European Reference Network
for rare or low prevalence complex diseases

Network Neuromuscular Diseases (ERN EURO-NMD)

Present and future of gene therapy in Neuromuscular Diseases

Satellite Scientific Symposium endorsed by ERN EURO-NMD

February, 22nd 2024

Muscle diseases (DMD, LGMD, MTM1)

Serge Braun, PharmD, PhD
Chief Scientific Officer

AFMTÉLÉTHON INNOVER POUR GUÉRIR

GENETHON CURE THROUGH INNOVATION

GenoSafe

GENETHON
CURE THROUGH INNOVATION

Human Molecular Genetics, 2011, Vol. 20, No. 4 **681–693**
doi:10.1093/hmg/ddq514
Advance Access published on November 30, 2010

Intravenous scAAV9 delivery of a codon-optimized *SMN1* sequence rescues SMA mice

Elisa Dominguez[1,†], Thibaut Marais[1,†], Nicolas Chatauret[1,2], Sofia Benkhelifa-Ziyyat[1], Sandra Duque[1], Philippe Ravassard[3,4,5], Romain Carcenac[1], Stéphanie Astord[1], Aurélie Pereira de Moura[1], Thomas Voit[1] and Martine Barkats[1,*]

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Received July 20, 2010; Revised and Accepted November 2

Out-licensed to Avexis

(19) Europäisches Patentamt / European Patent Office / Office européen des brevets

(11) **EP 2 212 424 B1**

(12) **EUROPEAN PATENT SPECIFICATION**

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(51) Int Cl.: ***C12N 15/864*** *(2006.01)* ***A61K 48/00*** *(2006.01)*

(86) International application number: **PCT/EP2008/063297**

(87) International publication number: **WO 2009/043936 (09.04.2009 Gazette 2009/15)**

(54) **WIDESPREAD GENE DELIVERY TO MOTOR NEURONS USING PERIPHERAL INJECTION OF AAV VECTORS**

WEITREICHENDE GENVERABREICHUNG AN MOTORNEURONEN UNTER VERWENDUNG EINER PERIPHEREN INJEKTION VON AAV-VEKTOREN

DÉLIVRANCE À LARGE DIFFUSION DE GÈNES À DES NEURONES MOTEURS PAR INJECTION PÉRIPHÉRIQUE DE VECTEURS AAV

US 20100130594A1

(19) **United States**

(12) **Patent Application Publication**
Barkats

(10) **Pub. No.: US 2010/0130594 A1**

(43) **Pub. Date: May 27, 2010**

(54) **CNS GENE DELIVERY USING PERIPHERAL ADMINISTRATION OF AAV VECTORS**

(76) Inventor: **Martine Barkats**, Charenton-le-Pont (FR)

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(21) Appl. No.: **12/452,789**

(22) PCT Filed: **Jul. 22, 2008**

(86) PCT No.: **PCT/EP2008/059595**

§ 371 (c)(1),
(2), (4) Date: **Jan. 22, 2010**

(30) **Foreign Application Priority Data**

Jul. 23, 2007 (EP) 07301263.5

Publication Classification

(51) **Int. Cl.**
A61K 31/711 (2006.01)
A61P 25/00 (2006.01)

(52) **U.S. Cl.** ..... **514/44 R**

(57) **ABSTRACT**

The present invention relates to compositions and methods for the delivery of therapeutic proteins to the CNS using recombinant AAV vectors. More specifically, the invention relates to compositions and methods for delivering proteins into the cerebrospinal fluid of mammalian subjects through peripheral administration of AAV vectors. The invention may be used to treat various disorders of the central nervous system, including degenerative diseases and motor neuron diseases.

PROGRAMS AT CLINICAL STAGE / MARKETED

13 ONGOING CLINICAL TRIALS
5 IN THE NEXT 3 YEARS

MUSCLE DISEASES

SMA (Sub-licensed to avexis / NOVARTIS)

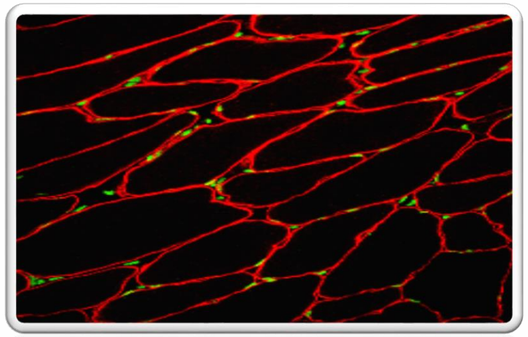
Myotubular myopathy (Sub-licensed to AUDENTES THERAPEUTICS / astellas GENE THERAPIES)

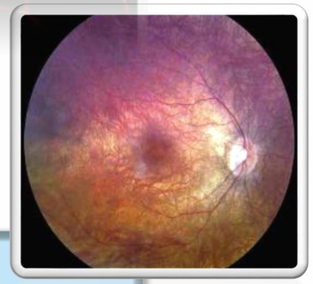
DMD (Phase1/2/3 ongoing)

LGMDs: **FKRP (Phase 2b ongoing)**

γ-sarcoglycanopathy (Phase 1 starting Q1 2024)

Pompe (Phase1/2 ongoing) Spark THERAPEUTICS



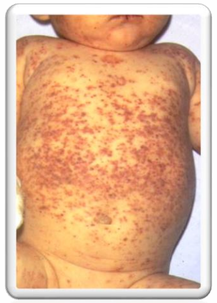


EYE DISEASES

Hereditary Neuropathy (LUMEVOQ® Pivotal trial) GenSight BIOLOGICS

LIVER DISEASES

Crigler-Najjar disease (Pivotal trial ongoing , Fr, It, Netherl.)



IMMUNE AND BLOOD disease

Orchard therapeutics

Ciemat Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas

Wiskott Aldrich (trial in France, US, UK)

Chronic Granulomatosis (trial in Fr US, UK)

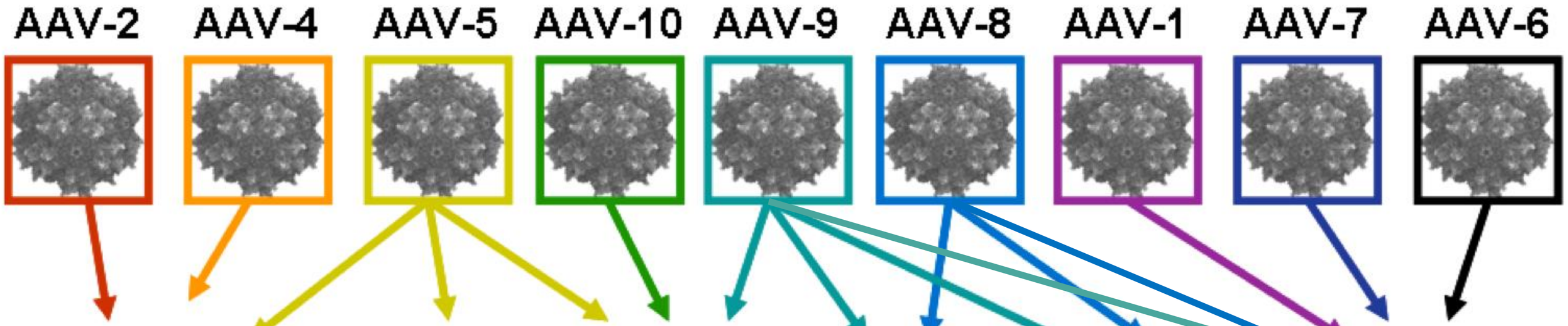
Fanconi anemia (trial in Spain)

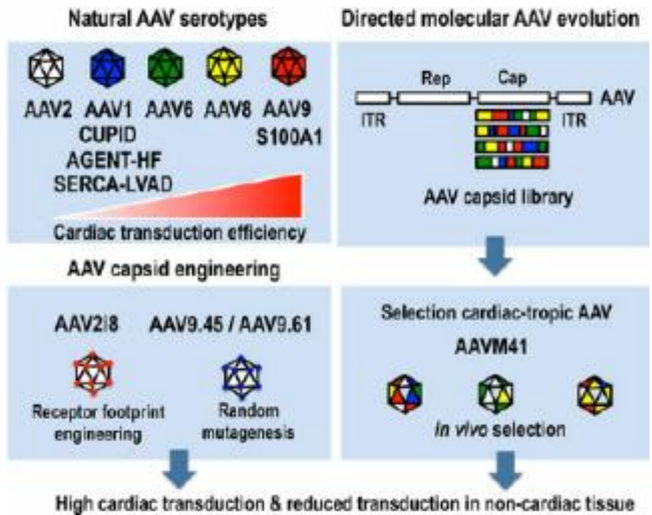
Severe X-linked immunodeficiencies (US trial)

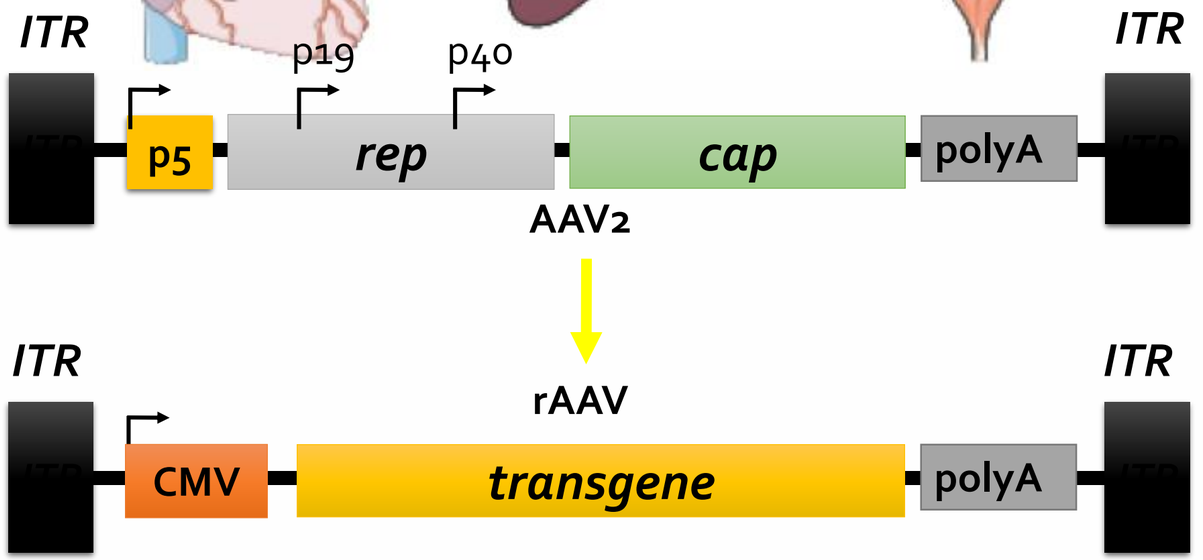
Artemis (Trial in France)

Sickle Cell Anemia

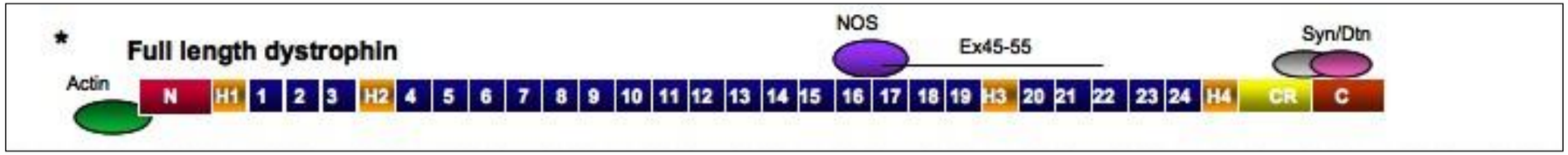
Tropisms


AAV-2
AAV-4
AAV-5
AAV-10
AAV-9
AAV-8
AAV-1
AAV-7
AAV-6

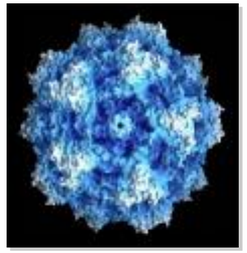

Natural AAV serotypes
Directed molecular AAV evolution
AAV2 AAV1 AAV6 AAV8 AAV9
CUPID
AGENT-HF
SERCA-LVAD
S100A1
Cardiac transduction efficiency
Rep
Cap
AAV
ITR
ITR
AAV capsid library
AAV capsid engineering
AAV2i8
AAV9.45 / AAV9.61
Receptor footprint engineering
Random mutagenesis
Selection cardiac-tropic AAV
AAVM41
in vivo selection
High cardiac transduction & reduced transduction in non-cardiac tissue


ITR
p19
p40
ITR
p5
rep
cap
polyA
AAV2
rAAV
ITR
ITR
CMV
transgene
polyA

GNT0004: codon-optimized human µDystrophin-AAV



Codon optimized µDystrophin (MD1)



rAAV-hMD1

- codon-optimized for tRNA frequency, GC content and mRNA stability
- consensus Kozak sequence to improve initiation of translation
- 30-fold increase in microdystrophin expression
- muscle- and heart-specific (spc5.12) promoter
- murine, canine and human sequence variants

(Foster et al. Mol Ther 2008)

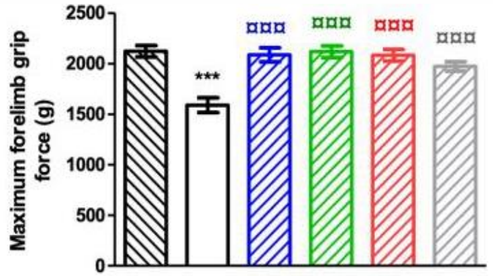
Pr. George Dickson
RHUL

Partnership SAREPTA THERAPEUTICS

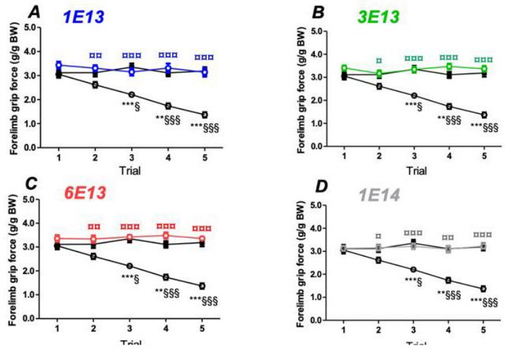
YPOSKESI | An SK pharmteco Company

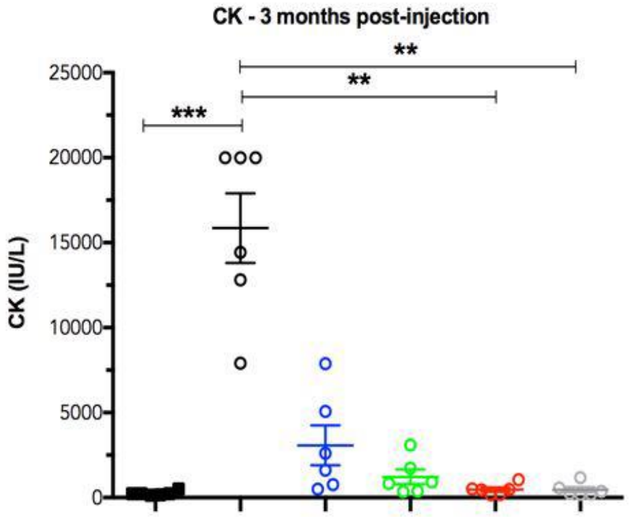
GENETHON
CURE THROUGH INNOVATION

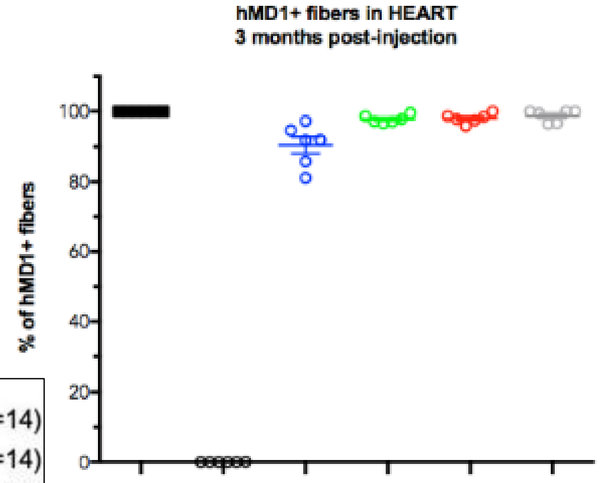
Very robust therapeutic effect in skeletal muscle an heart in rat *Dmd*

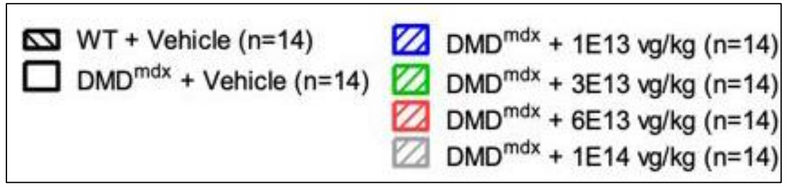


Strength (3 months p.i.)

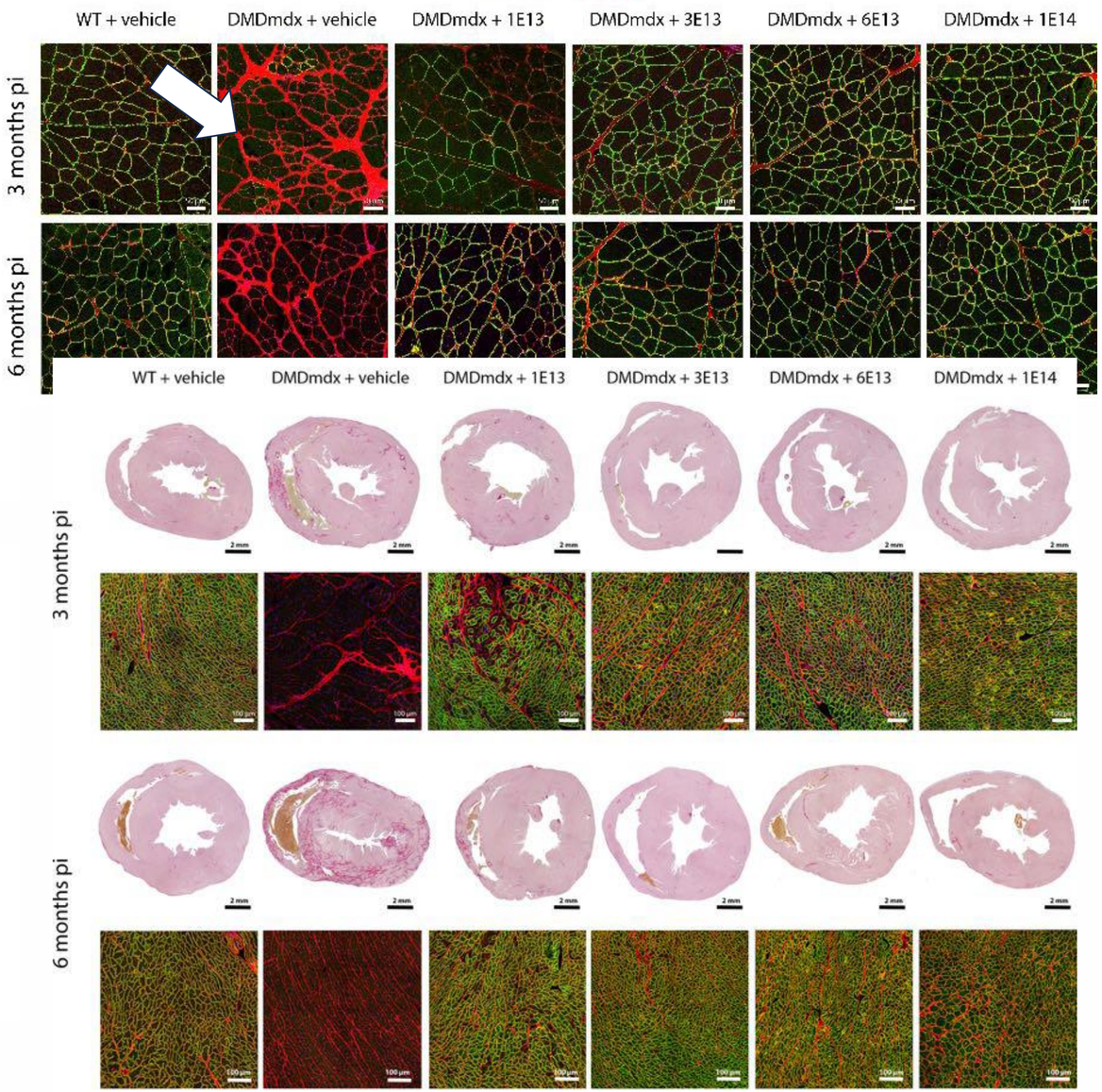








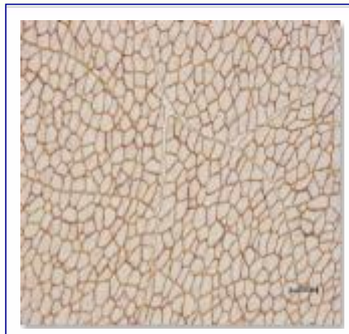
Fibrosis Microdystrophin



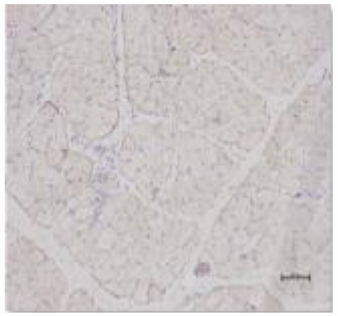
GNT0004 Non-Clinical Package

- **GRMD Dog** Locoregional (short term) and systemic (long-term) administration

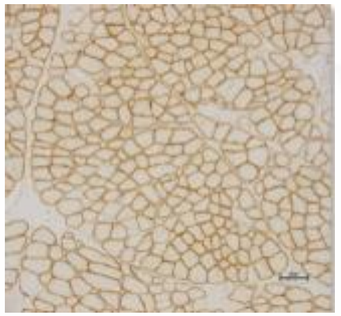
Effective, long-term, and safe
$2x10^{13}$ vg/kg and $1x10^{14}$vg/kg in GRMD dogs



WT dog
NCL-DysB

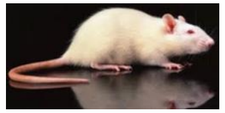


GRMD
+ excipient



GRMD
+ rAAV8-µDys

- **DMD^{mdx} Rat** IV injection, follow-up 3-6 months



Restores histology and function of skeletal muscles and heart

➢ Minimum Effective Dose : $1x10^{13}$ to $3x10^{13}$ vg/kg

➢ No Observed Adverse Effect Level (NOAEL) at $6.0x10^{13}$ vg/kg in WT and $2.1x10^{14}$vg/kg in DMD^{mdx} rats

Caroline LE GUINER

Stéphane BLOT, ENVA

Gernoux et al. 2021
Le Guiner et al. Nat. Comm. 2017
Larcher et al. 2014

Clinical development of GNT0004

A phase I-II-III study with a dose determination part followed by an efficacy and safety, quadruple blind placebo-controlled part, in ambulant boys aged 6 - 10 y.

Up to 5 y. follow-up

French ANSM : Nov 2020
CTA approval

UK MHRA: Q2 2022
CTA Approval

ANSM Q1 2022
Approval to resume trial

Today

Natural history study

Baseline
6-36 months
(F, UK)

Following dose selection

Baseline
6-36 months
(F, UK+ US + Israel)

Strasbourg, Brest, Lyon, Lille, Bordeaux, Paris

$1x10^{13}$ vg/kg

SUSAR/Hold
May 2021

$3x10^{13}$ vg/kg (Cohort 2)

Pivotal part (at selected dose, 42 pts)

$6x10^{13}$ optional cohort

NSAA at 52 w.
Microdystrophin expression at 8 W.

ActiMyo
Myotools (pintch, strength), ACTIVLIM, EQ5D
Muscle MRI
Heart function and respiratory function

The NEW ENGLAND JOURNAL of MEDICINE

CORRESPONDENCE

Dystrophin Immunity after Gene Therapy for Duchenne's Muscular Dystrophy

TO THE EDITOR: Duchenne's muscular dystrophy (DMD) is caused by loss-of-function mutations — often deletions — in *DMD* that lead to muscle dystrophin protein deficiency. Adeno-associated virus (AAV) gene therapy to deliver a shortened yet functional microdystrophin transgene that fits within the size constraints of AAV is under investigation in several studies. Mendell and colleagues[1] reported a strong T-cell immune response against epitopes encoded by an AAV-delivered microdystrophin after intramuscular delivery. The method of administration and the ubiquitous cytomegalovirus promoter that was used could have contributed to those findings.[2,3]

On the basis of recent investigations (performed with the assistance of clinical and other collaborators; see the Supplementary Appendix, available with the full text of this letter at NEJM.org), we now describe five boys with DMD, 7 to 9 years of age, who were enrolled in three separate trials evaluating investigational gene therapies (ClinicalTrials.gov numbers, NCT04281485 and NCT04626674, and Eudra-CT number, 2020-002093-27) and in whom strikingly similar suspected unexpected serious adverse reactions (i.e., unexpected adverse reactions that are considered likely to be related to the treatment) occurred. The three AAV products used in the three trials in which these adverse reactions occurred were different microdystrophin transgenes under different muscle-specific promoters, packaged in different AAV serotypes (AAV9, AAV8, and AAVrh74) and delivered intravenously at doses between 1×10^{13} and 2×10^{14} vector genomes per kilogram of body weight. Symptom onset occurred 3 to 6 weeks after administration: all five patients had severe weakness of the proximal and distal limb muscles that led to loss of ambulation, as well as weakness of the bulbar and respiratory muscles, which led to receipt of transient ventilatory support in three of the patients (two with noninvasive ventilation and one with endotracheal intubation). The presence of myositis in the five patients was supported by an increase in the creatine kinase level relative to the base-

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Disclosure forms provided by the authors are available with the full text of this letter at NEJM.org.

1. Mendell JR, Campbell K, Rodino-Klapac L, et al. Dystrophin immunity in Duchenne's muscular dystrophy. N Engl J Med 2010;363:1429-37.
2. Wilson JM. Autoimmunity, recessive diseases, and gene replacement therapy. Mol Ther 2010;18:2045-7.
3. Cordier L, Gao GP, Hack AA, et al. Muscle-specific promoters may be necessary for adeno-associated virus-mediated gene transfer in the treatment of muscular dystrophies. Hum Gene Ther 2001;12:205-15.

DOI: 10.1056/NEJMc2212912

N Engl J Med 2023; 388:2294-2296

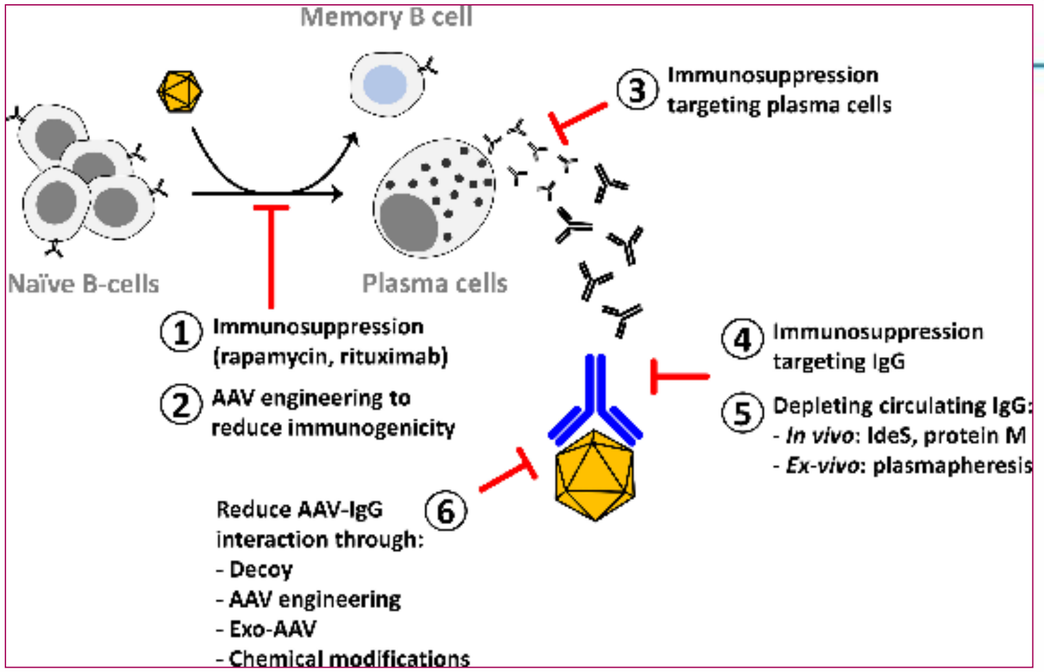
THIS WEEK'S LETTERS

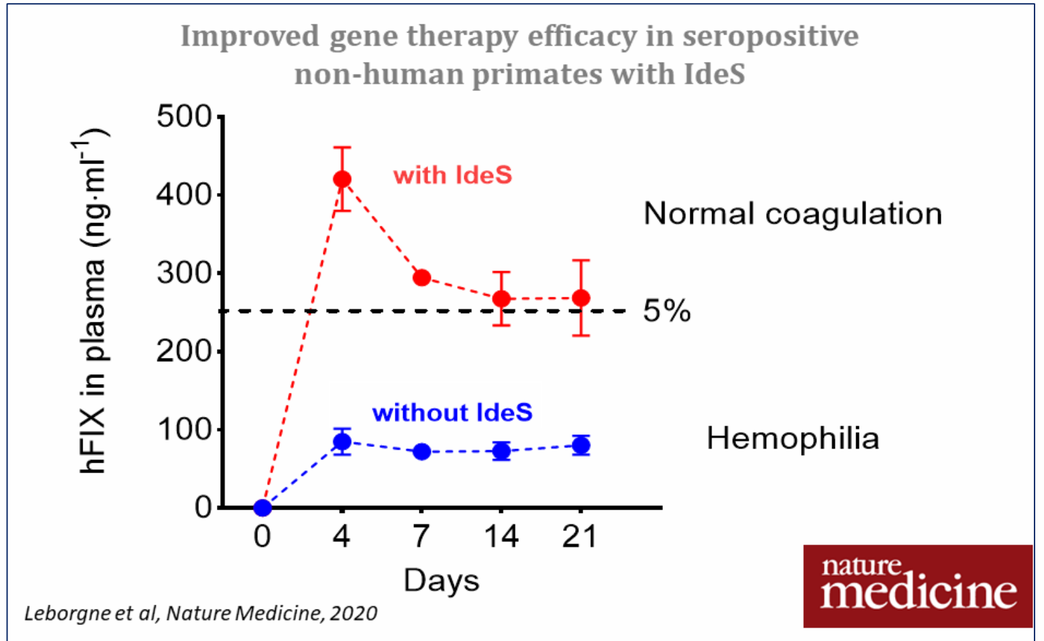
Approaches to reduce anti-capsid or anti-transgene immune responses

for re-dosing
+ non-eligible patients

- **Less immunogenic vectors**
  (ex capsid, promoters, miRT, innate motifs, …..)
- **Novel or immunomodulatory treatments**
  (ex. SVP-rapa, others ? **IDES**)
- **Repositioning existing immuno-modulatory/suppressive treatments in GT**
  (ex. methotrexate, others ?)
- **Deeper patient monitoring**

Gross et al. Frontiers in Immunology 2022

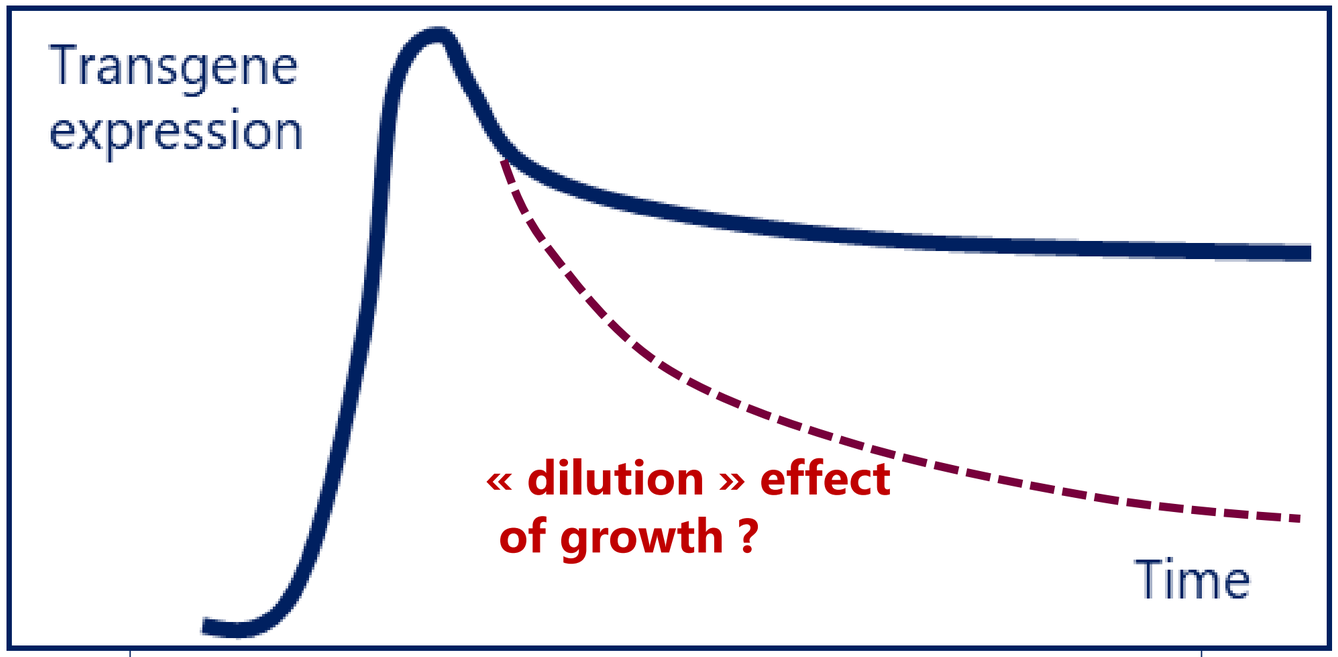




Leborgne et al, Nature Medicine, 2020

Need for re-dosing ?

(« stable » muscles vs regenerating vs growing)

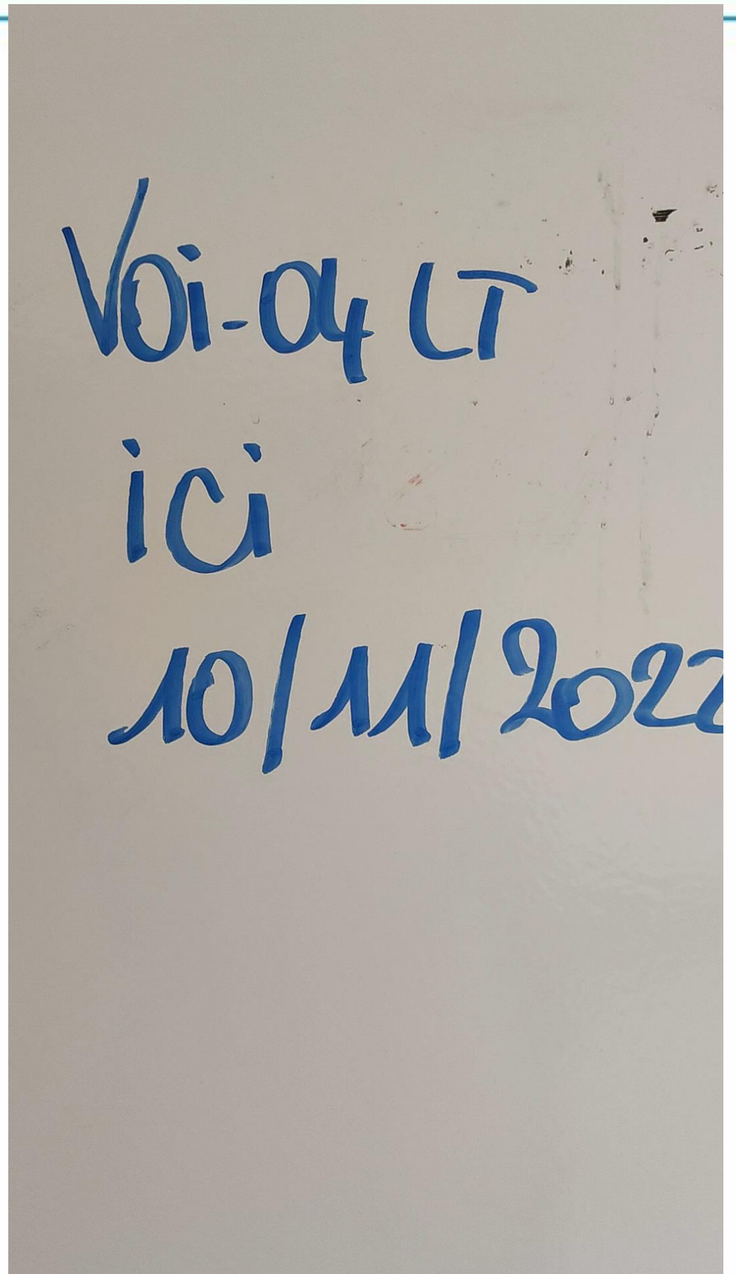


Hypothetical kinetics of AAV-induced transgene expression in skeletal muscles

Persistence of the therapeutic benefit

"ICI" (Golden retriever muscular dystrophy dog)

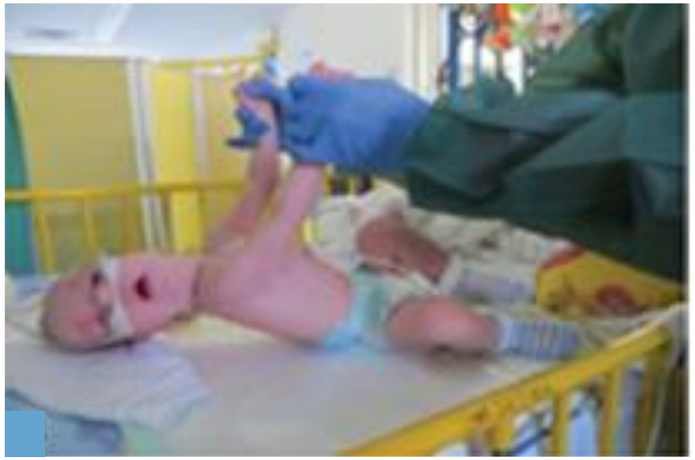
- **Born- Oct 2013**
- **AAV8-cMD1 gene therapy in 2014**
- **10 years later and still doing well !**



MYOTUBULAR MYOPATHY (XLMTM)

Ana BUJ-BELLO

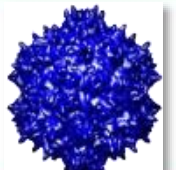
- Congenital myopathy : ~1/40-50,000 live-born males
- Hypotonia and generalized muscle weakness
- Respiratory insufficiency (ventilation)
- Severe prognosis: >50% die before 2 years of age
- Mutations in the *MTM1* gene, codes for myotubularin
- No approved disease-modifying therapy



Gangfuss et al. *J. Neuromusc. Dis.* 2021

ASPIRO CLINICAL TRIAL

GENETHON
CURE THROUGH INNOVATION



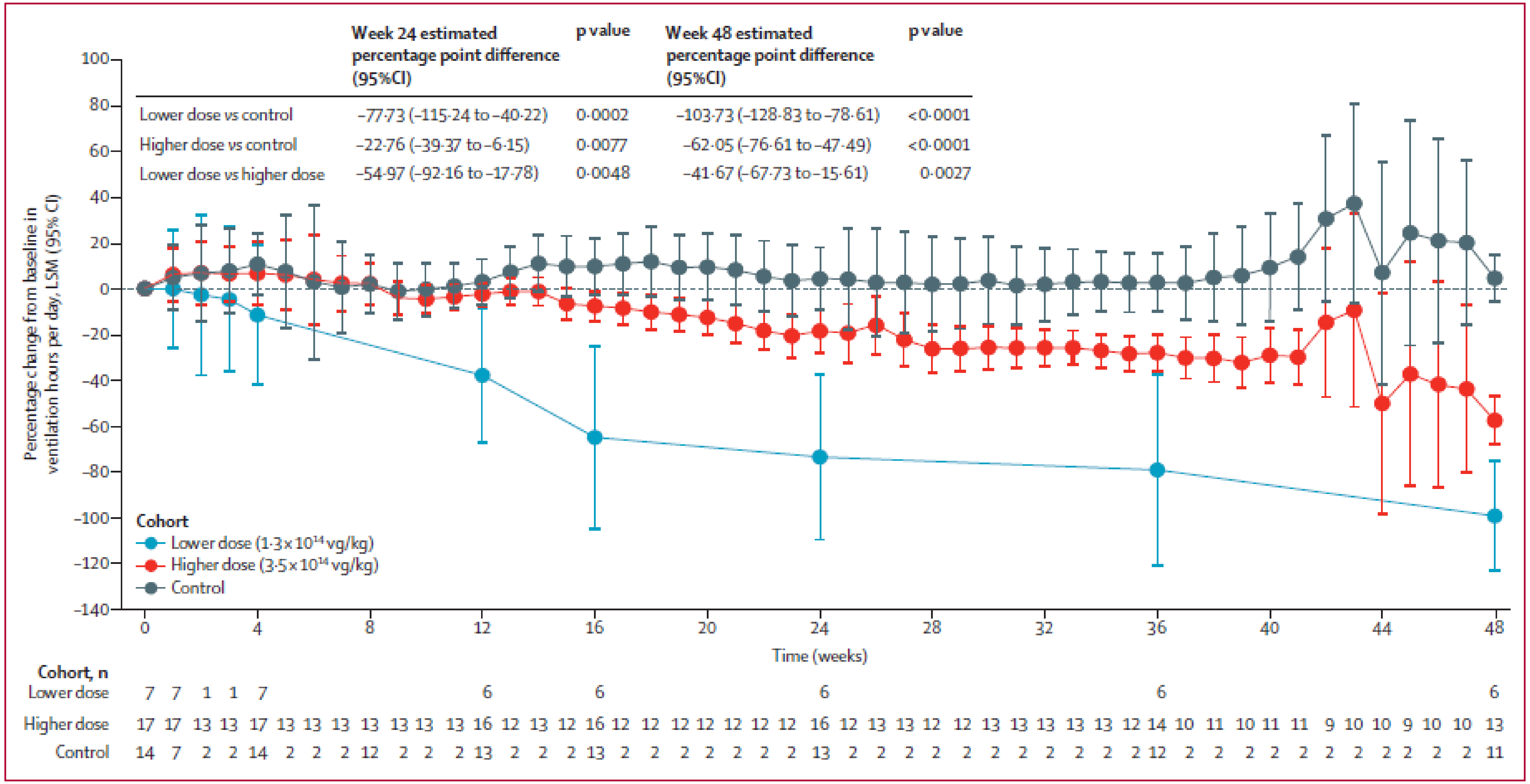
AAV8-MTM1
AT132

astellas
GENE THERAPIES
AUDENTES

USA, Canada, and Europe
Started 2017
24 treated patients
IV, 2 cohorts, 1 x10^{14} vg/kg, 3 x10^{14} vg/kg)

Respiratory outcomes

The Lancet Neurology, 2023



ASPIRO CLINICAL TRIAL

AAV8-MTM1

AT132

USA, Canada, and Europe

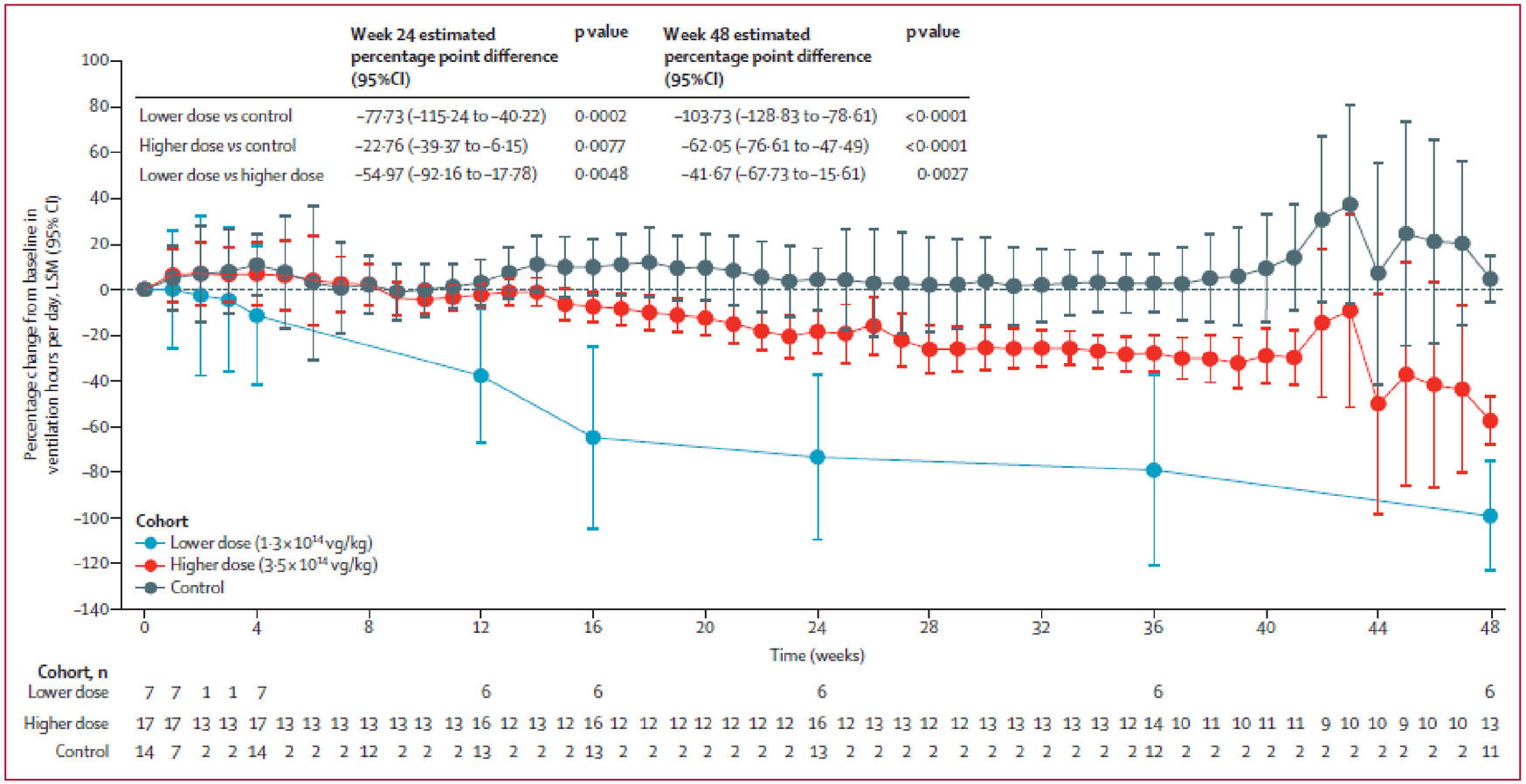
Started 2017

24 treated patients

IV, 2 cohorts, 1 x10^{14} vg/kg, 3 x10^{14} vg/kg)

Respiratory outcomes

The Lancet Neurology, 2023



- Decrease in daily ventilation hours
- **16 patients achieved ventilator independence**
- Increase in muscle strength and progressive acquisition of motor milestones
- **12 patients stand**
- **8 walk without support**

- **SAE and death in 4 patients → on hold**

XLMTM: LIVER PATHOLOGY

N=34 participants, age < 4 years
Duration on study: median 13 months (0.5-32.9 m)
Evaluation every 3 months

INCEPTUS Natural History, Run-in Study for Gene Replacement Clinical Trial in X-Linked Myotubular Myopathy

James J. Dowling[a,*], Wolfgang Müller-Felber[b], Barbara K. Smith[c], Carsten G. Bönnemann[d], Nancy L. Kuntz[e], Francesco Muntoni[f], Laurent Servais[g,h], Lindsay N. Alfano[i], Alan H. Beggs[j], Deborah A. Bilder[k], Astrid Blaschek[b], Tina Duong[l], Robert J. Graham[j], Minal Jain[m], Michael W. Lawlor[n], Jun Lee[o], Julie Coats[p], Charlotte Lilien[g], Linda P. Lowes[i], Victoria MacBean[q], Sarah Neuhaus[d], Mojtaba Noursalehi[o], Teresa Pitts[r], Caroline Finlay[r,o], Sarah Christensen[r,o], Gerrard Rafferty[s], Andreea M. Seferian[g], Etsuko Tsuchiya[a], Emma S. James[r,o], Weston Miller[p], Bryan Sepulveda[o], Maria Candida Vila[o], Suyash Prasad[o], Salvador Rico[o] and Perry B. Shieh[t] for the INCEPTUS investigators[1]

Journal of Neuromuscular Diseases 9 (2022) 503–516

24% of participants had history of hepatic disease at enrollment
85% - at least one elevated value of ALT
62% - at least one elevated value of AST
35% - at least one elevated value of total or direct bilirubin
62% - hepatic adverse events or ultrasonographic imaging abnormalities or both
50% received medications administrated for cholestatic or other hepatic disease

91% of participants had history of hepatobiliary disease at enrollment or showed at least one sign of hepatic disease

GTx of LGMDs and cardiomyopathies

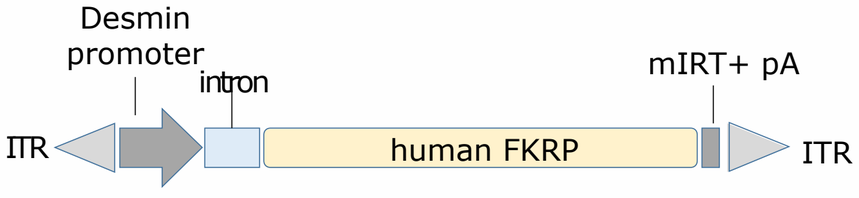
Isabelle RICHARD

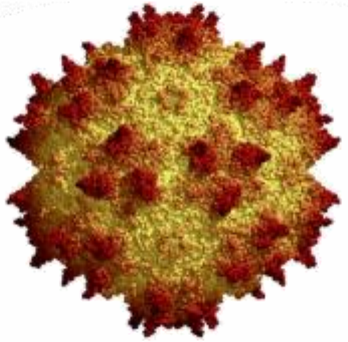
Technology Platforms

- Organ-sparing technologies
- New promoters
- New capsids

Research	Preclinical	Clinical trials
FKRP-deficiency– **LGMD-R9**		*Phase1b in Europe & US*
γ-sarcoglycanopathy – **LGMD-R5**		*CTA cleared – trial to start 1H 2024*
Calpainopathy – **LGMD-R1**		2025
Cardiomyopathies (Dilated CM) – several targets		

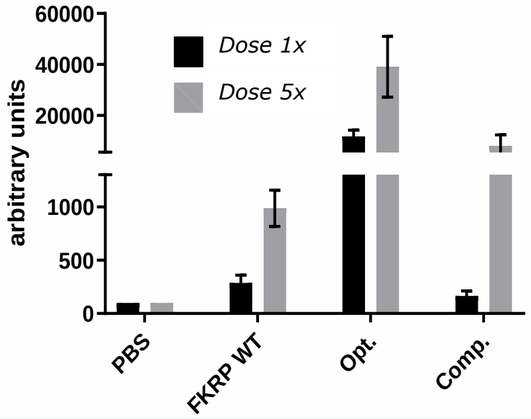
ATA-100: LGMD-R9 (FKRP-deficiency)





AAV9

FKRP proteic level after gene transfer



AAV9

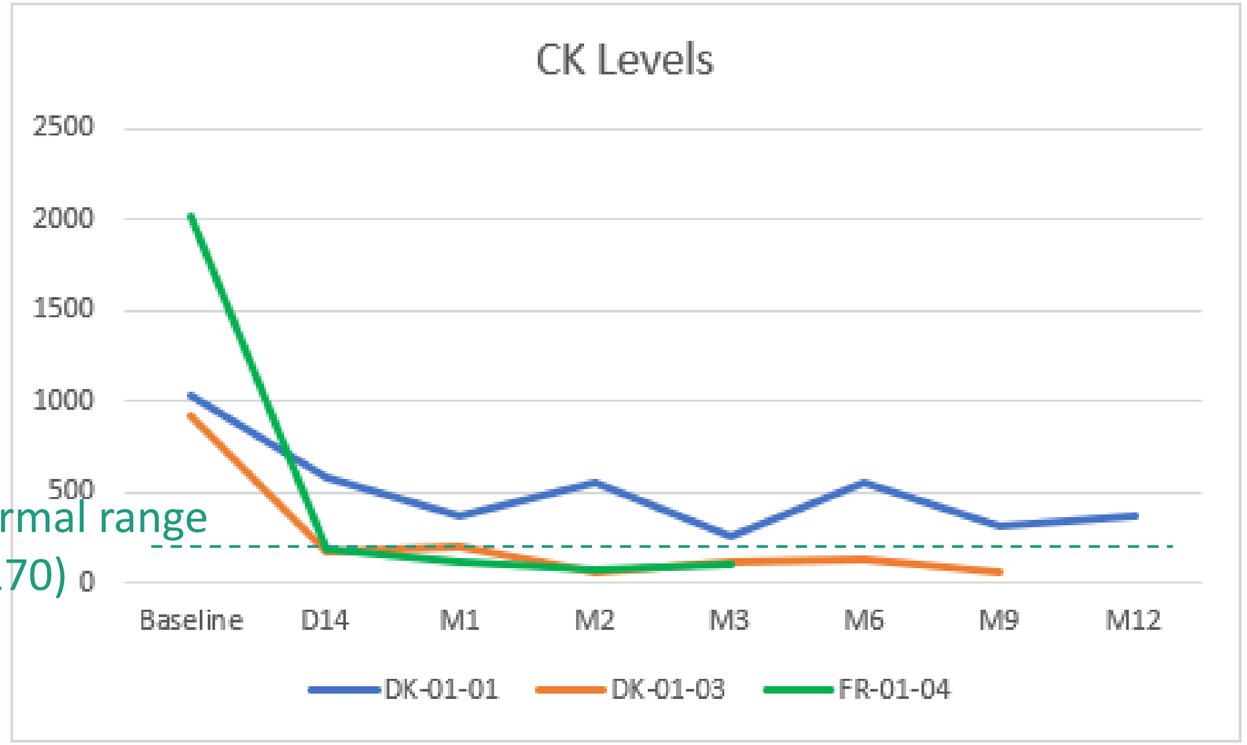
Codon-optimized sequence of FKRP improving expression by **5x ratio**

miRNA target sequence to modulate transgene expression to prevent cardiac toxicity

Total restoration (histological, functional) at 9E12 vg/kg (first clinical dose)

High safety margin (>30x)

All treated patients show marked decline in creatine kinase



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Patient Dk-01-01: 29 years old, 51 kg

	% Change from baseline at one year	% Change at one year from baseline in Natural History Study for FVC<80 (n=35)
Forced Vital Capacity (%)	+8%	-3.5%
Velocity (10MWT) m/s	+13%	-8.2%
Timed Up and Go (sec)	-11%	+2%
Activlim	+14%	-7.3%
Quality of Life (gNMD)	+54%	Not available
NSAD	-2%	-8.9%

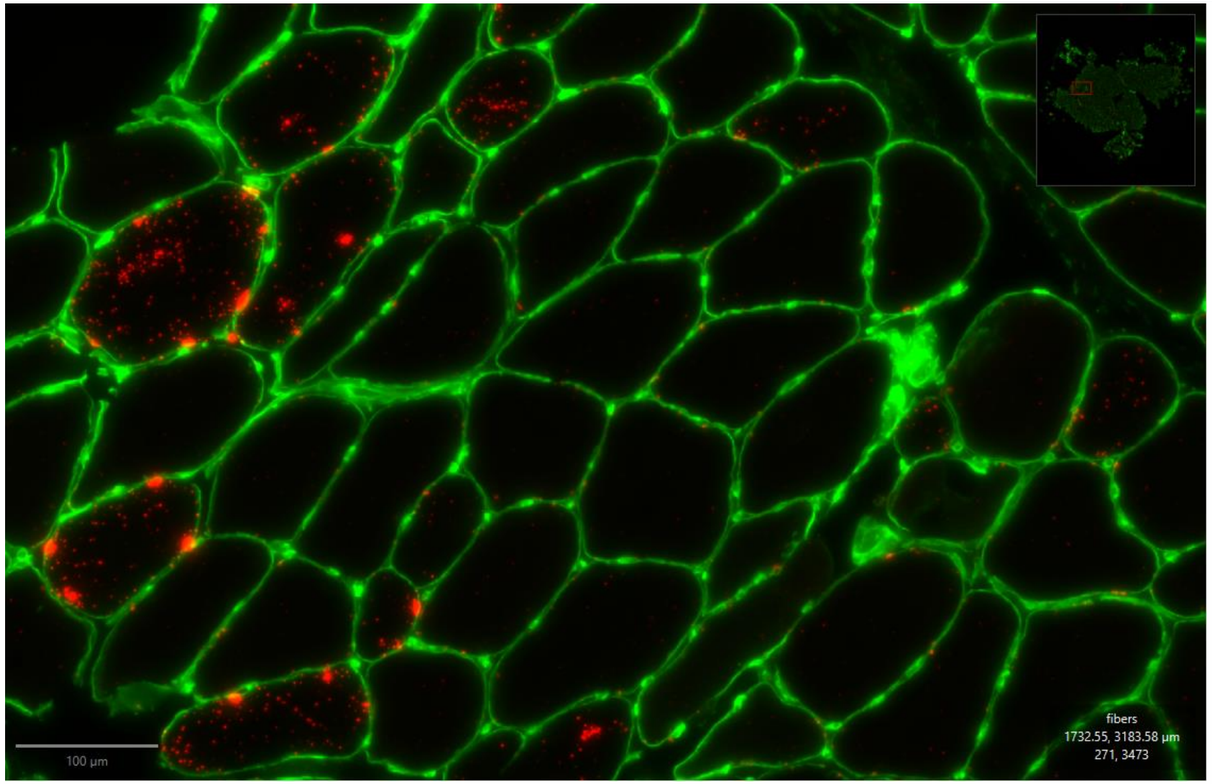
Patient Dk-01-03: 42 years old, 82 kg
Data at 6 months

Velocity (10MWT): +32%
TUG: -15%
Quality of Life: +7%

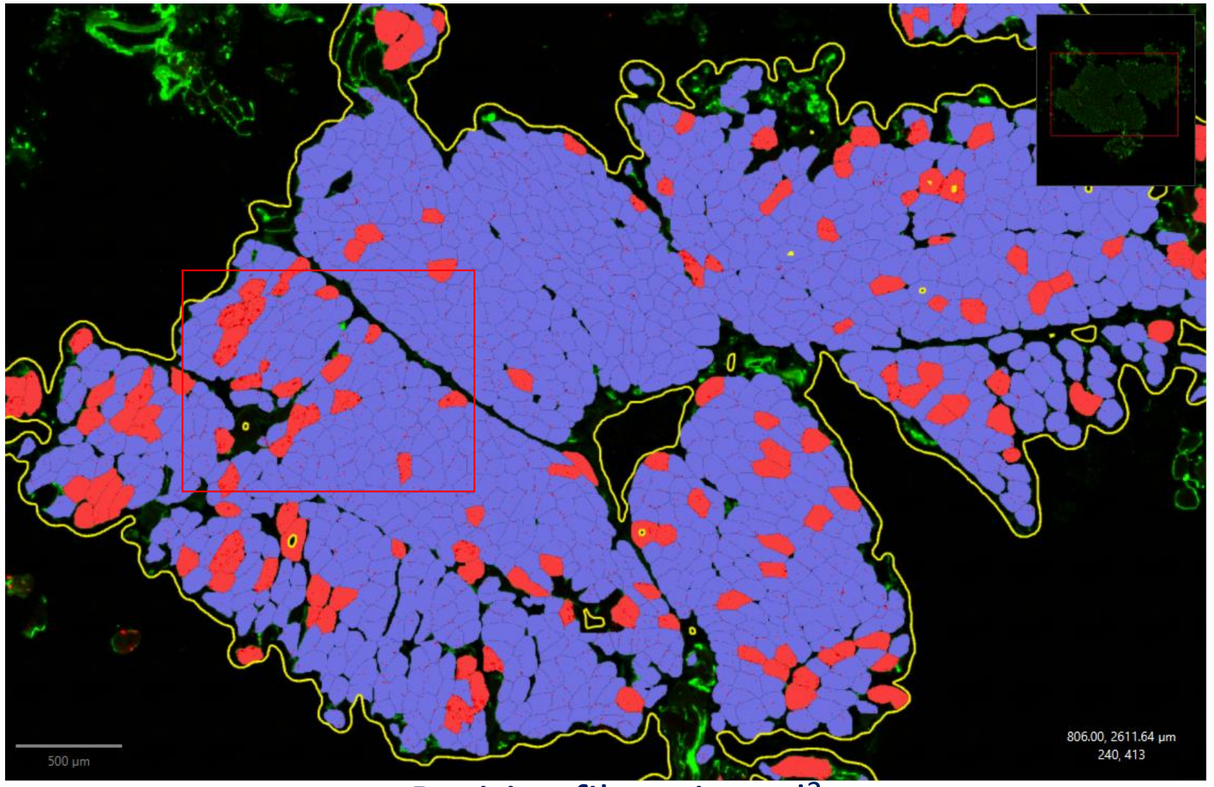
Evidence of transgene expression: FKRP-positive fibers (ACD)

KO mice : significant histological and functional correction
at doses ≥ $4.5x10^{12}$ vg/Kg with at least 10% FKRP-positive fibers

Patient 2 Muscle Biopsy at 3 months



IHF: muscle fiber membrane in green[1], FKRP mRNA spots in red



Positive fibers in red[2]

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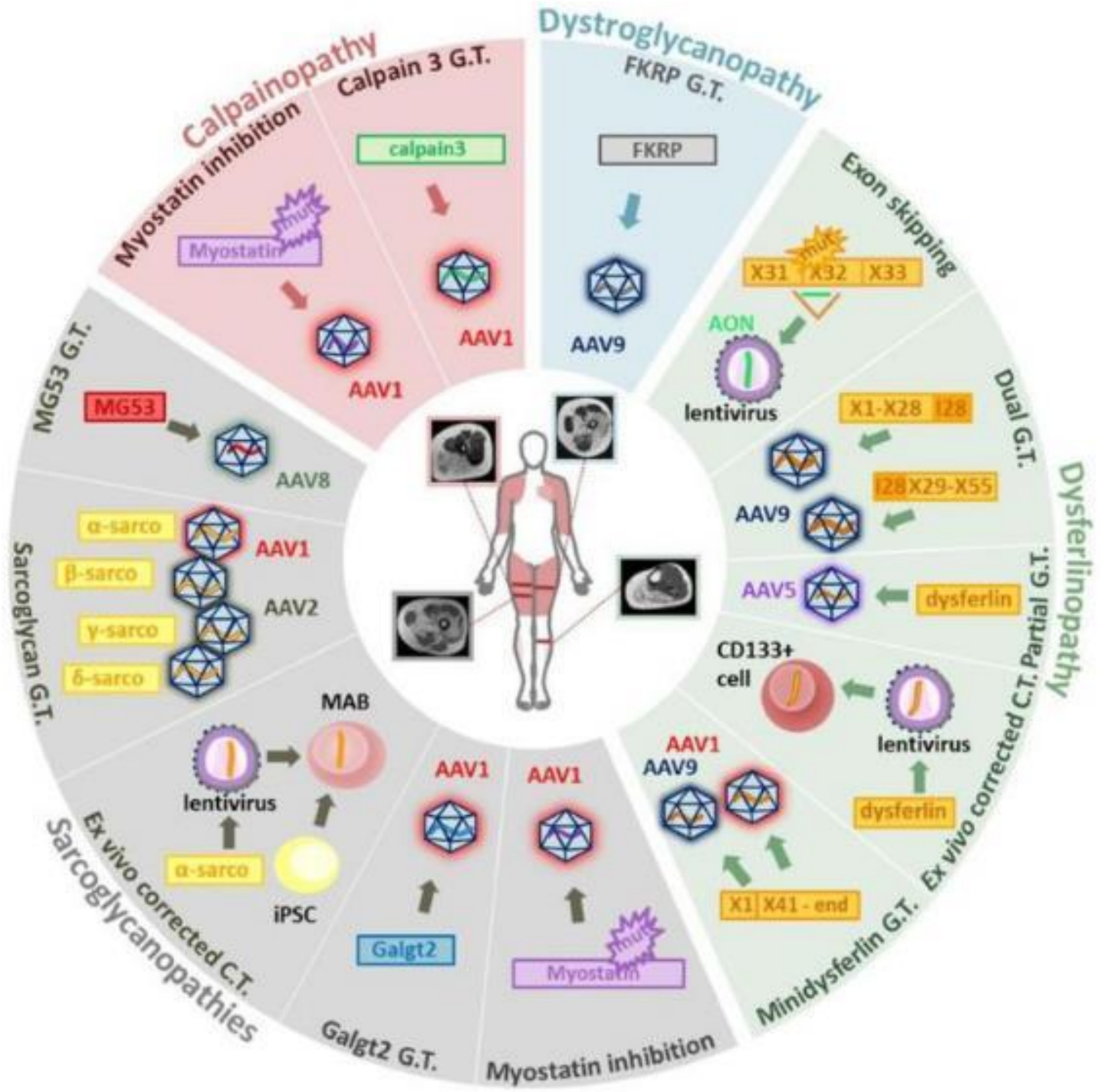

Gene therapies of LGMD

Gene therapies of Pompe disease

astellas

Spark THERAPEUTICS

AskBio

AAV2/8 α-glucosidase

GeneCradle 锦篮基因

Lacerta therapeutics

rAAV9-DES-hGAA injected intramuscularly into the TA
Rituximab + Sirolimus prior to second injection into the contraletral muscle

Table 2. Nucleic-acid-based molecules in preclinical studies and clinical trials for DM1.

Molecule Class	Target	Therapeutic Molecule	Mechanism	DDS	Admin. Route	Study Phase
ASOs	*DMPK* CUGexp	PMO-CAG25, 2′-OMe-CAG, LNA-CAG mixmers, all-LNA-CAG	MBNL1 binding block	Naked	IM	Preclinical
	DMPK CUGexp	PPMO-B, PPMO-K; Pip6a-PMO	MBNL1 binding block	CPP-conj	IM, IV	Preclinical
	DMPK CUGexp	miniPEG-γ PNA	MBNL1 binding block	Polymer-conj	SC	Preclinical
	DMPK 3′UTR	MOE gapmers, c-Et gapmers, LNA gapmers	*DMPK* mRNA degradation	Naked	IM, SC, ICV	Preclinical
	DMPK CUGexp	LNA gapmers, MOE gapmers	Mutated *DMPK* mRNA degradation	Naked	IM	Preclinical
	DMPK 3′UTR	IONIS-DMPKRx	*DMPK* mRNA degradation	Naked	SC	Clinical(completed)
	DMPK 3′UTR	palmitoyl-c-Et gapmers	*DMPK* mRNA degradation	Lipid-conj	SC	Preclinical
	miRNAs targeting *Mbnl1* mRNA	cholesterol-2′OMe-ASOs	AntagomiR	Lipid-conj	SC, IV	Preclinical
	Mbnl1 3′UTR	Pip9b2-PMO	BlockmiR	CPP-conj	IV	Preclinical
siRNA	*DMPK* CUGexp	siRNA-CAG	Mutated *DMPK* mRNA degradation	Nacked	IM	Preclinical
	DMPK mRNA	AOC 1001	*DMPK* mRNA degradation	Ab-conj	IV	Clinical (recruiting)
rAAV	*DMPK* downstream pathway	MBNL1	MBNL1 overexpression	rAAV1	IM	Preclinical
	DMPK downstream pathway	MBNL1	Competition for CUGexp interaction	rAAV9	IM	Preclinical
	DMPK CTG spanning region	Sa/eSpCas9-sgRNAs	CTGexp removal	rAAV9	IM	Preclinical
	DMPK CTGexp	dSaCas9-sgRNA	Transcription inhibition	rAAV6, rAAV9	IV	Preclinical
	DMPK CUGexp	RCas9-sgRNA	*DMPK* mRNA degradation	rAAV9	IV, TA	Preclinical

Abbreviations: Ab-conj = antibody-conjugated; Admin. Route = administration route; CPP = cell-penetrating peptide; CTGexp = CTG expansion; CUGexp = CUG expansion; DDS = drug delivery system; dSaCas9 = deactivated *Staphylococcus aureus* Cas9; ICV = intracerebroventricular; IM = intramuscular; IV = intravenous; Naked = not conjugated ASOs; rAAV = recombinant adeno-associated virus; RCas9 = RNA targeting Cas9; RO = retro orbital; eSpCas9-sgRNAs = enhanced *Streptococcus pyogenes Cas9*-single guide.

From Izzo et al.
Int. J. Mol. Sci. 2022

ELSEVIER
The Lancet Neurology
Volume 22, Issue 3, March 2023, Pages 218-228

Articles

Antisense oligonucleotide targeting *DMPK* in patients with myotonic dystrophy type 1: a multicentre, randomised, dose-escalation, placebo-controlled, phase 1/2a trial

Prof Charles A Thornton MD [Q], Prof Richard Thomas Moxley III MD [Q],

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IERAPEUTICS

s Announces Positive Initial Clinical Data from ACHIEVE Trial in DM1 Patients and MD Patients Demonstrating Promise of the FORCE™ Platform in Developing are Muscle Diseases

Avidity Biosciences Announces New Positive AOC 1001 Data Demonstrating Improvement in Multiple Additional Functional Endpoints and Favorable Long-term Safety and Tolerability in People with Myotonic Dystrophy Type 1

AVIDITY BIOSCIENCES

NEWS PROVIDED BY
Avidity Biosciences, Inc. →
07 Oct, 2023, 07:30 ET

Large-scale production, a key limiting factor…

… and an opportunity

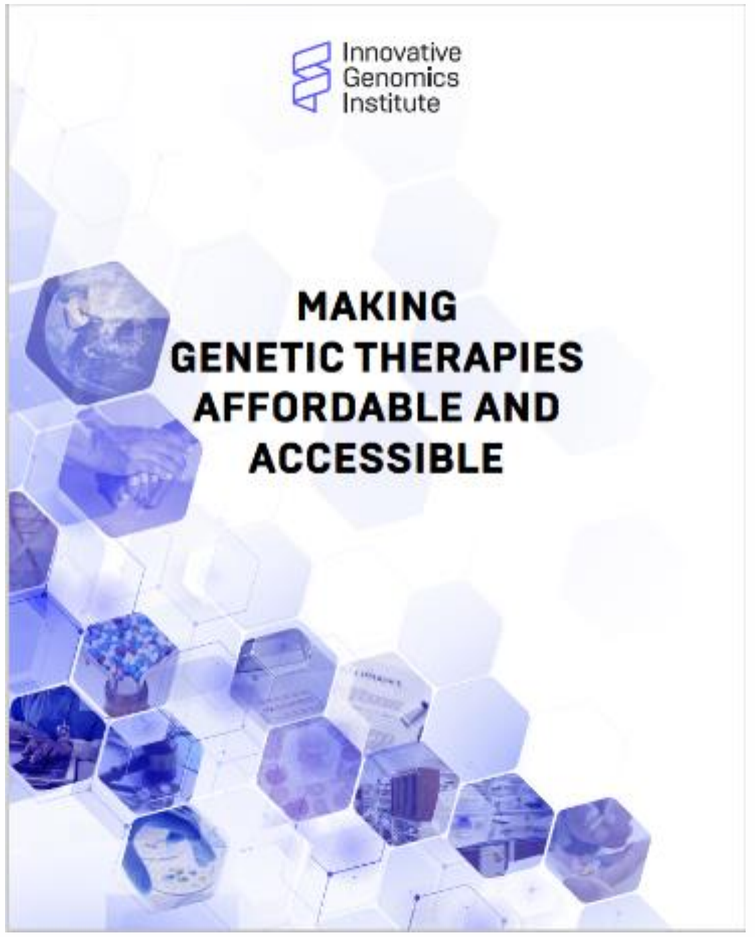
- **Vaccines:** $10^{7\text{-}10}$ vg / dose

- **Duchenne :** > 10^{15} vg / patient

  3 million L / 15 000 patients

Disruptive technologies are necessary

Jennifer Doudna



Now we are at an inflection point (...) While the therapeutic potential of genetic therapies is immense, their real-world impact will be limited if we do not secure access for everyone who stands to benefit