Ottawa Hospital Research Institute

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Institut de recherche de l'Hôpital d'Ottawa

Progress towards genetic therapies for Duchenne Muscular Dystrophy

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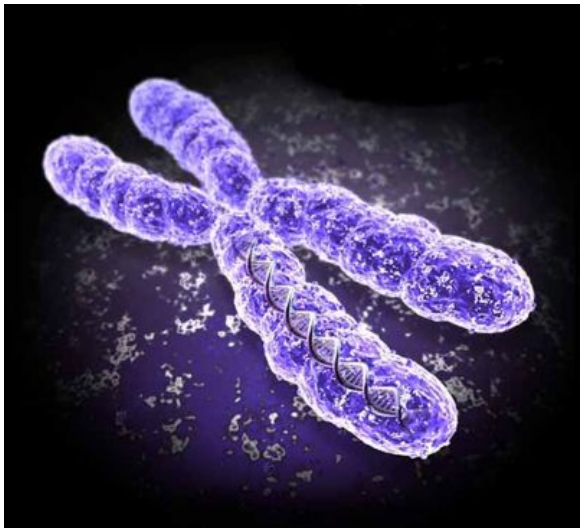
THE OTTAWA HOSPITAL L'HÔPITAL D'OTTAWA

uOttawa

99.9% of DNA is identical between individuals



Only 0.1% of our DNA is different

- Many of these differences result in subtle changes in how we look
  - for example, blue *versus* brown eyes
- Some of these changes lead to mutations
  - for example, mutations within the dystrophin gene cause Duchenne Muscular Dystrophy

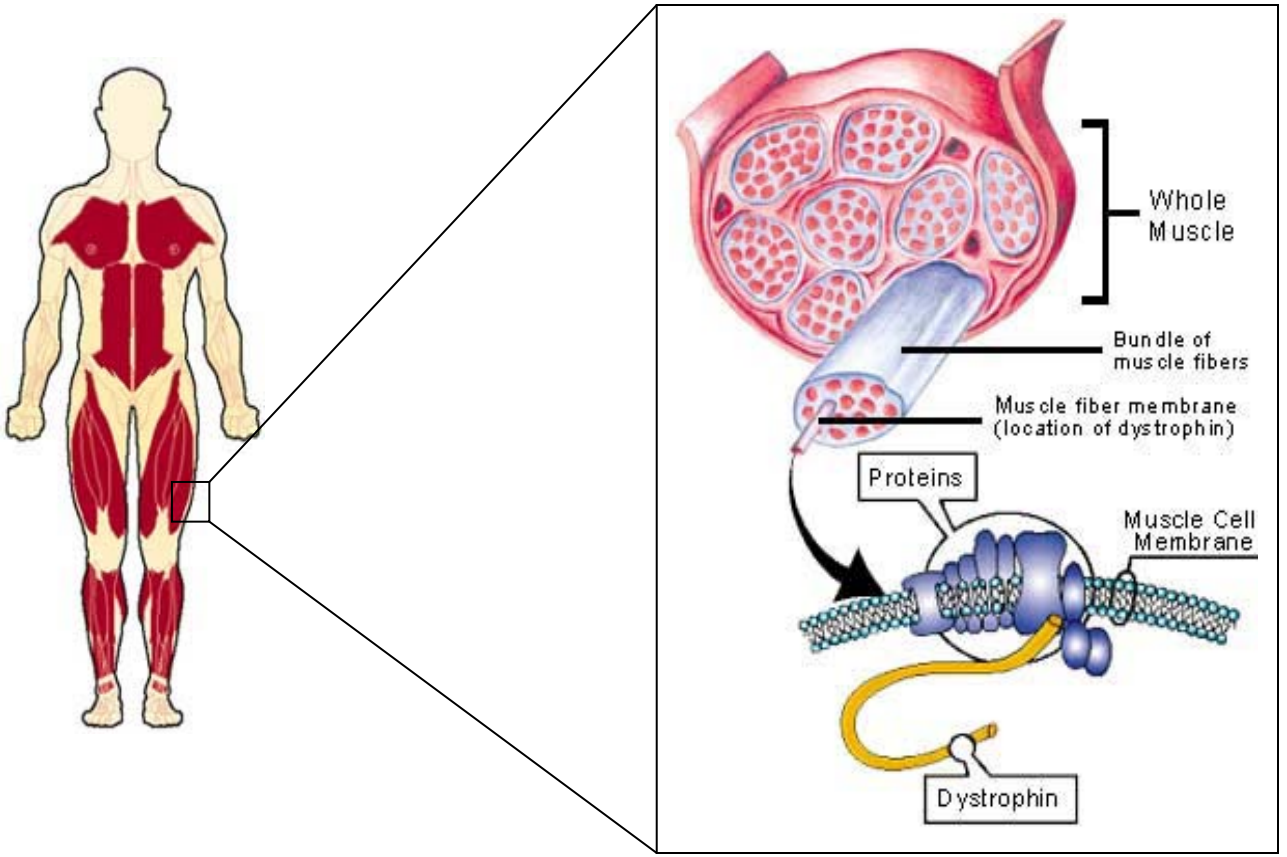
Duchenne Muscular Dystrophy

- Affects 1 in 3500 male children
- Characterized by progressive muscle wasting and weakness
- First symptoms appear as early as 3 years of age
  - Initially affects skeletal or voluntary muscles
- Patients lose the ability to walk by 7-12 years of age
- By early teens, heart and respiratory muscles are also affected
- Patients usually die in their 20's to 30's due to respiratory or cardiac failure

- No effective treatment is currently available

Duchenne Muscular Dystrophy

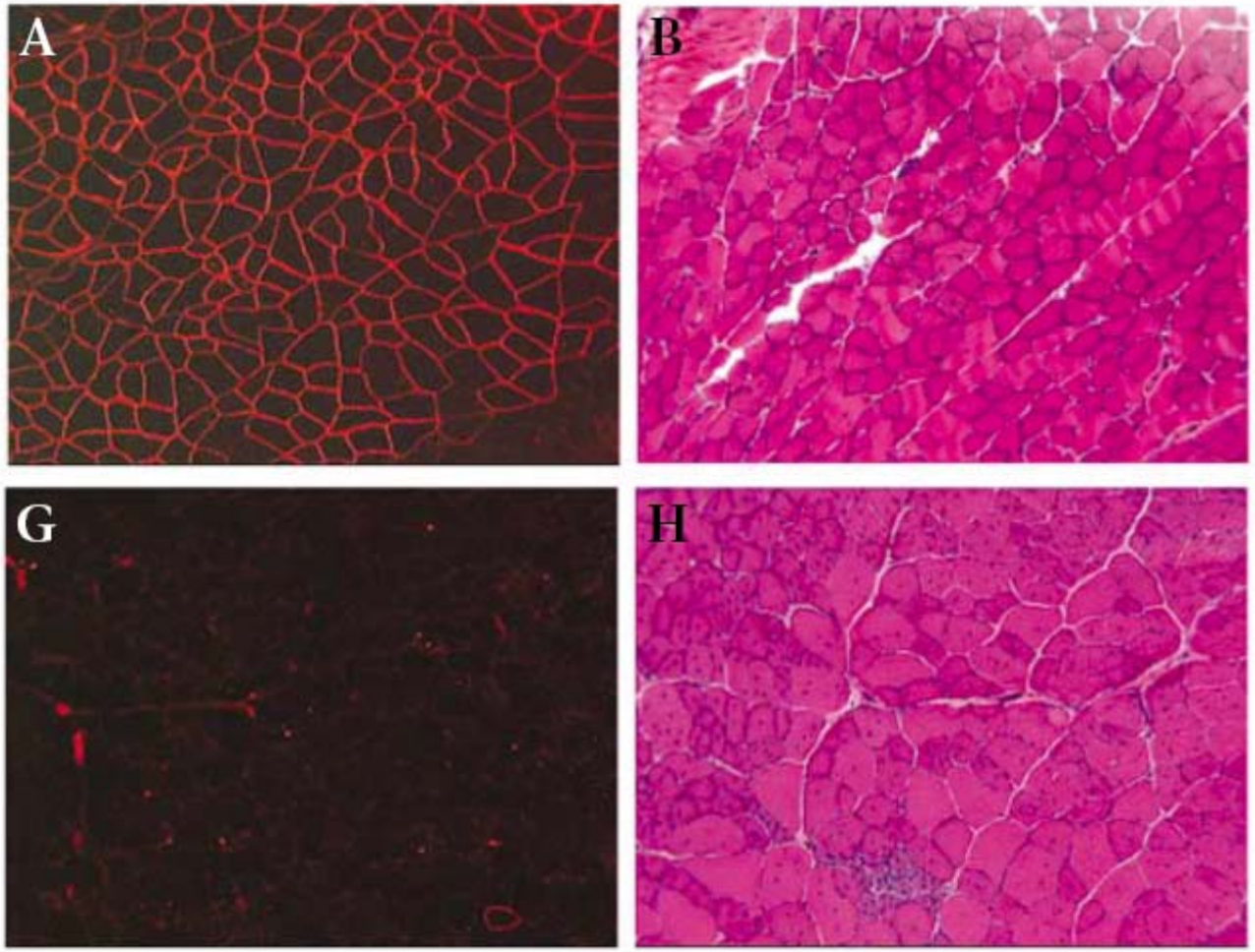
Duchenne muscular dystrophy is caused by mutation in the dystrophin gene



Patients with DMD do not produce dystrophin protein

Dystrophin is normally found around the edge of muscle cells

Patients with DMD do not produce dystrophin protein

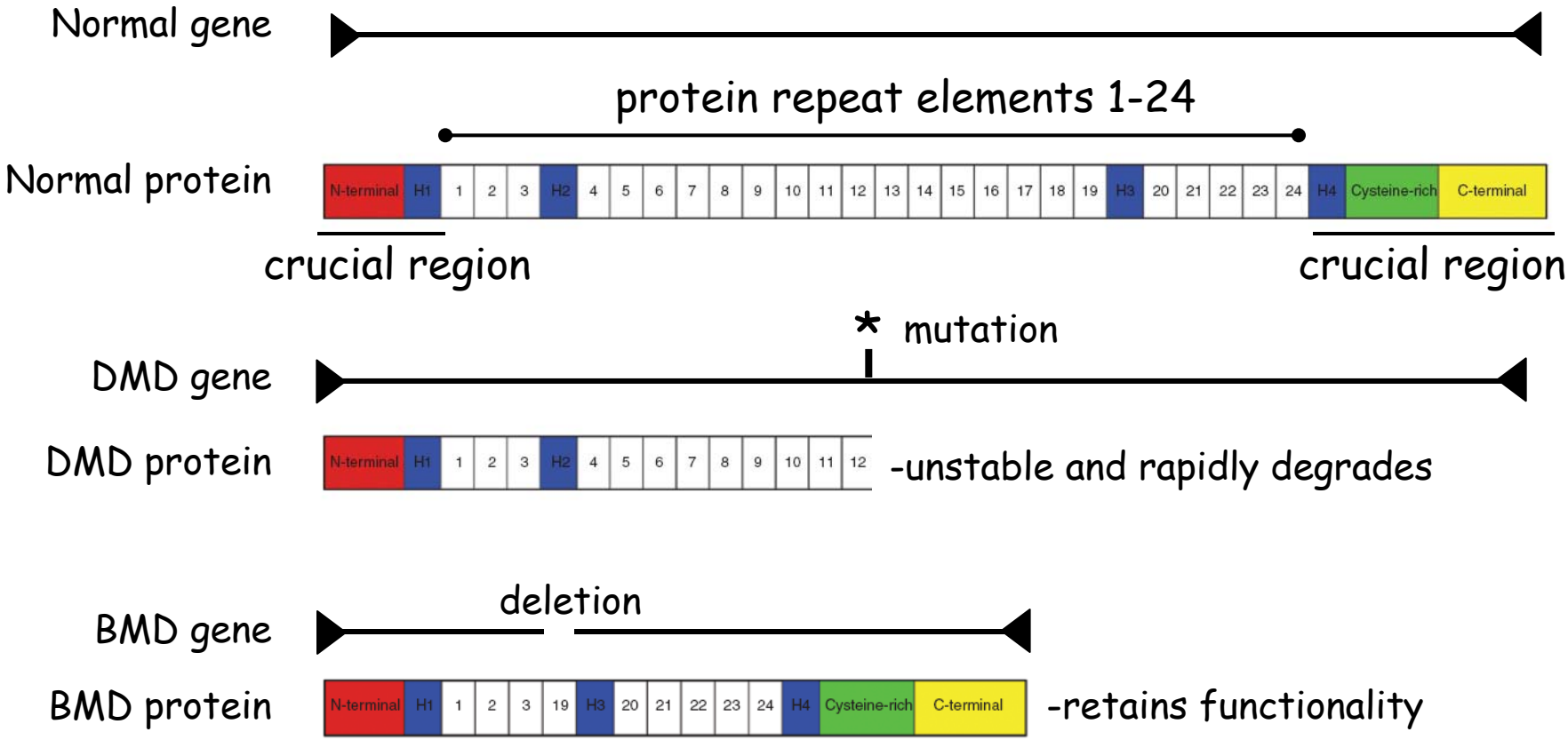


Duchenne vs. Becker muscular dystrophy

- Both are caused by mutations in the dystrophin gene
- In DMD – caused by premature "stop" signals
  - typically no protein is produced

- In BMD – caused by internal deletions within the gene which remove redundant elements
  - typically a smaller, but functional protein is produced

Duchenne vs. Becker muscular dystrophy



Conventional Therapeutic Options for Treatment of DMD

- Physiotherapy to prevent contractures
- Surgery to prevent spinal curvature
  - Insertion of a metal rod with hooks into the spine
- Medication
  - Anti-inflammatory drugs - prednisone or deflazacort
- Braces, standing frames and wheelchairs

From www.mda.org

Duchenne Muscular Dystrophy

Patients with DMD cannot produce dystrophin protein because of a "bad" gene.

Introduction of a "good" copy of the dystrophin gene into muscle cells of patients may lead to disease correction.

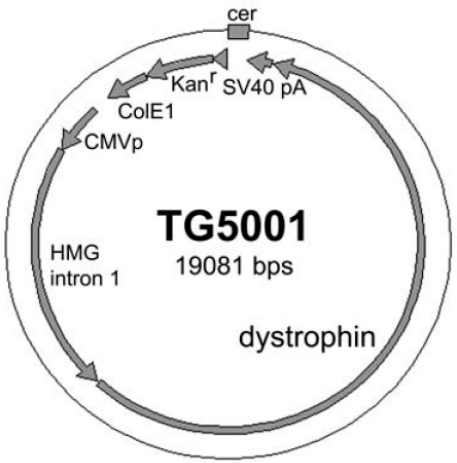
This is the basis of "Gene Therapy."

HUMAN GENE THERAPY 15:1065–1076 (November 2004)

Phase I Study of Dystrophin Plasmid-Based Gene Therapy in Duchenne/Becker Muscular Dystrophy

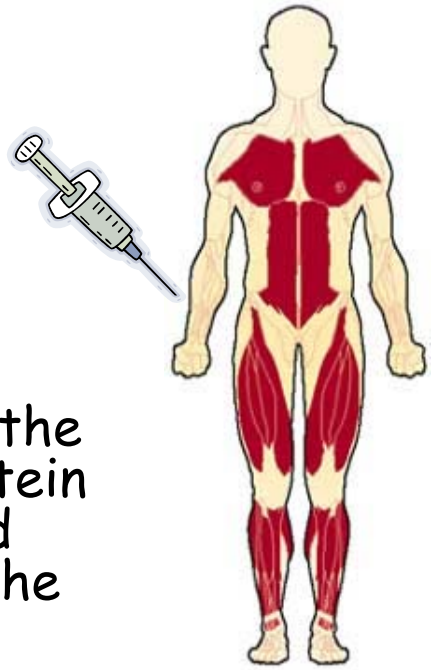
The first gene therapy clinical trial for DMD involved direct injection of a good copy of the gene into the radialis muscle

- 21 days later, muscle biopsies were examined for evidence of dystrophin expression



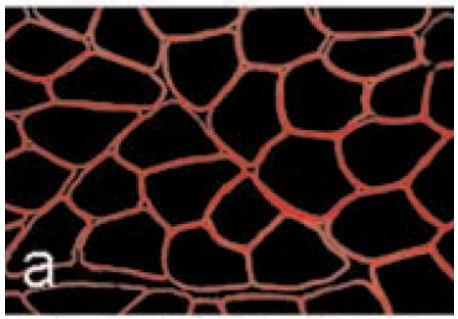
HUMAN GENE THERAPY 15:1065–1076 (November 2004)

Phase I Study of Dystrophin Plasmid-Based Gene Therapy in Duchenne/Becker Muscular Dystrophy

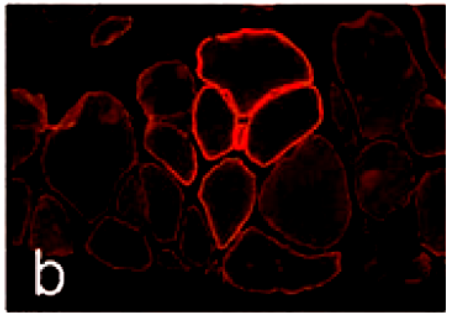


DNA encoding the dystrophin protein was injected directly into the arm muscle

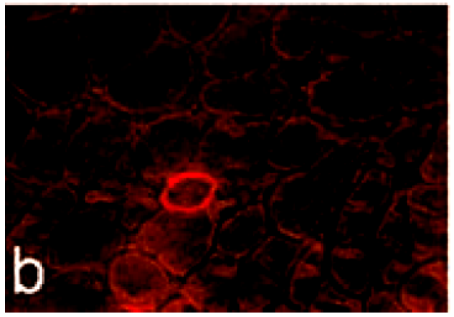
Unaffected Individual



Treated Patient



Treated Patient



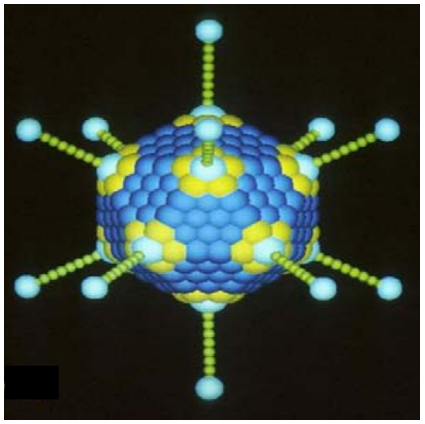
Result – although it worked, it was very inefficient

Gene Therapy for Muscle Disease

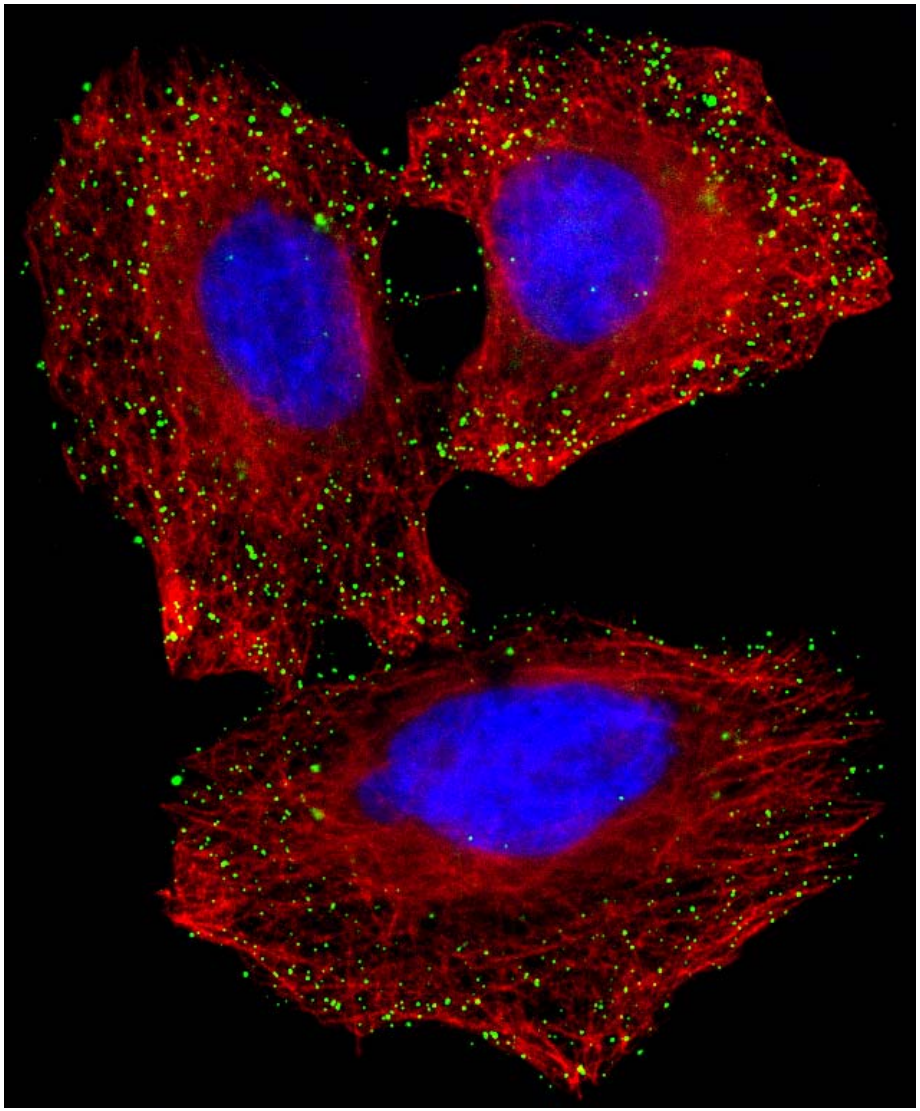
- Muscle cells do not take up naked DNA very efficiently
  - Only a few cells express the dystrophin protein
- To actually cure DMD, all muscle cells in the body must express dystrophin
- How can we improve the efficiency of gene delivery?

How do you improve the efficiency of gene delivery to a patient?

- Use a virus:
  - Viruses are basically parasites that invade living cells
    - Optimized over millions of years of evolution to deliver their DNA to the nucleus of cells



Viruses on the Cellular Super-Highway

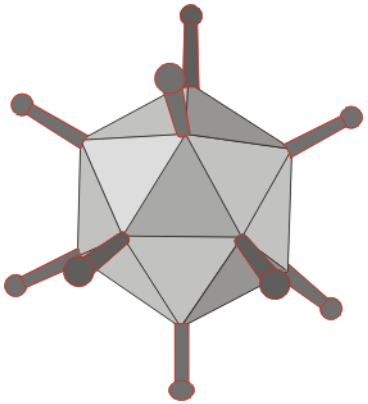


Green – Virus Blue - Cell Nucleus Red - Tubulin

Viruses: From Bad to Good

Problem: how do you turn a disease-causing virus into a therapeutic gene-delivery vehicle?

Viruses: From Bad to Good



Viral DNA

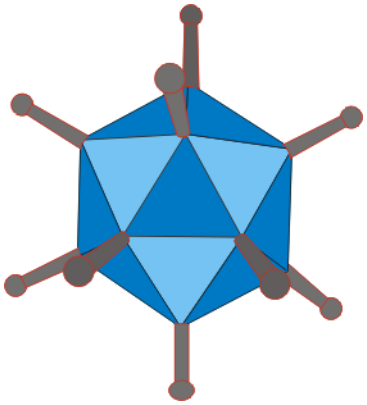
Contains genes required for virus growth and disease

Remove viral genes

Introduce therapeutic gene

Dystrophin

Viruses: From Bad to Good

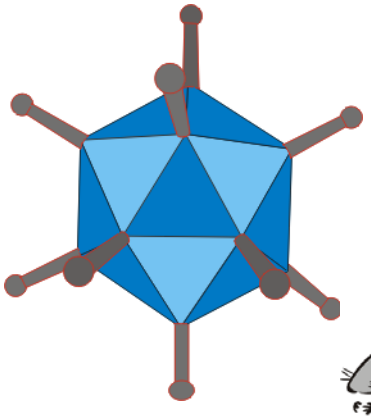


Reintroduce the modified DNA back into the virus

Dystrophin

This virus can still "infect" cells and deliver its DNA to the nucleus but cannot replicate or cause viral disease

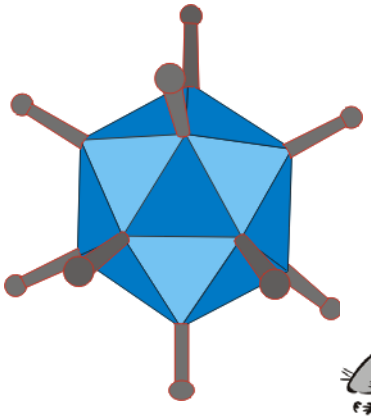
Viruses: From Bad to Good
…and their use in gene therapy

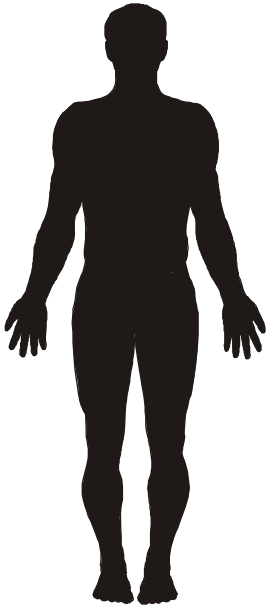


Test the therapeutic virus in animal models of human disease

Is it safe?
Is it effective?

Viruses: From Bad to Good
…and their use in gene therapy





Test the therapeutic virus in animal models of human disease

Test the therapeutic virus in human clinical trials

Can viruses be used to improve gene delivery to muscle?

Gene Therapy for DMD

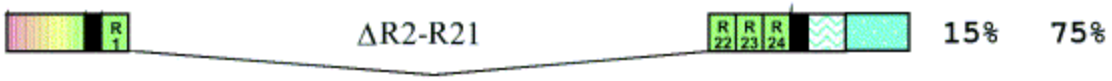
The cDNA for dystrophin is ~11 kb in length

Can accommodate ~5 kb

Adeno-associated Virus (AAV)

Because AAV is very small, it cannot accommodate the full length dystrophin gene

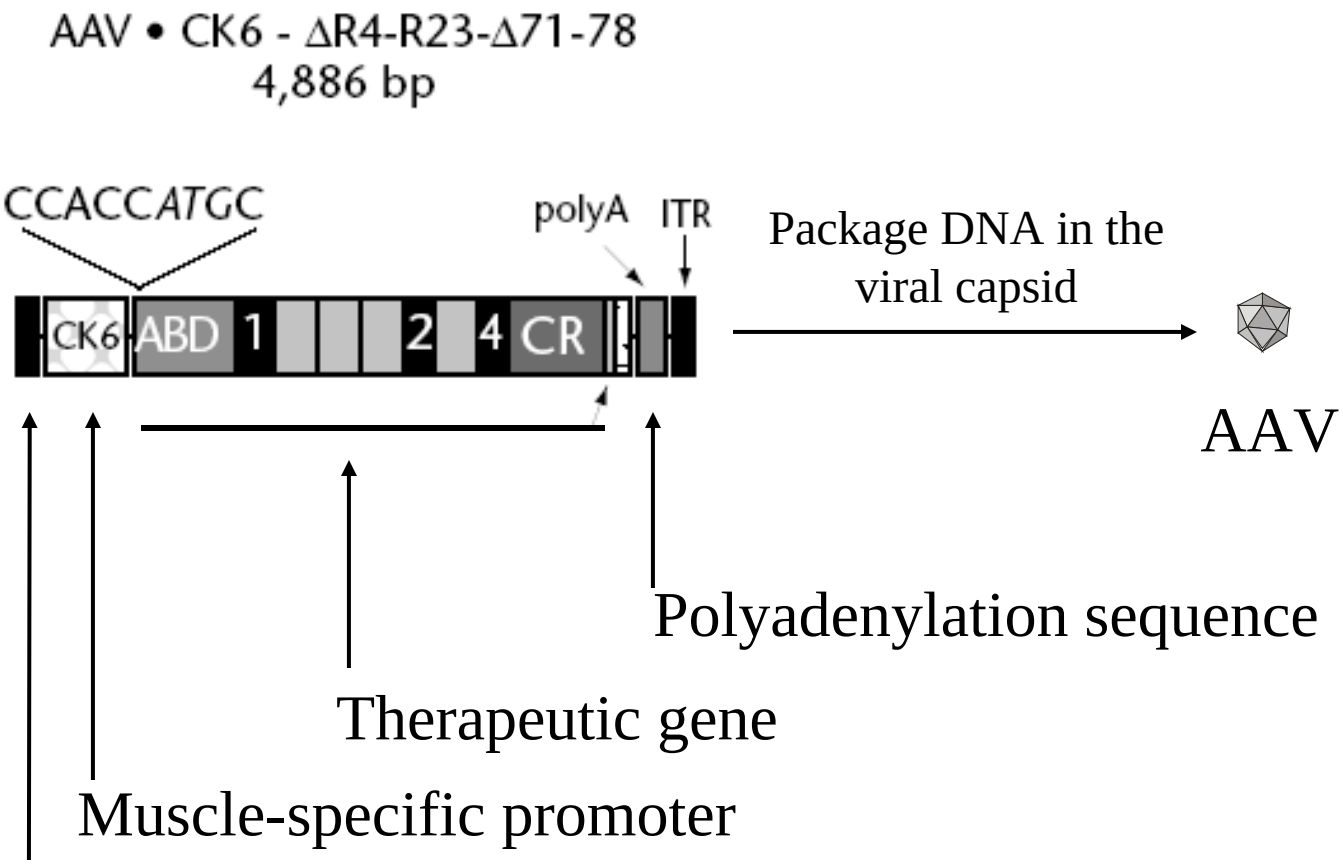
The dystrophin gene contains many repeated regions that can be removed without affecting its function



~5 kb in length

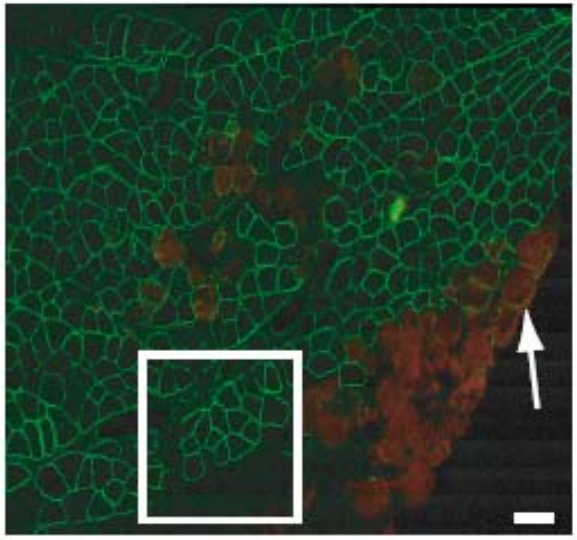
This “micro-dystrophin” gene is small enough to fit inside AAV and works almost as well as the normal dystrophin gene

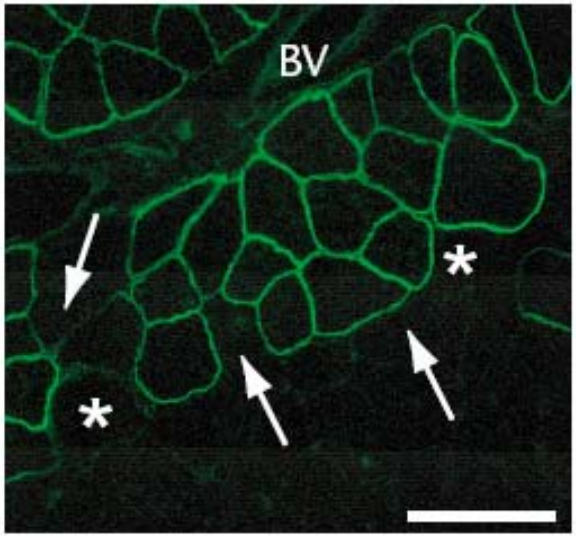
Gene Therapy for DMD - Vector Design

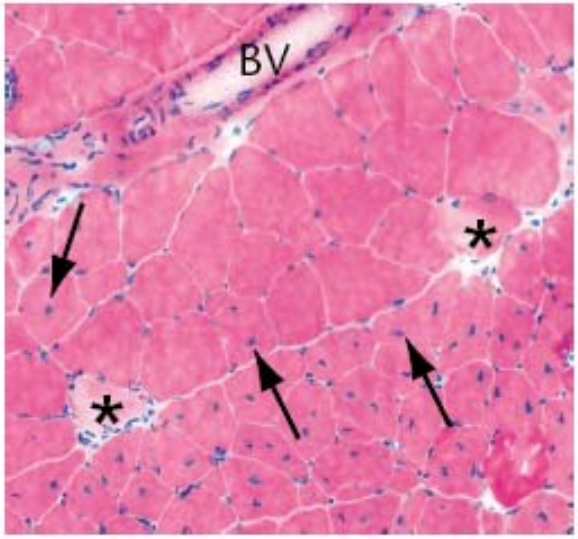


Direct injection of AAV into muscle

Localized injection of AAV into muscle results in extensive, but fairly localized transgene expression.



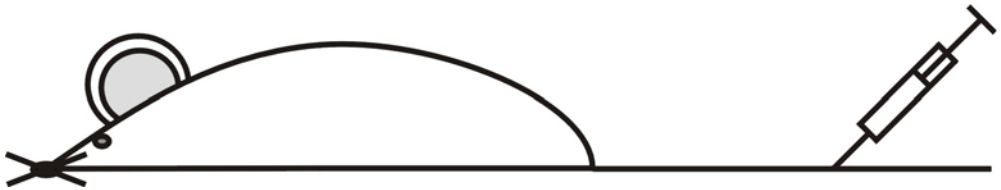




Systemic delivery of genes to striated muscles using adeno-associated viral vectors

VOLUME 10 | NUMBER 8 | AUGUST 2004 NATURE MEDICINE

Paul Gregorevic[1,4], Michael J Blankinship[1,4], James M Allen[2], Robert W Crawford[1], Leonard Meuse[1], Daniel G Miller[2], David W Russell[2,3] & Jeffrey S Chamberlain[1–3]



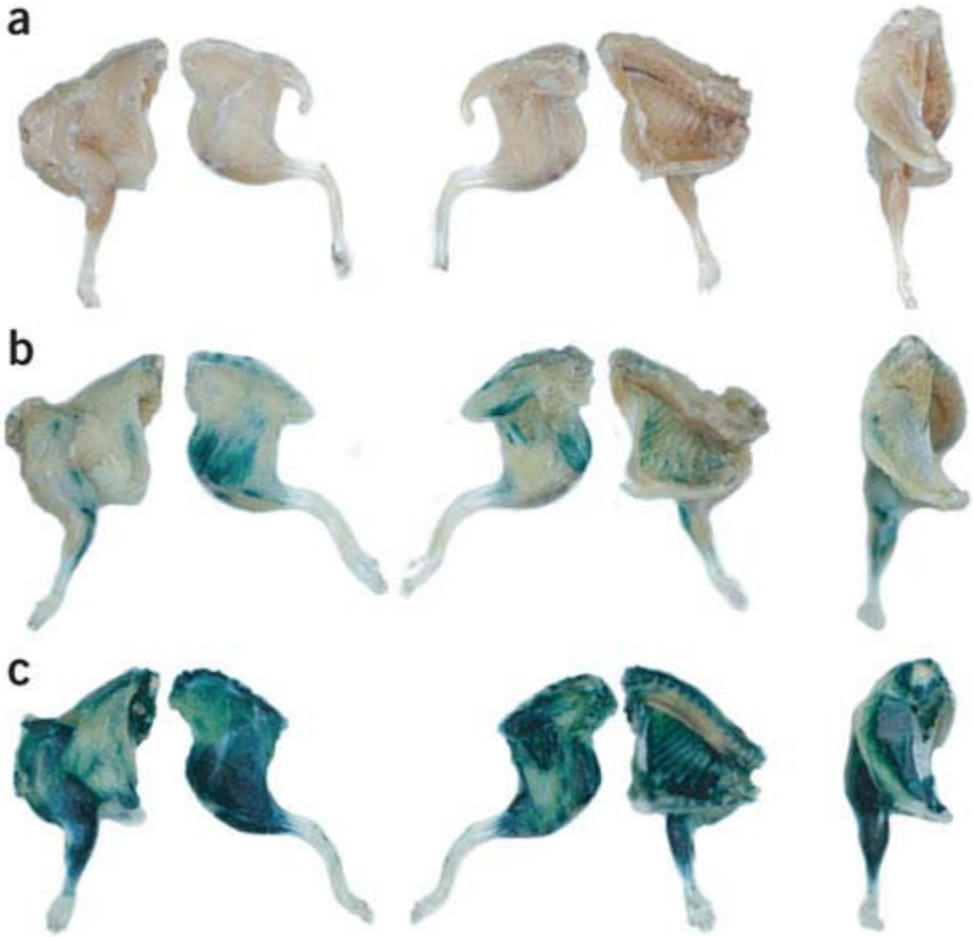
Injection through the tail vein provides an easy and effective method to deliver a drug systemically to a mouse

Gene Delivery using Viruses

Low Dose

Medium Dose

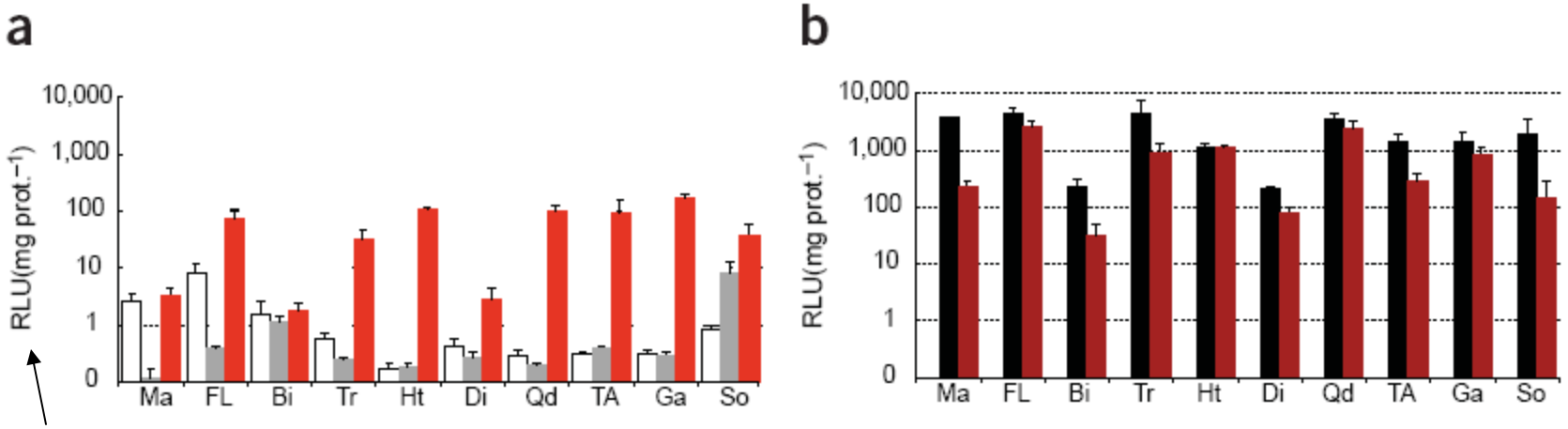
High Dose



Mice were given a single injection of "Blue" Virus

Jeff Chamberlain and coworkers
University of Washington

Gene Delivery using Viruses



Amount of blue in the muscle

Low dose of virus

White bars – untreated
Grey bars – treated
Red bars – treated + v.p.a.

High dose of virus

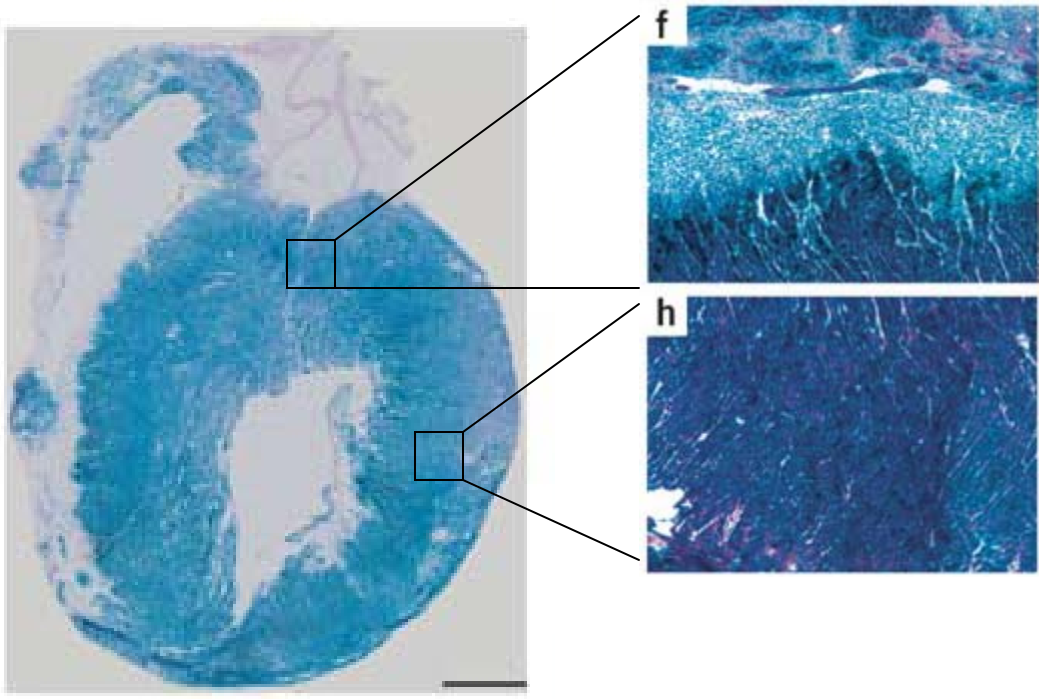
Black bars - treated
Red bars – treated + v.p.a.

Ma, masseter; FL, flexor digitorum profundus/carpi radialis; Bi, biceps; Tr, triceps; Ht, heart; Di, diaphragm; Qd, quadriceps; TA, tibialis anterior; Ga, gastrocnemius; So, soleus.

v.p.a. = vascular permeablizing agent

Gene Delivery using Viruses

..this same single injection gave high level gene expression in the heart.



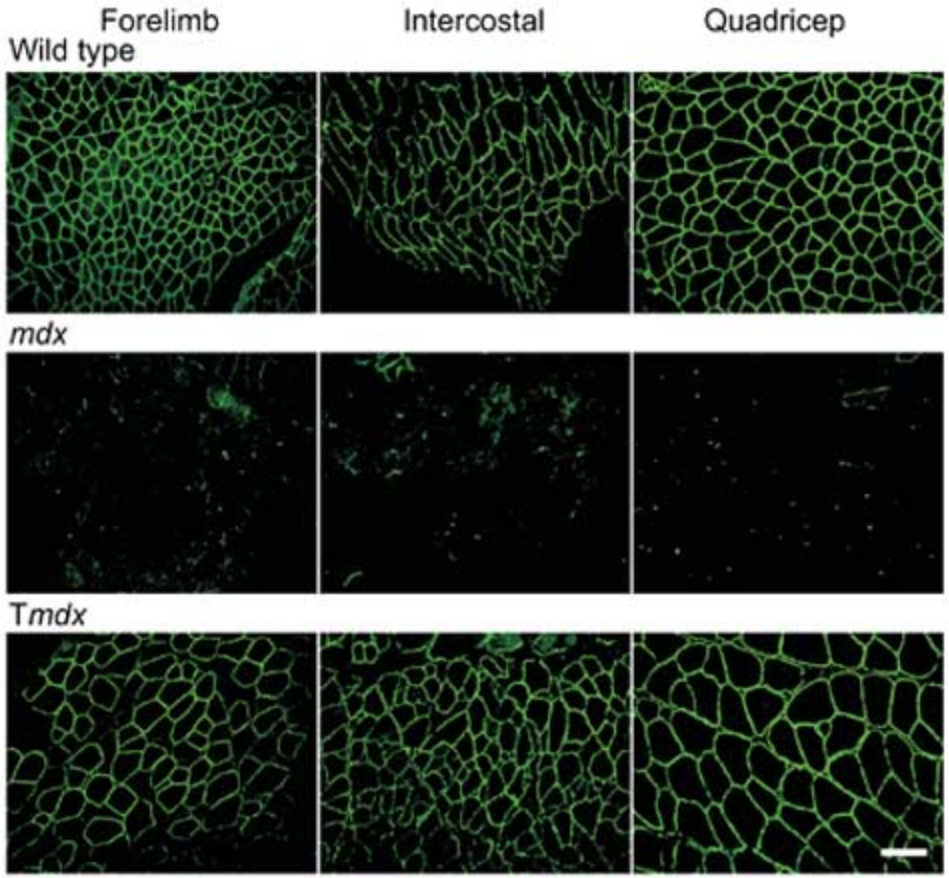
Mice were given a single injection of "Blue" Virus

Gene Delivery using Viruses

Untreated normal mice

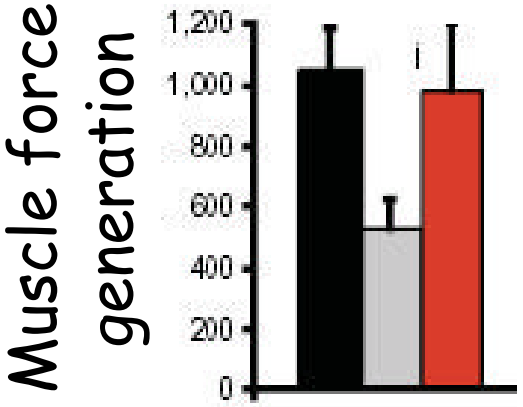
Untreated mdx mice
- mdx mice cannot produce dystrophin

Treated mdx mice



Injection of virus encoding the dystrophin gene gave high level gene expression in many muscle groups

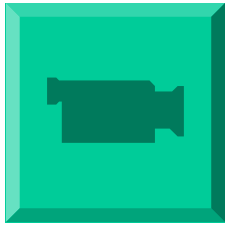
Gene Delivery using Viruses



The strength of the muscle was also improved

untreated normal mouse
untreated mdx mouse
treated mdx mouse

Does virus-mediated delivery of dystrophin to DMD mice ameliorate disease symptoms?



Movie 1

HUMAN GENE THERAPY 18:18–26 (January 2007)

DOI: 10.1089/hum.2006.093

Immunity to Adeno-Associated Virus-Mediated Gene Transfer in a Random-Bred Canine Model of Duchenne Muscular Dystrophy

ZEJING WANG,[1–3] JAMES M. ALLEN,[3] STANLEY R. RIDDELL,[4,5] PAUL GREGOREVIC,[3] RAINER STORB,[1,4] STEPHEN J. TAPSCOTT,[2,3] JEFFREY S. CHAMBERLAIN,[3,4,6] and CHRISTIAN S. KUHR[1,7,8]

Although gene therapy for DMD looked very promising in mouse studies, things seemed a little more complicated in larger animals

Sustained AAV-mediated Dystrophin Expression in a Canine Model of Duchenne Muscular Dystrophy with a Brief Course of Immunosuppression

Zejing Wang[1,2,3], Christian S Kuhr[1,4,5], James M Allen[3], Michael Blankinship[3], Paul Gregorevic[3], Jeffrey S Chamberlain[3,6,7], Stephen J Tapscott[2,3] and Rainer Storb[1,7]

Molecular Therapy vol. 15 no. 6 june 2007

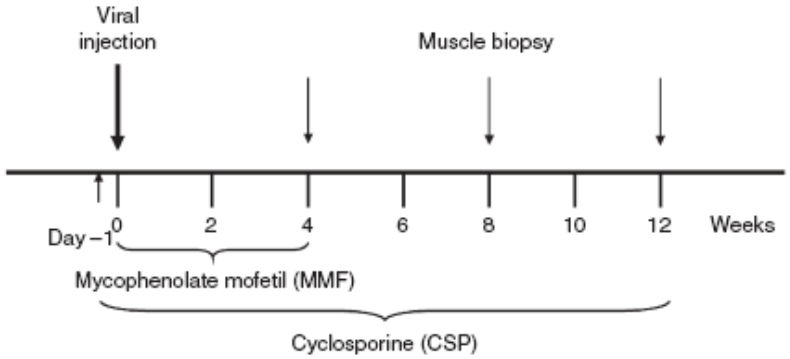
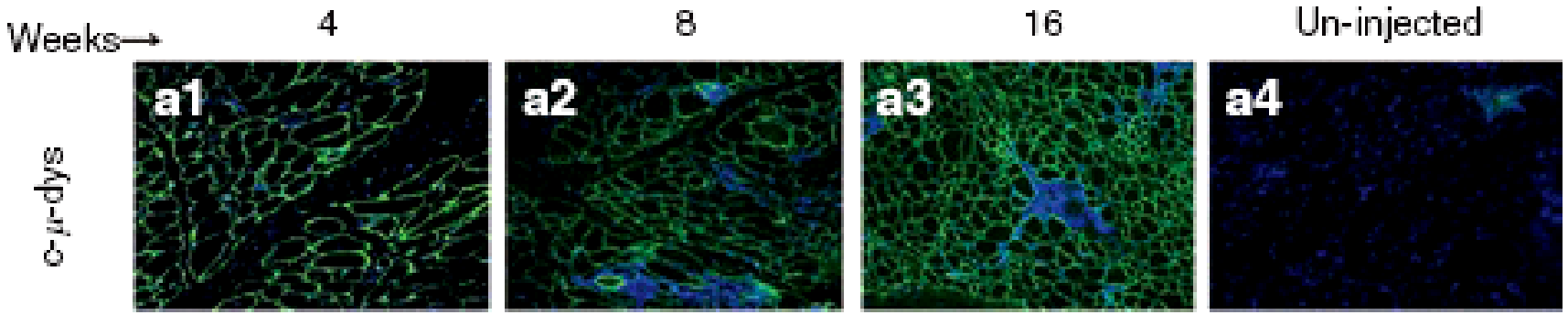


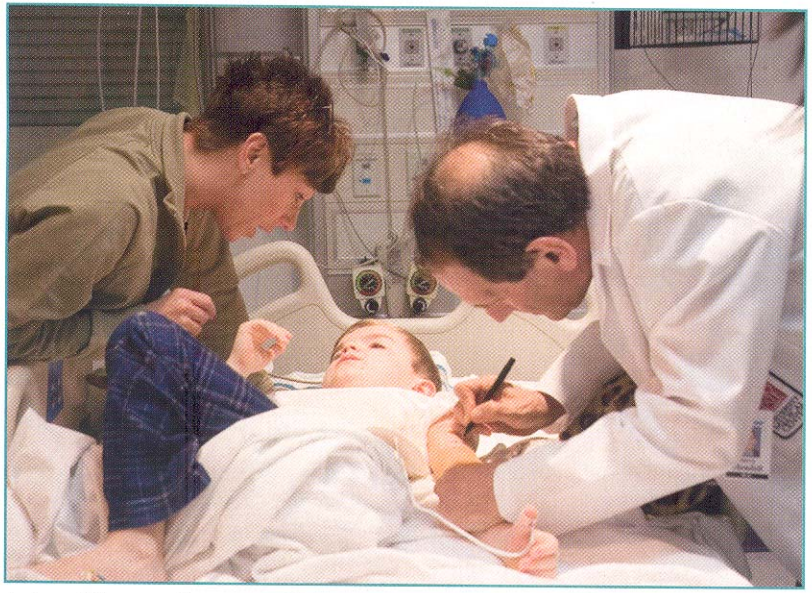
Figure 1 Schematic of intramuscular viral injection and immunosuppression with cyclosporine (CSP) and mycophenolate mofetil (MMF) in wild-type dogs. CSP was given orally from day –1 for 12 weeks at 5 mg/kg, twice daily, and MMF subcutaneously from day 0 for 4 weeks at 7.5 mg/kg twice daily. Muscle biopsies were taken 4, 8, and 12 weeks after viral injection.



Research UPDATES

MDA Begins First U.S. Duchenne Gene Therapy Trial

COLUMBUS, Ohio, March 29, 2006 — The first U.S. human gene therapy trial directed at Duchenne Muscular Dystrophy was launched at Columbus Children's Hospital.



"Neurologist Jerry Mendell administers gene therapy vector to Andrew Kilbarger, 8, of Lancaster, Ohio."

Dystrophin Immunity in Duchenne's Muscular Dystrophy

N ENGL J MED 363;15 NEJM.ORG OCTOBER 7, 2010

- Six boys at 5-11 years of age received $2x10^{10}$ viruses/kg or $1x10^{11}$viruses7kg injected into the bicep muscle

- Biopsy samples were removed at 42 or 60 days post-treatment

Table 1. Mild Adverse Events in Six Patients.*

Adverse Event	No. of Patients	No. of Events
Nausea or upset stomach	1	1
Macular rash	2	2
Fungal rash	1	1
Sore throat	2	2
Total†	5	6

* No moderate or severe adverse events were reported.
† One patient had both a macular rash and a fungal rash.

- two of four biopsies showed dystrophin-positive muscle fibers at 42 days
- no positive fibers were detected at 60 days (two samples)

Dystrophin Immunity in Duchenne's Muscular Dystrophy

N ENGL J MED 363;15 NEJM.ORG OCTOBER 7, 2010

- Six boys at 5-11 years of age received $2x10^{10}$ viruses/kg or $1x10^{11}$viruses7kg injected into the bicep muscle

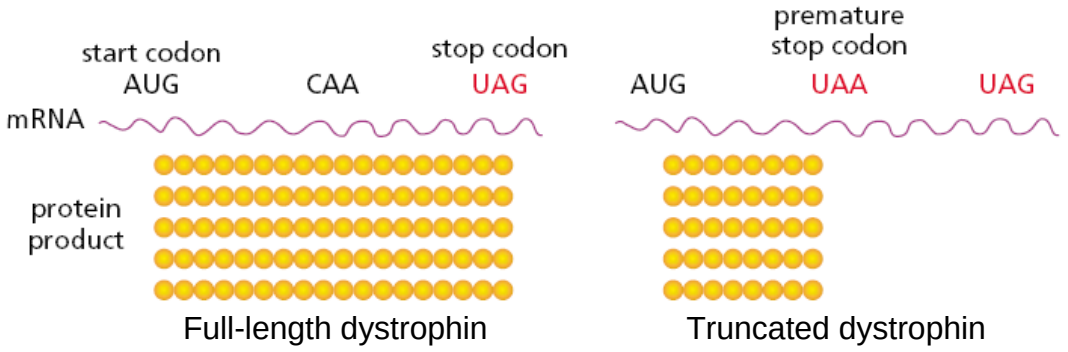
- Dystrophin-specific cytotoxic T-lymphocytes were detected after treatment
  - suggesting that the patients had developed an immune response to the "foreign" therapeutic protein

Status of gene therapy clinical trials using viral vectors for treating DMD

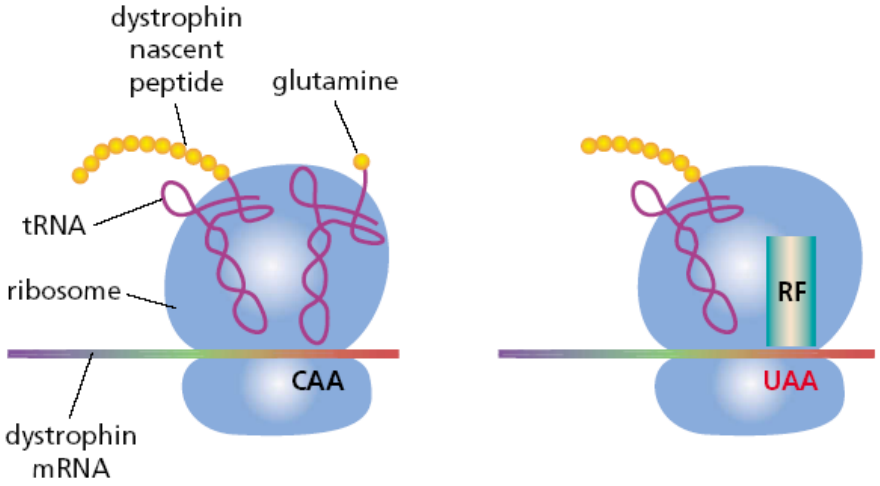
- Currently, the trials are on hold while these researchers evaluate what to do.
- At this time, no plans to proceed with another phase I or phase II trail.

Read-through of premature stop codons

- 15% of DMD patients have a premature stop codon and cannot produce a full-length dystrophin protein

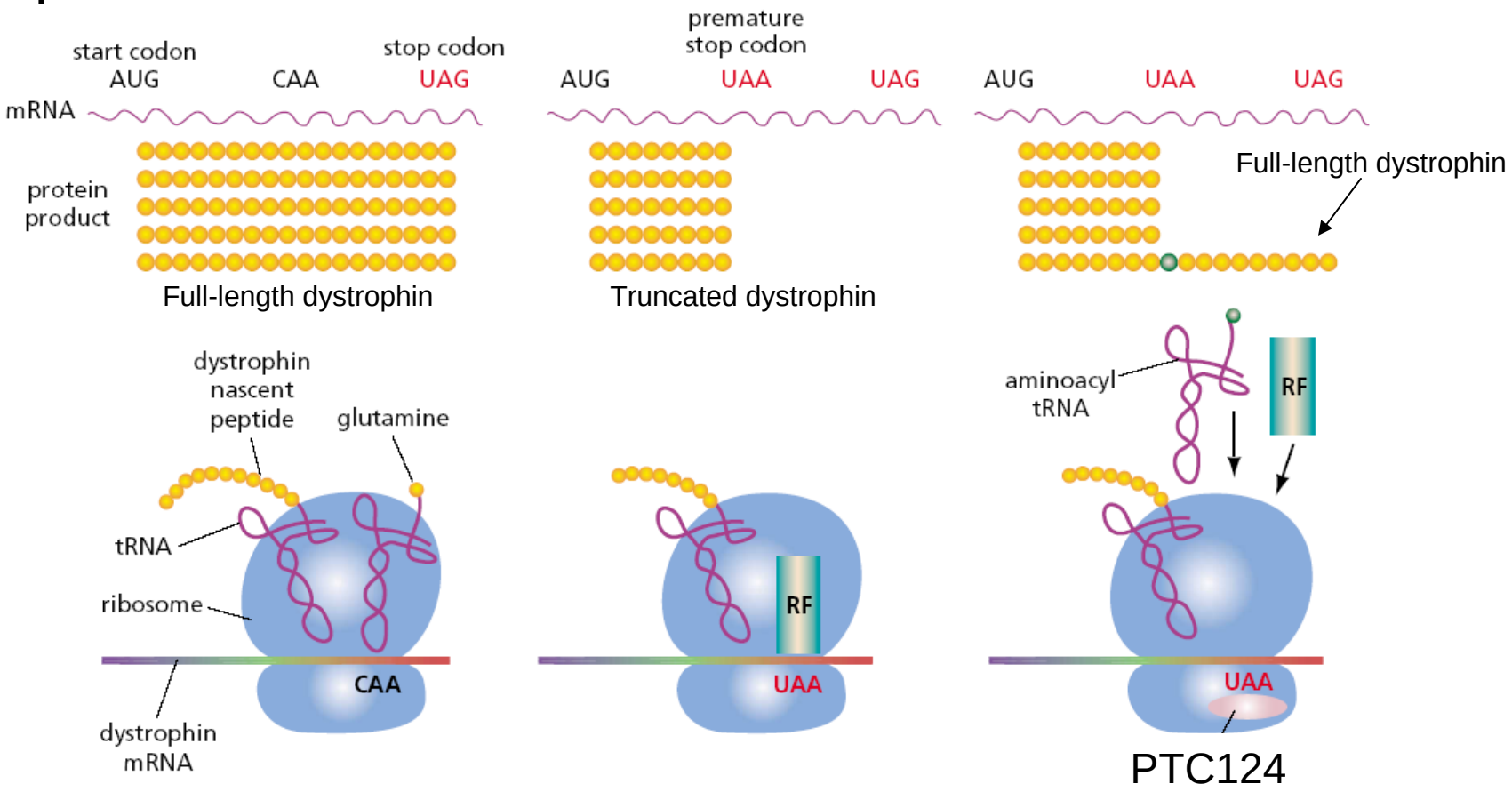


Mutation of ‘CAA’ to ‘UAA’ produces a premature stop codon.



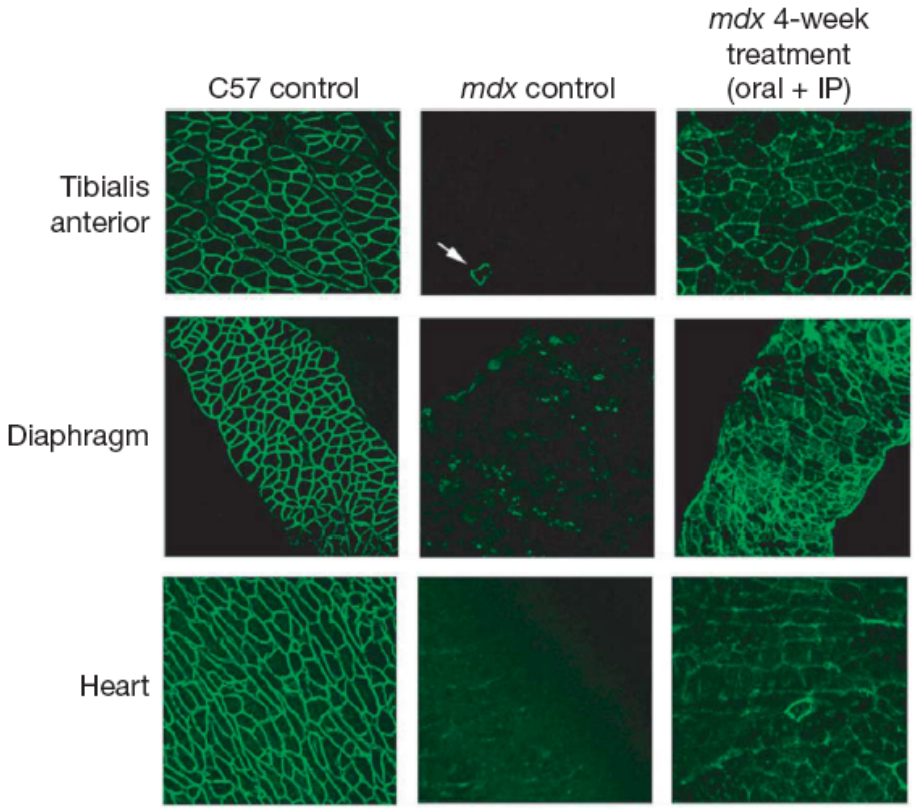
Read-through of premature stop codons

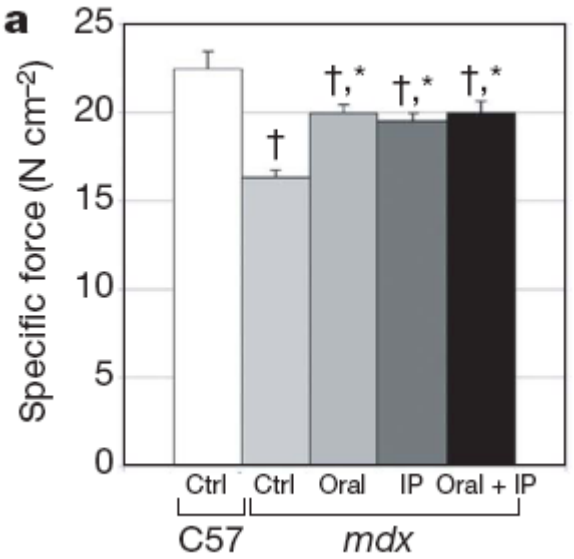
- The new drug PTC124 causes “read-through” of the premature stop codon, producing a full-length protein



Read-through of premature stop codons

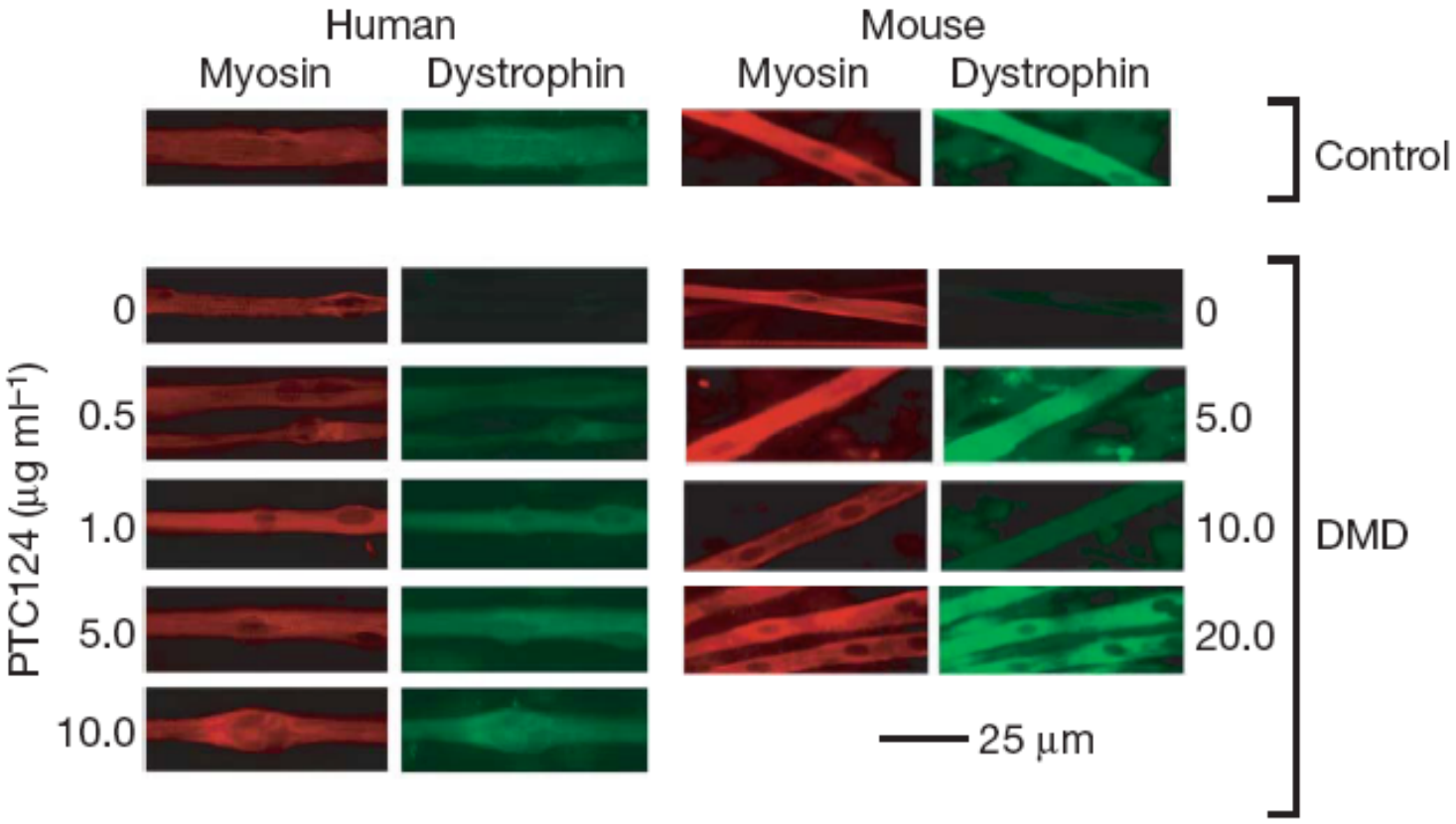
- DMD mice treated with PTC124 now express full-length dystrophin protein
  ….and have stronger muscles





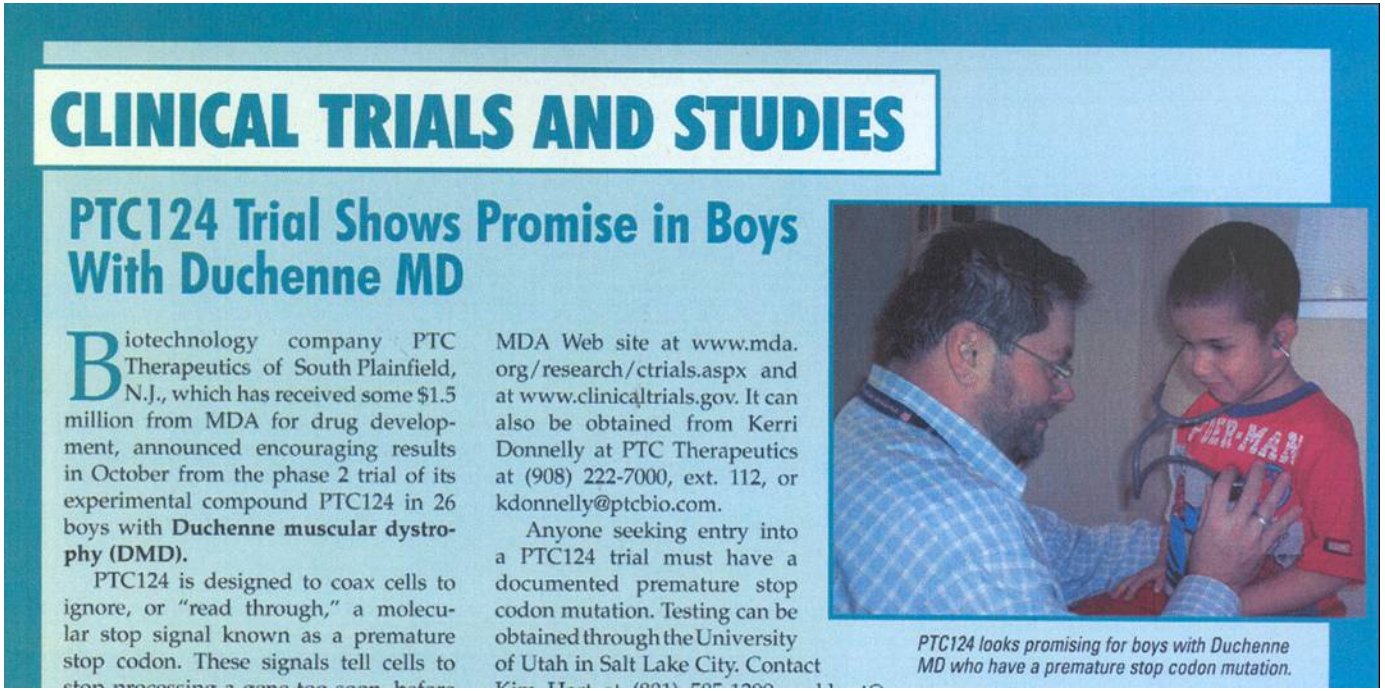
Read-through of premature stop codons

- Cells from patients with DMD also produce dystrophin after treatment with PTC124



Read-through of premature stop codons

- Early clinical results in DMD patients were very encouraging.



CLINICAL TRIALS AND STUDIES

PTC124 Trial Shows Promise in Boys With Duchenne MD

Biotechnology company PTC Therapeutics of South Plainfield, N.J., which has received some $1.5 million from MDA for drug development, announced encouraging results in October from the phase 2 trial of its experimental compound PTC124 in 26 boys with **Duchenne muscular dystrophy (DMD).**

PTC124 is designed to coax cells to ignore, or "read through," a molecular stop signal known as a premature stop codon. These signals tell cells to

MDA Web site at www.mda.org/research/ctrials.aspx and at www.clinicaltrials.gov. It can also be obtained from Kerri Donnelly at PTC Therapeutics at (908) 222-7000, ext. 112, or kdonnelly@ptcbio.com.

Anyone seeking entry into a PTC124 trial must have a documented premature stop codon mutation. Testing can be obtained through the University of Utah in Salt Lake City. Contact

PTC124 looks promising for boys with Duchenne MD who have a premature stop codon mutation.

Muscular Dystrophy Association – Quest: Jan 2007

"Several parents and teachers have reported that boys participating in the study have had improvements in terms of greater activity and increased endurance during treatment."

Current status of PTC124

- Trials of PTC124 delivery to boys with DMD have been halted.
  - no evidence of a therapeutic effect.
- PTC124 is still being pursued for other diseases
  - missense mutations in cystic fibrosis
    - “The published three-month data showed that treatment with PTC124 resulted in statistically significant improvements in chloride channel activity, CF-related cough and positive trends in lung function.”

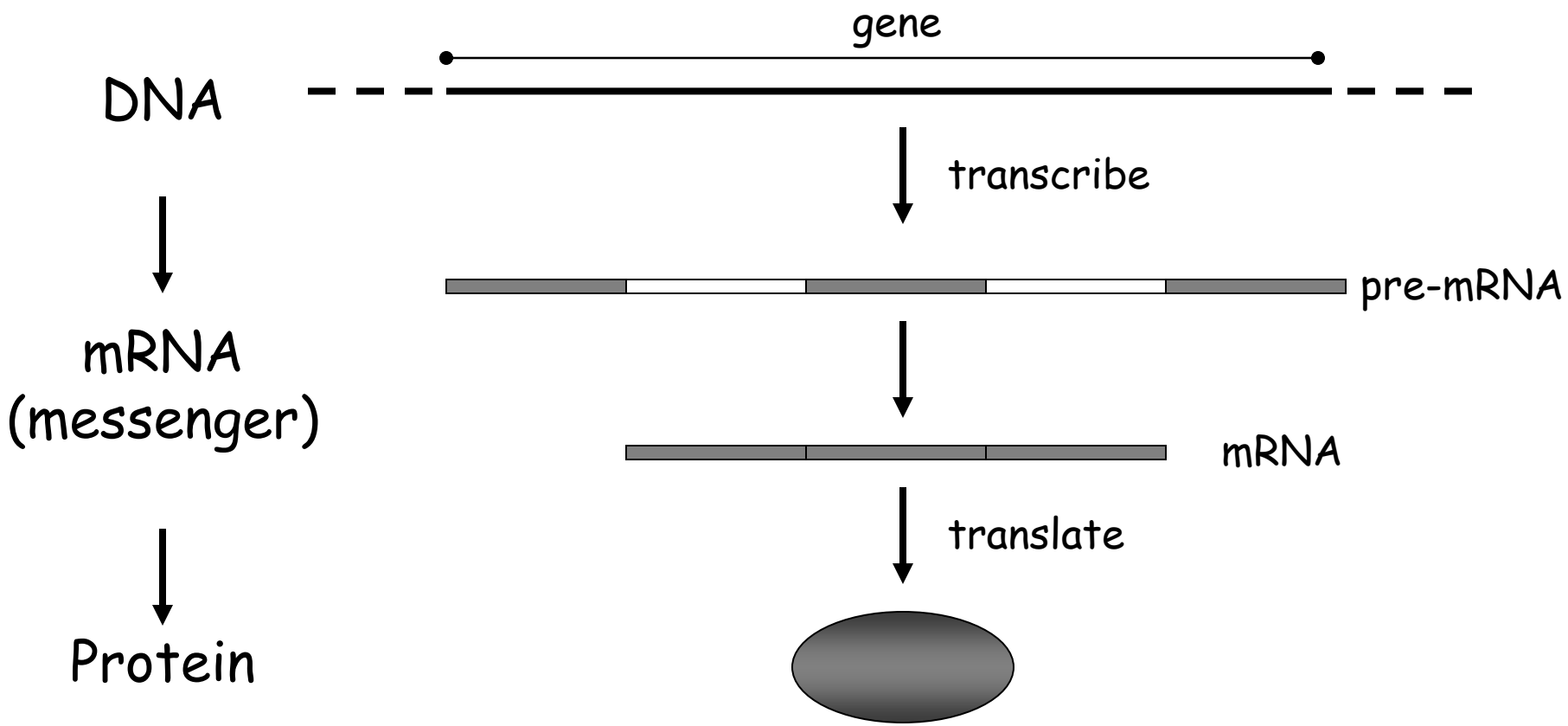
Taking a lesson from nature

- BMD patients produce a smaller dystrophin protein that is still functional
  - deleted of an internal, non-essential region
- Can a similar approach be used to "bypass" the mutated region of dystrophin in DMD patients?
  - produce a smaller, functional protein similar to BMD

Molecular Biology 101: The Central Dogma

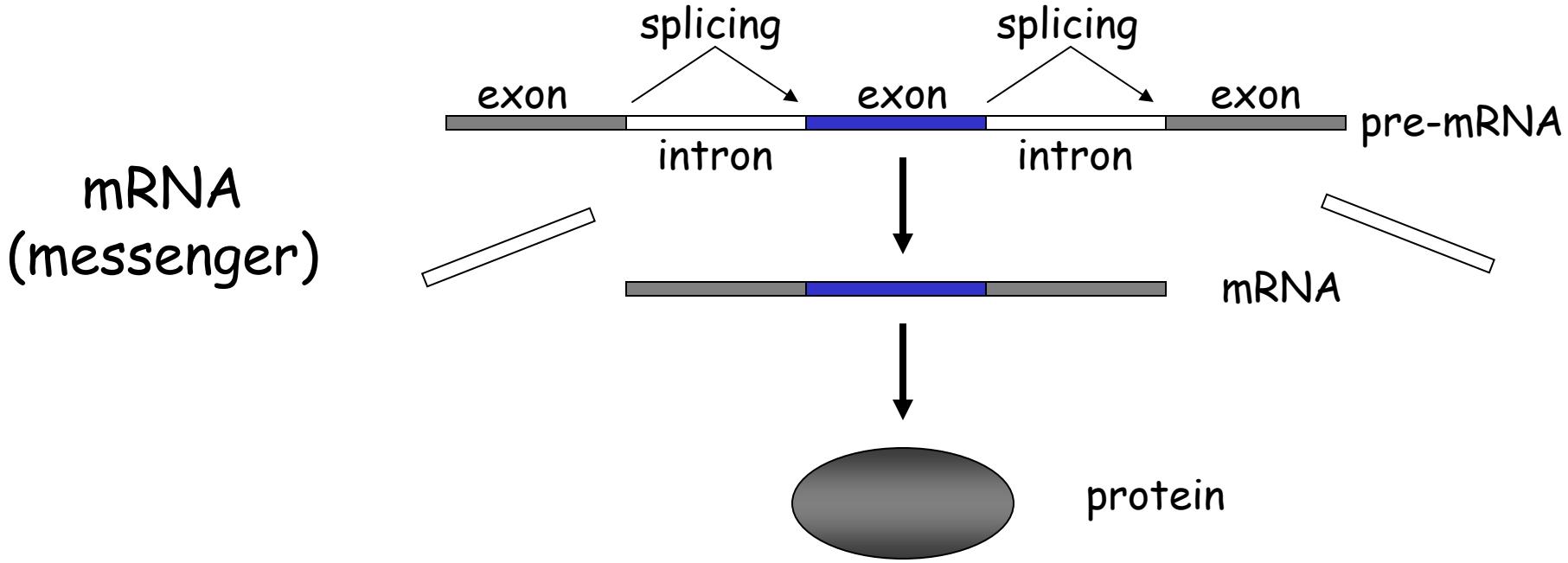
DNA	A cookbook (DNA) in the library (nucleus)
↓	
mRNA (messenger)	A photocopy of a recipe
↓	
Protein	The completed dish (lasagna)

Molecular Biology 101: The Central Dogma



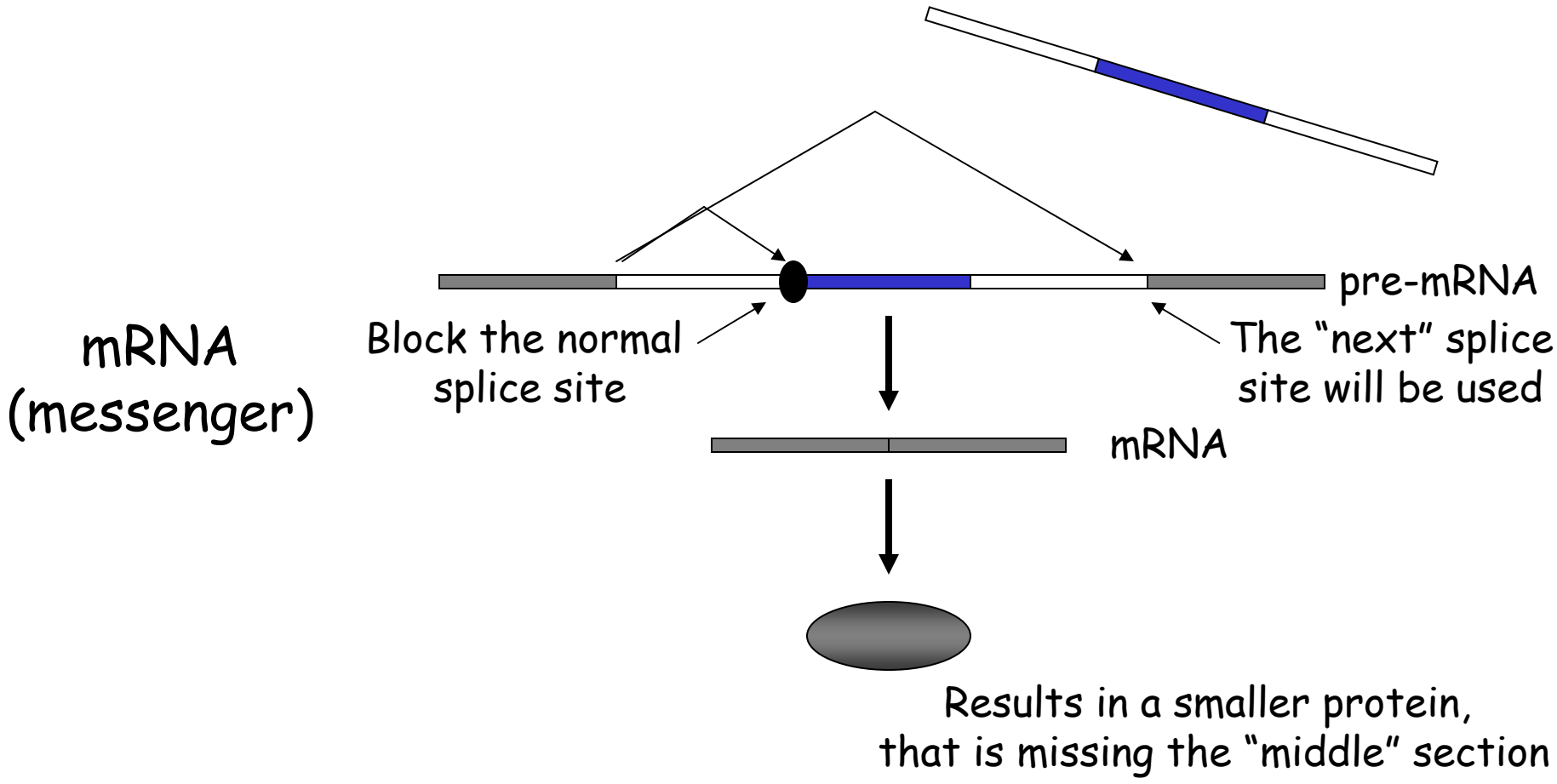
Molecular Biology 101: The Central Dogma

The "photocopy" contains blank pages (introns) that must be removed

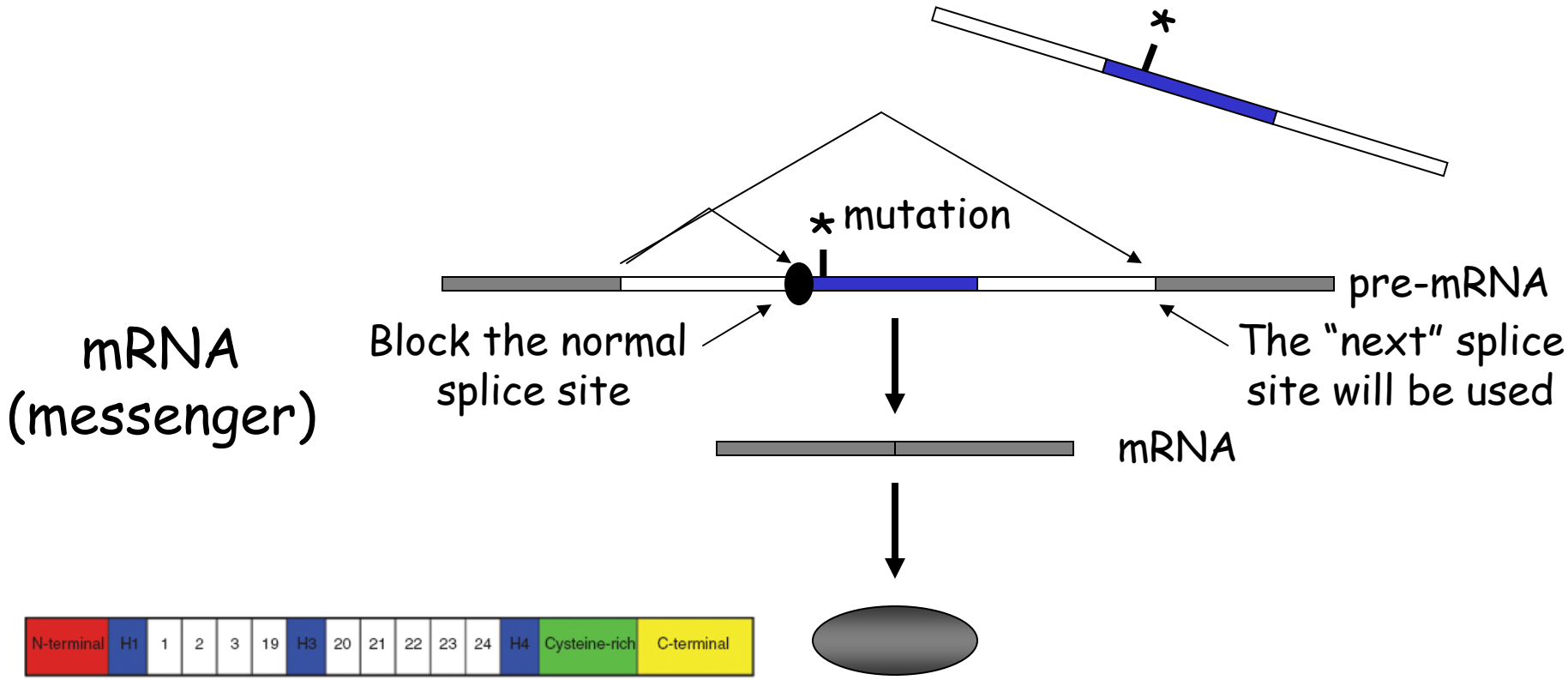


intron = intervening sequence, mostly junk DNA/RNA

Manipulating the central dogma: exon skipping



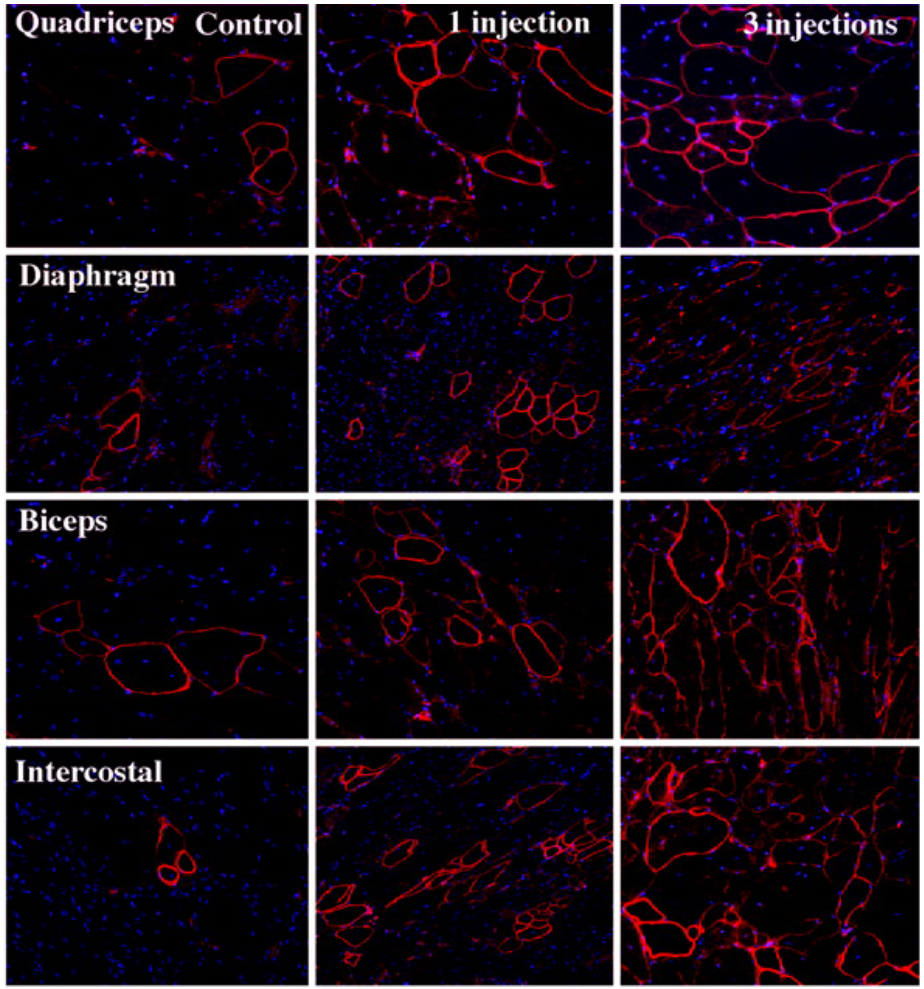
Manipulating the central dogma: exon skipping



Systemic delivery of antisense oligoribonucleotide restores dystrophin expression in body-wide skeletal muscles

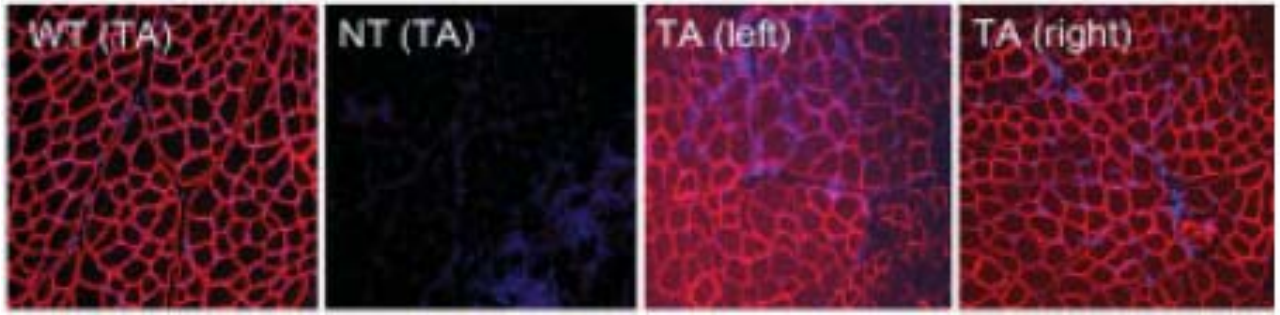
198–203 | PNAS | January 4, 2005 | vol. 102 | no. 1

An antisense oligoribonucleotide (AO) is a very small DNA molecule that binds to the mRNA and induces exon skipping
-mice were injected systemically with AO and evaluated 2 weeks later for dystrophin expression



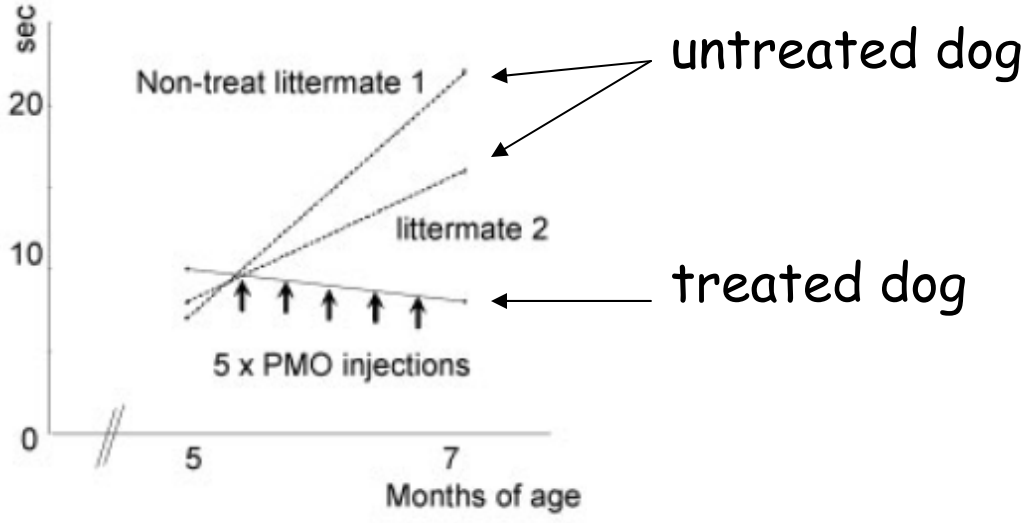
Efficiency of exon skipping induced by antisense oligonucleotide in the dystrophic dog model

Ann Neurol 2009;65:667–676



untreated normal dog | untreated dystrophic dog | treated dystrophic dog

15 meter run test



Efficiency of exon skipping induced by antisense oligonucleotide in the dystrophic dog model

Ann Neurol 2009;65:667–676

Untreated dystrophic dog

http://onlinelibrary.wiley.com/store/10.1002/ana.21627/asset/supinfo/ANA_21627_sm_SupVideo2.mov?v=1&s=ca6527e587c06b2f4278873df8677036554e9500

Treated dystrophic dog

http://onlinelibrary.wiley.com/store/10.1002/ana.21627/asset/supinfo/ANA_21627_sm_SupVideo5.mov?v=1&s=ced43e70ac5e578540a88366a5b4f1788cbe59fd

Human clinical trial for exon skipping in DMD

Systemic Administration of PRO051 in Duchenne's Muscular Dystrophy

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

- Twelve patients received weekly abdominal subcutaneous injections of AO (0.5 to 6.0 mg/kg body weight) for 5 weeks
- Results
  - well tolerated
  - half-life of PRO051 was 29 days
  - dystrophin expression was observed in 60-100% of muscle fibers in 10/12 patients
  - improvement in 6 minute walk test

Human clinical trial for exon skipping in DMD

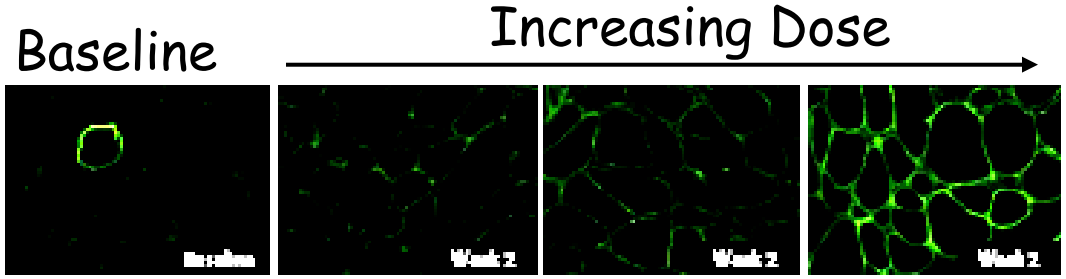
Systemic Administration of PRO051 in Duchenne's Muscular Dystrophy

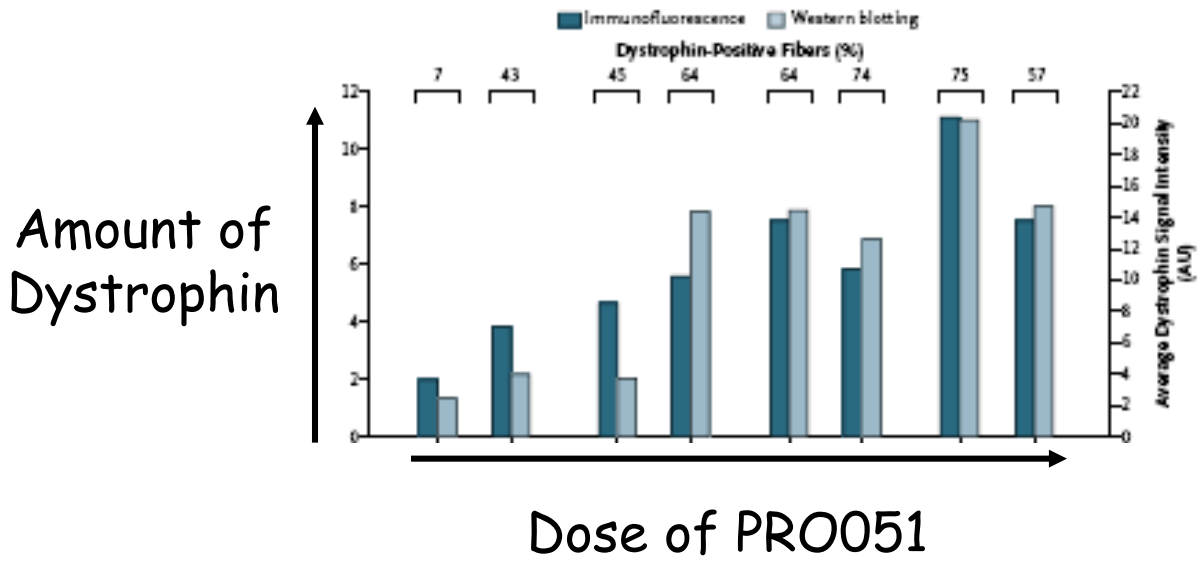
Table 1. Adverse Events That Occurred in More Than 2 Patients during the 12-Week Extension Phase.

Event	No. of Patients
Proteinuria	12
Elevated urinary α_1-microglobulin levels	11
Injection site	
Erythema and inflammation	9
Hematoma or bruising	6
Tenderness	5
Irritation or itching	3
Moderate pain during injection	4
Common cold	4
Gastroenteritis	4
Pain*	3

Human clinical trial for exon skipping in DMD

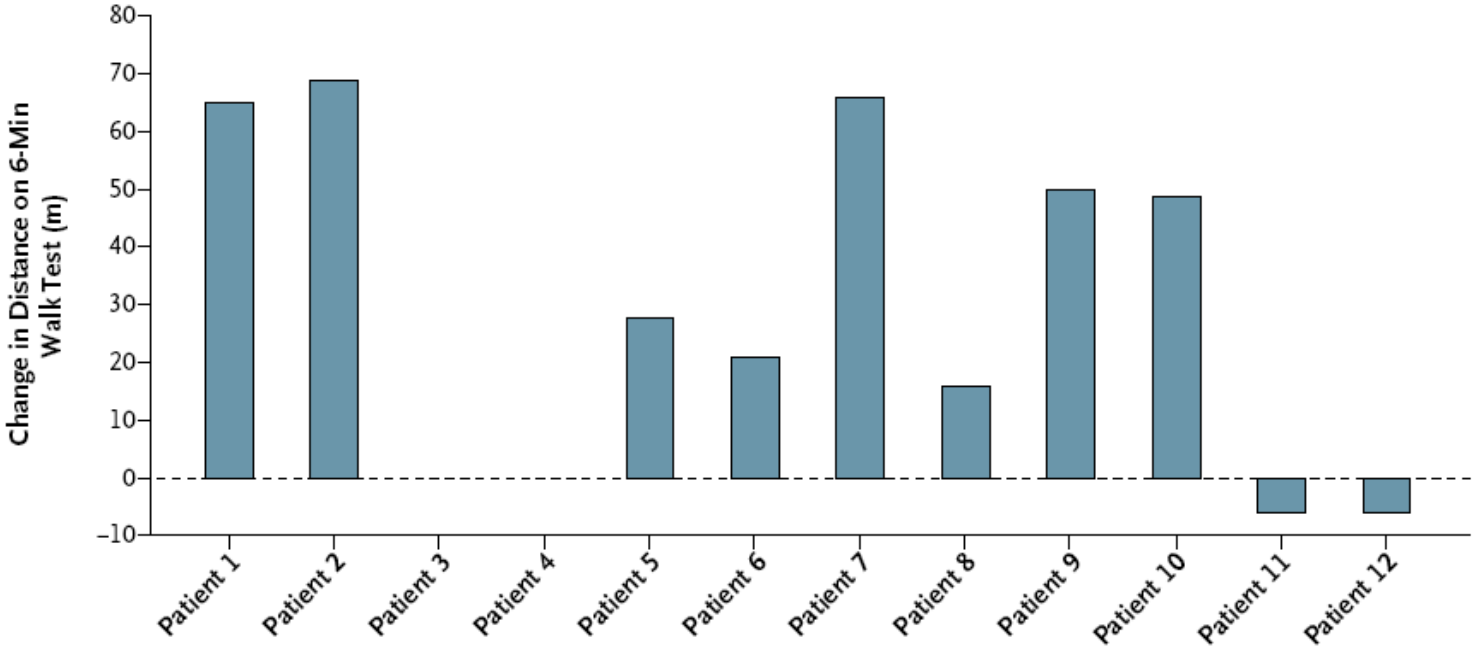
Systemic Administration of PRO051 in Duchenne's Muscular Dystrophy





Human clinical trial for exon skipping in DMD

Systemic Administration of PRO051 in Duchenne's Muscular Dystrophy



The average distance for these patients before the trial was ~400 meters

Current status of exon skipping for treatment of DMD

- Overall, very encouraging results have been achieved in human clinical trials
- Two different "chemistries" are being investigated
  - 2-OMP has entered phase III
  - PMO has entered phase II
- Note: there has been some toxicity observed in baboons - concerns about toxicity in humans

Take home message

- There are a variety of different strategies being investigated to treat DMD
- Many of these treatments have shown very good results in mice
- Translating these results to larger animal models (dogs) is more problematic
- Some of these therapies have moved into clinical trials, and very encouraging results have been achieved

Thank you – Questions?