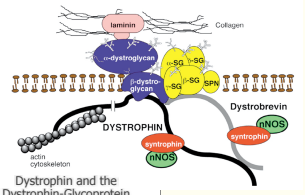
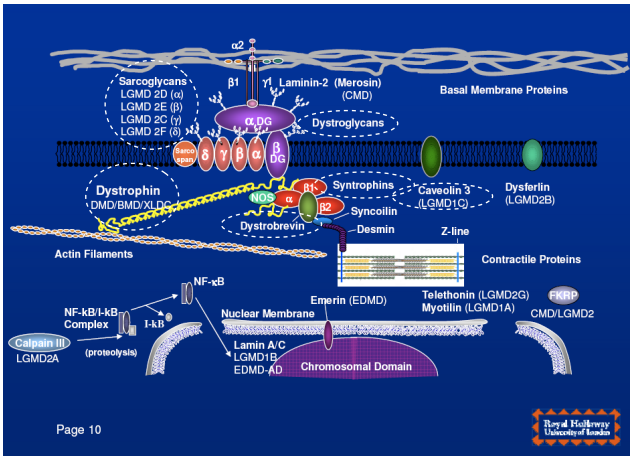
# Distrofie muscolari

| 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) |

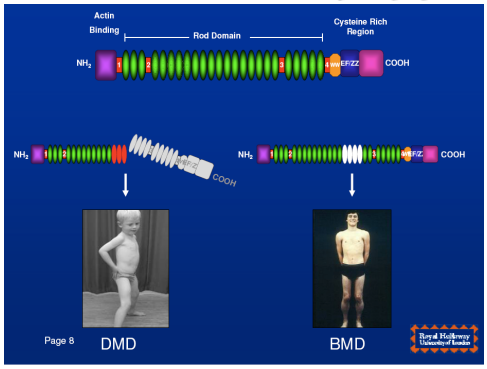


Dystrophin and the Dystrophin-Glycoprotein Complex (DCG)

## 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

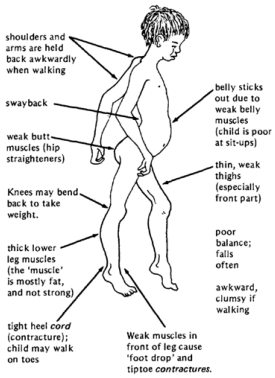
## DMD: il problema clinico

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

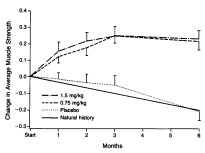
Only glucocorticoids have consistently demonstrated efficacy in DMD


1552 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. Amato, and B. Gelber

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.

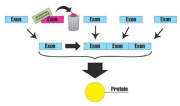
Side effects:
- weight gain with cushingoid appearance, high risk for hypertension, cataract, loss of bone density, vertebral compression fractures and long bone fractures
- long term administration limited by steroid-induced behavioral problems

## DMD: emerging drugs or small molecule therapies

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

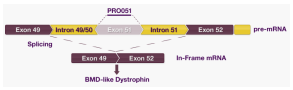
## Exon skipping

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

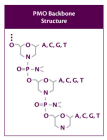
original article

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

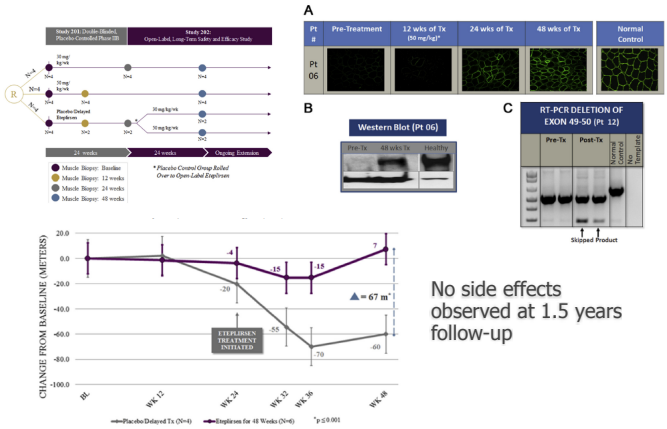



**Exon skipping and dystrophin restoration in patients with Duchenne muscular dystrophy after systemic phosphorodiamidate morpholino oligomer treatment: an open-label, phase 2, dose-escalation study**

Sebahattin Cirak*, Virginia Arechavala-Gomeza*, Michela Guglieri, Lucy Feng, Silvia Torelli, Karen Anthony, Stephen Abbs, Maria Elena Garralda, John Bourke, Dominic J Wells, George Dickson, Matthew J A Wood, Steve D Wilton, Volker Straub, Ryszard Kole, Stephen D 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

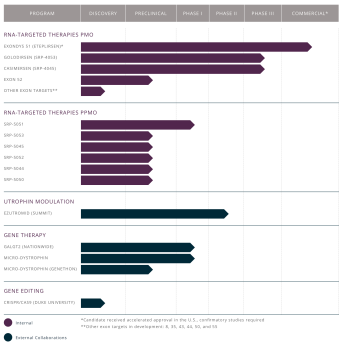
## Accelerated FDA approval of the exon-skipping drug eteplirsen

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

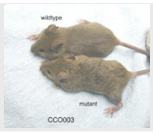
Gene therapy requires delivery of a new gene to the vast majority of muscles in the body and to the heart - a daunting challenge, since muscle tissues makes up **>40% of body mass**

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

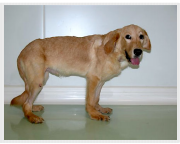
Muscle transduction must not trigger toxic or immunological reactions that are harmful to the patient or that lead to further muscle damage

# Duchenne muscular dystrophy - natural animal models

mdx mice (point mutation leading to a premature truncation)

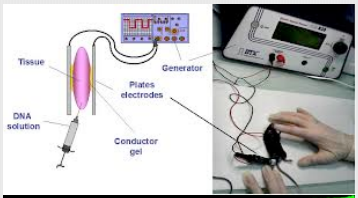


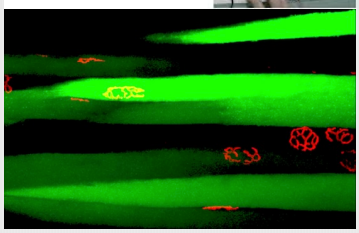

xdm golden retriever dog (exon 7 skipping)



## 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]

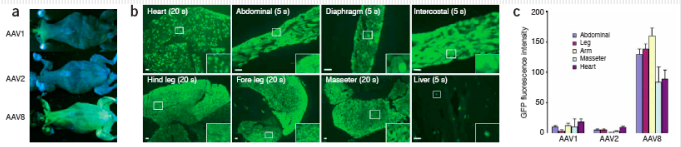



**Figure 2** Systemic gene delivery to muscle of neonatal mice by different AAV serotypes via i.v. injection. (**a**) Whole-body fluorescent photography taken one month after i.v. (temporal vein) injection of $2 \times 10^{11}$ v.g. of various dsAAV-CB-GFP at 3 d of age. (**b**) GFP expression seen in 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

*R254* *Human Molecular Genetics, 2006, Vol. 15, Review Issue No. 2*

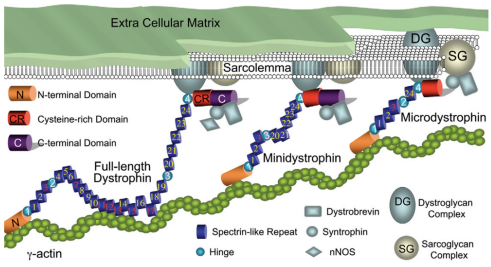



**Figure 1.** Schematic outline of full-length dystrophin, minidystrophin and microdystrophin and their interaction with other cellular proteins. Spectrin-like repeats are numbered from 1 to 24 (positively charged repeats are in red color, other repeats are in yellow color). Proline-rich hinges are numbered 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.

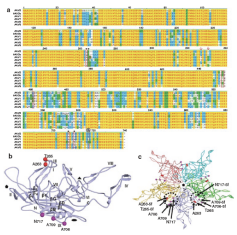
Principal Investigator:
Jerry R. Mendell, MD
Nationwide Children's Hospital

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[3], Jade J Samulski[2], Angelique S Camp[4], Juan Li[3], Bing Wang[3], Paul E Monahan[3], Joseph E Rabinowitz[4], Joshua C Grieger[4], Lakshmanan Govindasamy[3], Mavis Agbandje-McKenna[3], 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]Asklepios 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**

| 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 boys
Good safety
Unsuccessful transgene expression

*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


## GNT 0004 (Genethon)

- First patient dosed in April 2021
- The trial aims to enroll boys aged 6 to 10 suffering from DMD who are still able to walk. The trial was approved in France, in the UK, and submissions are ongoing in the USA and Israel.
- Now developed jointly in the clinical phase with Sarepta Therapeutics.

## PF-06939926 (Pfizer)

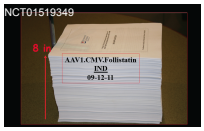
- An adeno-associated virus serotype 9 (AAV9) capsid to deliver a mini-dystrophin gene
- Ongoing Phase 1b trial in boys ages 5-12 with DMD who can still walk supports safety, sustained production of the the mini-dystrophin protein and improvement of motor function
- A randomized, placebo-controlled Phase 3 trial, called **CIFFREO**, is now enrolling up to 99 DMD boys, ages 4 to 7, at sites in Italy, Israel, and Spain, and is expected to expand to 55 sites in 15 countries.

## 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

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Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205

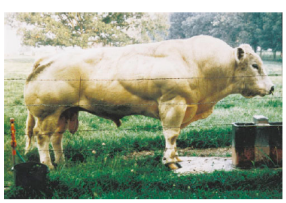

FIG. 2. A fullblood Belgian Blue bull showing the double muscling phenotype.

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

Alexandra C. McPherron and Se-Jin Lee

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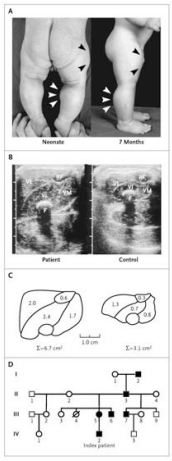
Address correspondence to: Se-Jin Lee, Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, 725 N. Wolfe Street, Baltimore, Maryland 21205, USA. Phone: (410) 614-0198; Fax: (410) 955-0831; E-mail: sjlee@jhmi.edu.

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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SCIENCE DIRECT
Clinical Biochemistry 38 (2005) 999–965
CLINICAL BIOCHEMISTRY

Review

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

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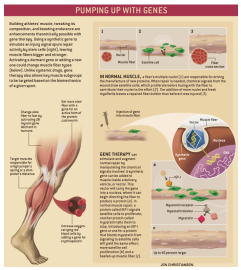
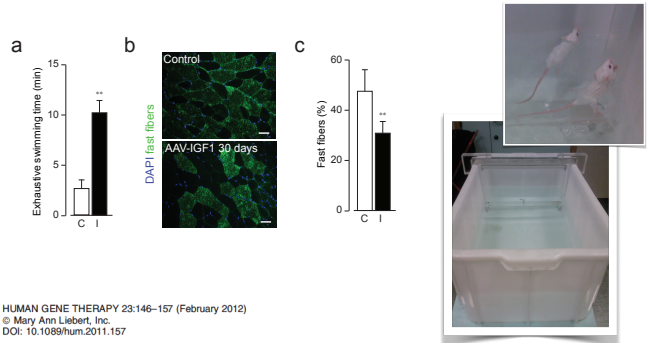



Table 1
Candidate genes for sports doping

| 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 |

## 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]



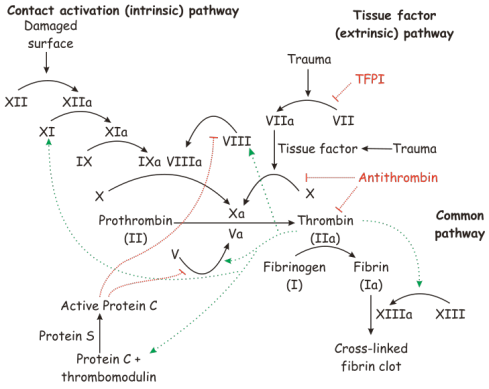

HUMAN GENE THERAPY 23:146–157 (February 2012)

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

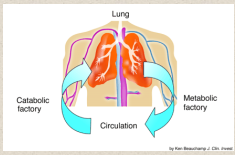
## Blood coagulation

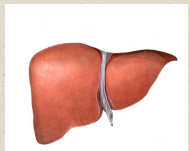



## Genetically engineered metabolic factory to treat genetic diseases

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

## 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?

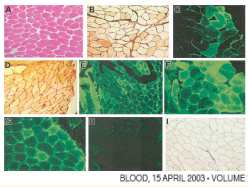
One patient so far.
At 7 days: persistent elevation of transaminases = low therapeutic index
No additional patients enrolled

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* | immunohistochemistry | F.IX | Max circ of F.IX | Decrease in F.IX infusion |
|---|---|---|---|---|---|---|
| A | Pos† | Neg | Neg | Pos | 1.40% | 50% |
| B | Pos† | ND | Pos | Pos | <1% | 50% |
| C | Pos† | Pos (4) | Neg | Pos | <1% | None |
| D | Pos | Pos (1.5) | Pos | Pos | <1% | None |
| | Pos | Pos (2.5) | Pos | Pos | <1% | None |
| E | Neg | Neg | Pos | Pos | <1% | None |
| F | Neg | Neg | Pos | Pos | 1% | None |
| | Pos | Pos (0.5) | Pos | Pos | 1% | None |
| G | Pos | Neg | Pos | Pos | <1% | None |
| H | Pos | Pos (0.5) | Pos | 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

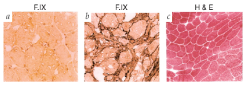



Fig. 1 Histochemical analysis of muscle biopsy. Immunoperoxidase staining of F.IX is shown for cross-sections of muscle tissue of a negative control (a) and a vector-injected patient (d). The dark brown staining for F.IX is seen in the extracellular matrix surrounding the muscle fibers. Original magnification ×100. (b, c, e, f) Hematoxylin and eosin-stained cross-section of muscle tissue from a patient injected with vector. Original magnification ×100. Muscle biopsies were performed 2–3 months after vector administration.

Table 2 • Coagulation data* for patients A and B

| | Patient A† | | | Patient 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 | 0.8% | 53.7 | | | |
| Week 24 | 0.5% | 52.1 | | 0.8% | 65.5 |

*Unless otherwise noted, all data points were drawn at least 14 d after the most recent factor transfusion. †Data generated in CHOP Clinical Coagulation Laboratory. ‡Data generated in Stanford University Clinical Coagulation Laboratory.

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

| Dose | F.IX level in vivo* | F.IX level in dogs† | Predicted level in humans‡ | Predicted % normal levels in humans |
|---|---|---|---|---|
| $2\times10^{11}$ vg/kg | 6 ng/ml | 2–4 ng/ml | 2–4 ng/ml | ≤0.1% |
| $2\times10^{12}$ vg/kg | 60 ng/ml | 16 ng/ml | 16–40 ng/ml | 0.3–1.2% |
| $1\times10^{13}$ vg/kg | 300 ng/ml | 80 ng/ml | 80–300 ng/ml | 1.8–6% |

*Predicted plasma F.IX level in mice based on mouse-experimental data†. *Predicted plasma F.IX level in dogs based on canine experimental data†. ‡Extrapolated from studies in animals.

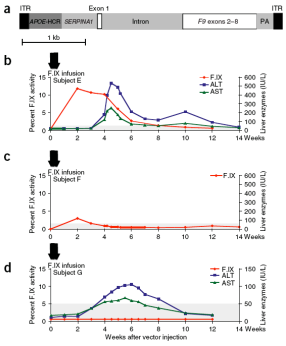
M. Kay, K. High

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

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

MARCH 2006 NATURE MEDICINE

Catherine S Manno[1,2,15], Glenn F Pierce[3,15], Valder R Arruda[1,2,15], Bertil Glader[4,15], Margaret Ragni[5], John J E Rasko[6], Margareth C Ozelo[7], Keith Hoots[8], Philip Blatt[9], Barbara Konkle[10], Michael Dake[4], Robin Kaye[1,2], Mahmood Razavi[4], Albert Zajko[10], James Zehnder[4], Pradip K Rustagi[11], Hiroyuki Nakai[4], Amy Chew[1,3], Debra Leonard[2,12], J Fraser Wright[3], Ruth R Lessard[3], Jürg M Sommer[3], Michael Tigges[3], Denise Sabatino[1], Alvin Luk[3], Haiyan Jiang[3], Federico Mingozzi[1], Linda Couto[3], Hildegund C Ertl[1,13], Katherine A High[1,2,3,14] & Mark A Kay[4]




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

## 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., Amullo 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

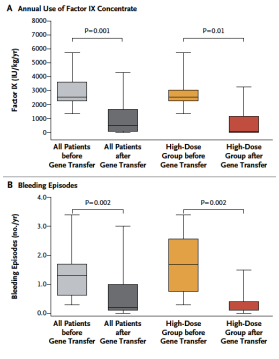
Subjects received low (2 · 10^11 vg/kg), intermediate (6 · 10^11 vg/kg), or high (2 · 10^12 vg/kg) scAAV8-FIX vector doses, with two participants in each cohort.
**All subjects expressed FIX above the 1% threshold for several months (FIX levels varied between 2% and 11%)**
**Four discontinued FIX prophylaxis 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.

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.

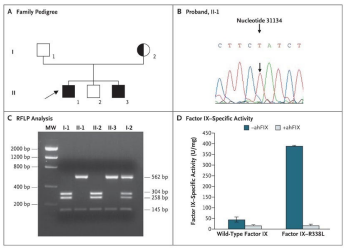
METHODS
We evaluated the stability of transgene expression and long-term safety in 10 patients with severe hemophilia B: 6 patients who had been enrolled in an initial phase 1 dose-escalation trial, with 2 patients each receiving a low, intermediate, or high dose, and 4 additional patients who received 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.)

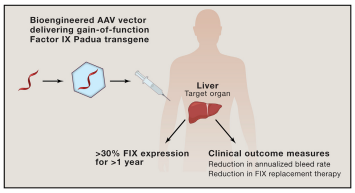
# Factor IX Padua variant

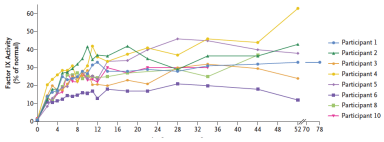
- single point mutation, R338L
- initially discovered in a thrombophilic patient
- possesses increased procoagulant activity due to an enhanced incorporation into the intrinsic tenase complex in the clotting cascade
- pre-clinical studies of FIX Padua in gene therapy confirmed its benefit




# 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

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

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 Therapeutics
*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***

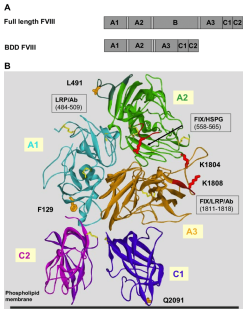
# AAV5–Factor VIII Gene Transfer in Severe Hemophilia A

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)**




# Gene Therapy for patients with haemophilia A

2-year and 3-year safety and efficacy data from 15 patients with severe hemophilia A in a manufacturer-sponsored phase 1/2 dose-escalation study involving a single infusion of the non-FDA-approved therapy AAV5-hFVIII-SQ (valoctocogene roxaparvovec). 4 cohorts, dose-escalating.


ORIGINAL ARTICLE
Multiyear Follow-up of AAV5-hFVIII-SQ Gene Therapy for Hemophilia A
K. John Pasi, M.B., Ch.B., Ph.D., Savita Rangarajan, M.B., B.S., Nina Mitchell, M.B., B.Chir., Will Lester, M.B., Ch.B., Ph.D., Emily Symington, M.B., B.S., Bella Madan, M.D., Michael Laffan, D.M., Chris B. Russell, Ph.D., Mingjin Li, M.Sc., Glenn F. Pierce, M.D., Ph.D., and Wing Y. Wong, M.D. et al.
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N Engl J Med 2020; 382:29-40
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- Key findings:
- An increase in ALT was the most common adverse event, but was mild (grade 1).
- At 3 years, patients in lower dose cohorts 1 and 2 (1 patient each) had factor VIII levels below 1 IU/dL.
- At 3 years, the seven patients in cohort 3 had a median factor VIII activity level of 20 IU/dL, along with a 96% decrease in the bleeding rate and a 96% decrease in use of factor VIII infusions. All 7 experienced resolution of target joint bleeding.
- At 2 years, the 6 patients in cohort 4 had a median factor VIII activity level of 13 IU/dL, along with a 92% decrease in annualized bleeding rate and a 95% decrease in use of factor VIII infusions. Five had resolution of target joint bleeding.
- No patients developed a factor VIII inhibitor.