = low therapeutic index
No additional patients enrolled

AAV-mediated factor IX gene transfer to skeletal muscle in patients with severe hemophilia B

Catherine S. Manno, Amy J. Chew, Sylvia Hutchison, Peter J. Larson, Roland W. Herzog, Valder R. Arruda, Shing Jen Tai, Margaret V. Ragni, Arthur Thompson, Margareth Ozelo, Linda B. Couto, Debra G. B. Leonard, Frederick A. Johnson, Alan McClelland, Ciaran Scallan, Erik Skarsgard, Alan W. Flake, Mark A. Kay, Katherine A. High, and Berti Glader

Table 8. Bioactivity and efficacy studies in subjects treated with intramuscular AAV-hFIX

| | PCR on muscle biopsy | Southern blot on muscle biopsy[a] | F.IX immunohistochemistry | Max circ of F.IX | Decrease in F.IX infusion |
|---|---|---|---|---|---|
| A | Pos† | Neg | Neg | 1.40% | 50% |
| B | Pos† | ND | Pos | <1% | 50% |
| C | Pos† | Pos (4) | Neg | <1% | None |
| D | Pos | Pos (1.5) | Pos | <1% | None |
| | Pos | Pos (2.5) | Pos | <1% | None |
| E | Neg | Neg | Pos | <1% | None |
| F | Neg | Neg | Pos | 1% | None |
| | Pos | Pos (0.5) | Pos | 1% | None |
| G | Pos | Neg | Pos | <1% | None |
| H | Pos | Pos (0.5) | Pos | <1% | None |



BLOOD, 15 APRIL 2003 • VOLUME 101, NUMBER 8

Phase I Trial: 8 adult men with severe hemophilia B (<1% baseline F.IX)

F.IX expression close to the site of injection up to 10 months after treatment; no evidence for inflammation

Circulating levels of F.IX were less than what is required for therapeutic effect

## Evidence for gene transfer and expression of factor IX in haemophilia B patients treated with an AAV vector

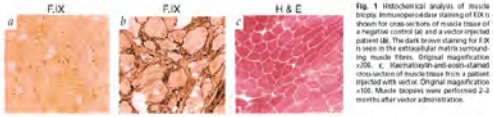



Table 2 • Coagulation data[a] for patients A and B

| | Patient A[b] | | | Patient B[b] | |
|---|---|---|---|---|---|
| | F.IX | aPTT | | F.IX | aPTT |
| Baseline | <1% | | | <0.3% | |
| Week 6 | <0.3% | 82.9 | | | |
| Week 8 | 1% | 61 | | <0.3% | 102 |
| Week 10 | 1.6% | 48 | | 0.3% | 91.2 |
| Week 12 | 1.4% | 46.8 | | 0.3% | 102.3 |
| Week 14 | 3.7% | 41.0 | (post-F.IX infusion) | 3.0% | 52.6 (post-F.IX infusion) |
| Week 17 | 1.3% | 50.6 | | 0.4% | 72 |
| Week 18 | 0.8% | 49.4 | | | |
| Week 20 | 0.5% | 54.1 | | 0.4% | 107 |
| Week 22 | 0.9% | 53.7 | | | |
| Week 24 | 0.5% | 52.1 | | 0.8% | 65.5 |

[a]Unless otherwise noted, all data points were drawn at least 14 d after the most recent factor transfusion. [b]Data generated in CHOP Clinical Coagulation Laboratory. [c]Data generated in Stanford University Clinical Coagulation Laboratory.

Table 3 • Predicted levels of circulating F.IX in humans

| Dose | F.IX level in mice[a] | F.IX level in dogs[b] | Predicted level in humans[c] | Predicted % normal levels in humans |
|---|---|---|---|---|
| $2\times10^{11}$ vg/kg | 6 ng/ml | 2–4 ng/ml | 2–6 ng/ml | ≤0.1% |
| $2\times10^{12}$ vg/kg | 60 ng/ml | 16 ng/ml | 16–60 ng/ml | 0.3–1.2% |
| $1\times10^{13}$ vg/kg | 300 ng/ml | 80 ng/ml | 80–300 ng/ml | 1.8–6% |

[a]Predicted plasma F.IX level in mice based on mouse experimental data[1]. [b]Predicted plasma F.IX level in dogs based on canine experimental data[2]. [c]Extrapolated from studies in animals.

- Transient expression of FIX
- Partial correction of bleeding time
- Detection of anti-AAV2 antibodies

M. Kay, K. High

# Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response



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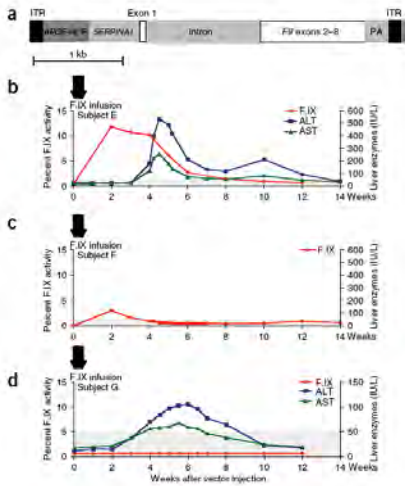


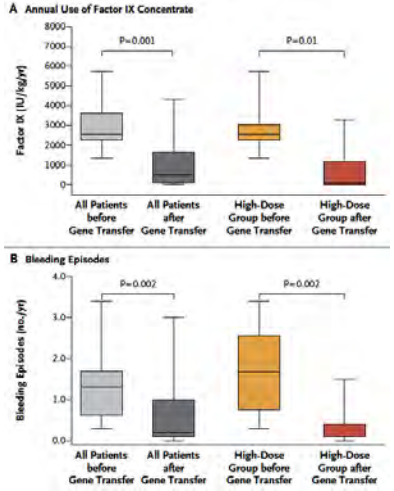

F.IX activity assay and transaminase levels (AST, ALT) plotted as a function of time in weeks after vector administration in subjects E, F and G

ORIGINAL ARTICLE

## Long-Term Safety and Efficacy of Factor IX Gene Therapy in Hemophilia B

A.C. Nathwani, U.M. Reiss, E.G.D. Tuddenham, C. Rosales, P. Chowdary, J. McIntosh, M. Della Peruta, E. Lheriteau, N. Patel, D. Raj, A. Riddell, J. Pie, S. Rangarajan, D. Bevan, M. Recht, Y.-M. Shen, K.G. Halka, E. Basner-Tschakarjan, F. Mingozzi, K.A. High, J. Allay, M.A. Kay, C.Y.C. Ng, J. Zhou, M. Cancio, C.L. Morton, J.T. Gray, D. Srivastava, A.W. Nienhuis, and A.M. Davidoff

N ENGL J MED 371;21 NEJM.ORG NOVEMBER 20, 2014




ABSTRACT

BACKGROUND

In patients with severe hemophilia B, gene therapy that is mediated by a novel self-complementary adeno-associated virus serotype 8 (AAV8) vector has been shown to raise factor IX levels for periods of up to 16 months. We wanted to determine the durability of transgene expression, the vector dose–response relationship, and the level of persistent or late toxicity.

METHODS

We evaluated the stability of transgene expression and long-term safety in 10 patients with severe hemophilia B: 6 patients who had been enrolled in an initial phase 1 dose-escalation trial, with 2 patients each receiving a low, intermediate, or high dose, and 4 additional patients who received the high dose ($2\times10^{12}$ vector genomes per kilogram of body weight). The patients subsequently underwent extensive clinical and laboratory monitoring.

RESULTS

A single intravenous infusion of vector in all 10 patients with severe hemophilia B resulted in a dose-dependent increase in circulating factor IX to a level that was 1 to 6% of the normal value over a median period of 3.2 years, with observation ongoing. In the high-dose group, a consistent increase in the factor IX level to a mean (±SD) of 5.1±1.7% was observed in all 6 patients, which resulted in a reduction of more than 90% in both bleeding episodes and the use of prophylactic factor IX concentrate. A transient increase in the mean alanine aminotransferase level to 86 IU per liter (range, 36 to 202) occurred between week 7 and week 10 in 4 of the 6 patients in the high-dose group but resolved over a median of 5 days (range, 2 to 35) after prednisolone treatment.

CONCLUSIONS

In 10 patients with severe hemophilia B, the infusion of a single dose of AAV8 vector resulted in long-term therapeutic factor IX expression associated with clinical improvement. With a follow-up period of up to 3 years, no late toxic effects from the therapy were reported. (Funded by the National Heart, Lung, and Blood Institute and others; ClinicalTrials.gov number, NCT00979238.)

# Merry christmas for patients with hemophilia B.

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.

N Engl J Med 2011; 365:2357-2365 | December 22, 2011

Patients suffering from severe hemophilia B ( < 1% FIX) were injected by peripheral vein administration with an AAV serotype 8 vector (AAV8) encoding a codon-optimized FIX

**AAV8** can efficiently transduce hepatocytes, does not interact as efficiently with antigen-presenting cells as AAV2, and has limited cross-reactivity with preexisting anti-AAV2 antibodies

This **scAAV** design is more efficient possibly because it obviates the need for second-strand synthesis or reannealing of positive and negative AAV strands to generate transcription-competent double-stranded DNA templates

Subjects received low (2 · 10^11 vg/kg), intermediate (6 · 10^11 vg/kg), or high (2 · 10^12 vg/kg) scAAV8-FIX vector doses, with two participants in each cohort.
**All subjects expressed FIX above the 1% threshold for several months (FIX levels varied between 2% and 11%)**
**Four discontinued FIX prophylaxis and remained free of spontaneous bleeding episodes, although most of these subjects required prophylaxis to prevent bleeding upon trauma**

One subject who received the highest vector dose developed grade III liver toxicity related to the vector itself, resulting in a significant increase in serum transaminase levels and a concomitant decrease of FIX levels from 7% to 3%. This was associated with the detection of AAV8 capsid-specific T-cells.
The other subject had a slight increase in liver enzyme levels concomitant with an increase in AAV8 capsid-specific T-cells and a slight decrease in FIX level.

## Seven different pharma companies are currently developing gene therapy treatments for hemophilia:

1) uniQure
January 7th, 2016

UniQure presented **results from an early study of AMT-060** (**AAV5-hFIX**). The first 2 hemophilia patients treated with AMT-060, about 12 and 20 weeks after treatment, are now producing 4.5 percent and 5.5 percent, respectively, of normal Factor IX. To put that in context, these patients have severe or moderately severe hemophilia, meaning they typically produce less than 1 to 2 percent of these levels, and rely on frequent infusions to get those numbers up.

2) Baxalta
June 24, 2015

Baxalta reported continued progress on the Phase 1/2 open-label clinical trial assessing the safety and optimal dosing level of BAX 335, an advanced **rAAV8**-based gene therapy technology for **FIX** expression.
Some FIX expression was observed in the lowest dosing cohort ($2x10^{11}$ vg/kg). In the second dosing cohort ($1x10^{12}$ vg/kg), two patients have experienced no bleeds without regular infusions of FIX and one of these patients has had sustained FIX expression levels of 20-25 % for 12 months. In the highest dose cohort ($3x10^{12}$ vg/kg), expression levels have peaked above 50 %, though the two patients in this cohort experienced an immune response which has led to decreased FIX expression, with one patient resuming regular FIX infusions.

3) DIMENSION THERAPEUTICS

**AAV8 and AAVrh10** are two forms of AAV that selectively target liver cells and have been optimized to deliver missing intact genes in diseases associated with the liver

4) Spark

*With Pfizer we are developing novel bio-engineered **AAV** vectors utilizing a **high-activity factor IX** transgene and a treatment protocol designed to mitigate immune responses seen in other hemophilia B gene therapy trials, including our own, that have limited the duration of efficacy. We initiated a Phase 1/2 trial in June of 2015.*

5) BIOMARIN®

September 28, 2015. BioMarin Enrolls First Patient in Phase 1/2 Trial of Gene Therapy Drug Candidate BMN 270 for the Treatment of Hemophilia A

The Phase 1/2 study will evaluate the safety and efficacy of BMN 270 gene therapy in up to 12 patients with severe Hemophilia A. The primary endpoints are to assess the safety of a single intravenous administration of a recombinant **AAV**, human-coagulation **Factor VIII** vector and to determine the change from baseline of Factor VIII expression level at 16 weeks after infusion.

6) SANGAMO

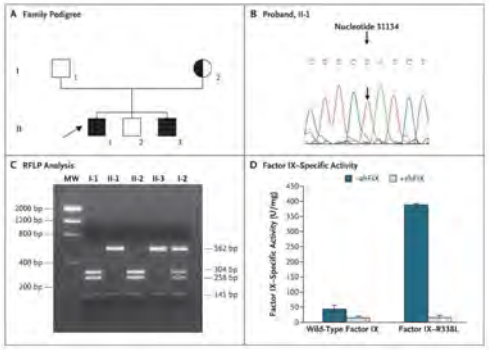
Sangam Biosciences is developing a **ZFN–mediated genome editing approach to hemophilia A and B** using our proprietary In Vivo Protein Replacement Platform (IVPRP).

7) biogen idec.

Biogen Idec, Fondazione Telethon and Ospedale San Raffaele Announce Global Collaboration to Develop Gene Therapies for Hemophilia
***Programs to apply lentiviral vector technology to hemophilia A and B***

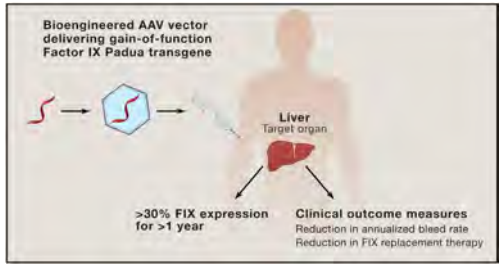
# Factor IX Padua variant

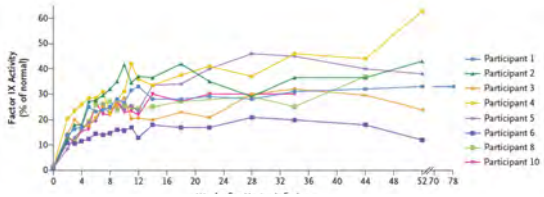
- single point mutation, R338L
- initially discovered in a thrombophilic patient
- possesses increased procoagulant activity due to an enhanced incorporation into the intrinsic tenase complex in the clotting cascade
- pre-clinical studies of FIX Padua in gene therapy confirmed its benefit




# Hemophilia B Gene Therapy with a High-Specific-Activity Factor IX Variant

L.A. George, S.K. Sullivan, A. Giermasz, J.E.J. Rasko, B.J. Samelson-Jones, J. Ducore, A. Cuker, L.M. Sullivan, S. Majumdar, J. Teitel, C.E. McGuinn, M.V. Ragni, A.Y. Luk, D. Hui, J.F. Wright, Y. Chen, Y. Liu, K. Wachtel, A. Winters, S. Tiefenbacher, V.R. Arruda, J.C.M. van der Loo, O. Zelenaia, D. Takefman, M.E. Carr, L.B. Couto, X.M. Anguela, and K.A. High




- use of a gain-of-function FIX transgene, the Padua variant that results in a 7-fold increase in specific coagulant activity of the protein
- FIX levels not only sufficient to eliminate the risk of spontaneous bleeding but also to minimize the risk of bleeding from interventions and trauma
- persistence of FIX expression out to beyond one year - could a single administration of AAV-mediated gene therapy be curative for hemophilia?
- novel AAV vector that has bioengineered changes to the vector capsid to avoid pre-existing immunity

Spark

N Engl J Med 2017;377:2215-27.
DOI: 10.1056/NEJMoa1708538

# AAV5–Factor VIII Gene Transfer in Severe Hemophilia A

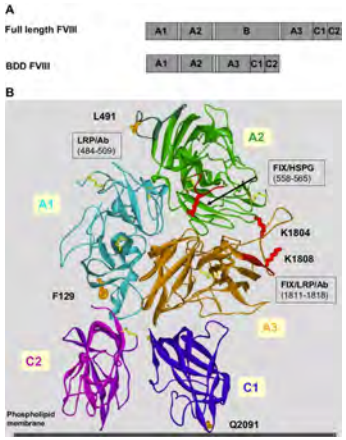
Savita Rangarajan, M.B., B.S., Liron Walsh, M.D., Will Lester, M.B., Ch.B., Ph.D., David Perry, M.D., Ph.D., Bella Madan, M.D., Michael Laffan, D.M., Hua Yu, Ph.D., Christian Vettermann, Ph.D., Glenn F. Pierce, M.D., Ph.D., Wing Y. Wong, M.D., and K. John Pasi, M.B., Ch.B., Ph.D.et al.

CONCLUSIONS The infusion of AAV5-hFVIII-SQ was associated with the sustained normalization of factor VIII activity level over a period of 1 year in six of seven participants who received a high dose, with stabilization of hemostasis and a profound reduction in factor VIII use in all seven participants. In this small study, no safety events were noted, but no safety conclusions can be drawn. (Funded by BioMarin Pharmaceutical; ClinicalTrials.gov number, NCT02576795; EudraCT number, 2014-003880-38.)

BIOMARIN®

December 28, 2017
N Engl J Med 2017; 377:2519-2530

Infusion of a **single** intravenous dose of a codon-optimized adeno-associated virus serotype 5 (AAV5) vector encoding a B-domain–deleted human factor VIII (AAV5-hFVIII-SQ) in nine men with severe **hemophilia A (the most common type)**




**CASE STUDY - Jake Omer, 29**

James lives in Billericay and is married with two children, aged 3 years and a baby of 5 weeks. Diagnosed at two years old, he has had frequent injections of factor VIII to prevent bleeds ever since. Before he was treated with the gene therapy, Jake would wake up early before work to inject three times a week as well as injecting whenever he had an injury to stop the bleeding. As a result of of repeated bleeding Jake has arthritis in his ankles. His father is a Turk-Cypriot and as a child he and his family could never travel to visit relatives in case he needed medical care as the facilities wouldn't have been available.

"The gene therapy has changed my life. I now have hope for my future. It is incredible to now hope that I can play with my kids, kick a ball around and climb trees well into my kids' teenage years and beyond. The arthritis in my ankles meant I used to worry how far I would be able to walk once I turned 40. At 23 I struggled to run 100m to catch a bus; now at 29 I'm walking two miles every day which I just couldn't have done before having the gene therapy treatment.
"It's really strange to not have to worry about bleeding or swellings. The first time I noticed a difference was about four months after the treatment when I dropped a weight in the gym, bashing my elbow really badly. I started to panic thinking this is going to be really bad, but after icing it that night I woke up and it looked normal. That was the moment I saw proof and knew that the gene therapy had worked."

The five UK trial sites included: The Royal London, Guys and St Thomas', Birmingham, Cambridge and Hampshire hospitals.

**Table 1.** Active hemophilia A & B AAV gene therapy clinical studies (43)

| Sponsor Factor IX | NCT | Study | AAV | Vector Dose (vg/kg) | Phase | Patients (target) | ED | AAV Ab | Status |
|---|---|---|---|---|---|---|---|---|---|
| SJCRH/UCL | NCT00979238 | n/a | scAAV2/8-LP1-FIXco-wt | 2e11, 6e11, 2e12 | 1 | 14 | ≥50 | Negative | Not recruiting |
| Shire (Baxalta) | NCT01687608 | AskBio009 | AAV8.sc-TTR-FIXco-Padua | 2e11, 1e12, 3e12 | 1, 2 | 30 | n/a | Negative | Not recruiting |
| Uniqure | NCT02396342 | AMT-060-01 | AAV5-FIXco-wt | 5e12, 2e13 | 1, 2 | 10 | >150 | Negative | Not recruiting |
| | NCT03489291 | AMT-061-01 | AAV5-FIXco-Padua | 2e13 | 2 | 3 | >20 | Included | Not recruiting |
| | NCT03569891 | AMT-061-02 (HOPE-B) | AAV5-FIXco-Padua | 2e13 | 3 | 56 | >150 | Included | Recruiting |
| Pfizer (Spark) | NCT02484092 | SPK-9001-101 | AAV-SPARK100-FIXco-Padua | 5e11 | 2 | 15 | ≥50 | Negative | Not recruiting |
| | NCT03307980 | SPK-9001-LTFU-101 | | SPK-9001 extension study | 2 | 20 | n/a | n/a | Recruiting |
| | NCT03861273 | BENEGENE-2 | | n/a | 3 | 55 | ≥50 | < 1:1 | Not yet recruiting |
| UCL Freeline | NCT03369444 | FLT-180a | AAVS3-FIXco-Padua | 6e11, 2e12 | 1 | 18 | >150 | Negative | Recruiting |
| | NCT03641703 | | | FLT180a extension study | 2, 3 | 50 | n/a | n/a | Recruiting |
| Factor VIII | | | | | | | | | |
| Biomarin | NCT02576795 | BMN-270-201 | AAV5-FVIII-BDD | 6e12, 2e13, 6e13 | 1, 2 | 15 | >150 | Negative | Not recruiting |
| | NCT03392974 | BMN-270-302 | | 4e13 | 3 | 40 | >150 | Negative | Recruiting |
| | NCT03370913 | BMN-270-301 | | 6e13 | 3 | 130 | >150 | Negative | Recruiting |
| | NCT03520712 | BMN-270-203 | | 6e13 | 1, 2 | 10 | >150 | Included | By invitation |
| Spark | NCT03003533 | SPK-8011-101 | AAV-SPARK200-FVIII-BDD | 5e11, 1e12, 2e12 | 1, 2 | 30 | >150 | Negative | Recruiting |
| | NCT03432520 | SPK-8011-LTFU | n/a | SPK-8011 extension study | 1,2 | 100 | n/a | n/a | By invitation |
| | NCT03734588 | SPK-8016-101 | | Dose finding pre FVIII inhibitor study | 1, 2 | 30 | > 150 | Negative | Recruiting |
| UCL | NCT03001830 | GO-8 | AAV2/8-HLP-FVIII-V3 | 6e11, 2e12, 6e12 | 1 | 18 | >50 | Negative | Recruiting |
| Sangamo | NCT03061201 | SB-525-1603 | AAV2/6-FVIII-BDD | 9e11, 2e12, 1e13, 3e13 | 1, 2 | 20 | >150 | Negative | Recruiting |
| Shire (Baxalta) | NCT03370172 | BAX-888 | AAV8-FVIII-BDD | n/a | 1, 2 | 10 | >150 | <1:5 | Recruiting |
| Bayer | NCT03588299 | BAY2599023 (DTX201) | n/a | n/a | 1, 2 | 18 | >150 | Negative | Recruiting |

Ab, antibody; co, codon optimized; ED, exposure days to factor concentrate; sc, self-complementary; SJCRH, St. Jude Children's Research Hospital; UCL, University College London; vg, vector genomes; wt, wild-type; FVIII-BDD, B-domain deleted FVIII.