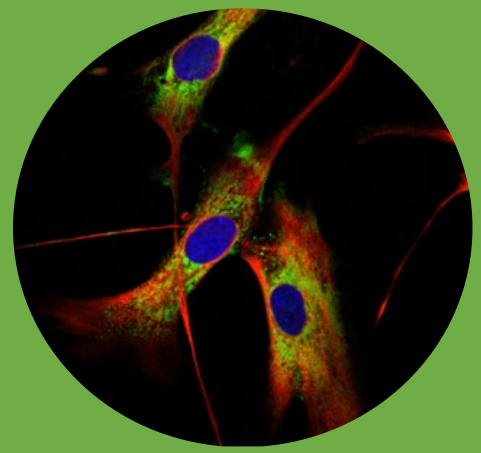
Gene edited human muscle stem cells as Advanced Therapies

Prof. Dr. Simone Spuler

Frontiers in Translational Medicine _ Scientific and Structural Challenges _ 23 April 2021

CHARITÉ
UNIVERSITÄTSMEDIZIN BERLIN

MDC
MAX-DELBRÜCK-CENTRUM
FÜR MOLEKULARE MEDIZIN
BERLIN-BUCH
IN DER HELMHOLTZ-GEMEINSCHAFT e.V.



Muscle Research Unit

https://www.mdc-berlin.de/spuler

Patient care

2500 patients in Charité muscle outpatient clinic

Diagnosis and follow-up

Supportive care

Clinical trials

Translational Research

Human muscle stem cells

ATMP– new therapies

Muscular dystrophy

Gene editing

UNTERSTÜTZT DURCH DEN IMPULS- UND VERNETZUNGSFONDS VON HELMHOLTZ

Bundesministerium für Bildung und Forschung

DFG Deutsche Forschungsgemeinschaft

SGK STIFTUNG GISELA KREBS

SPARK BIH

BERLIN INSTITUTE OF HEALTH

Else Kröner Fresenius Stiftung

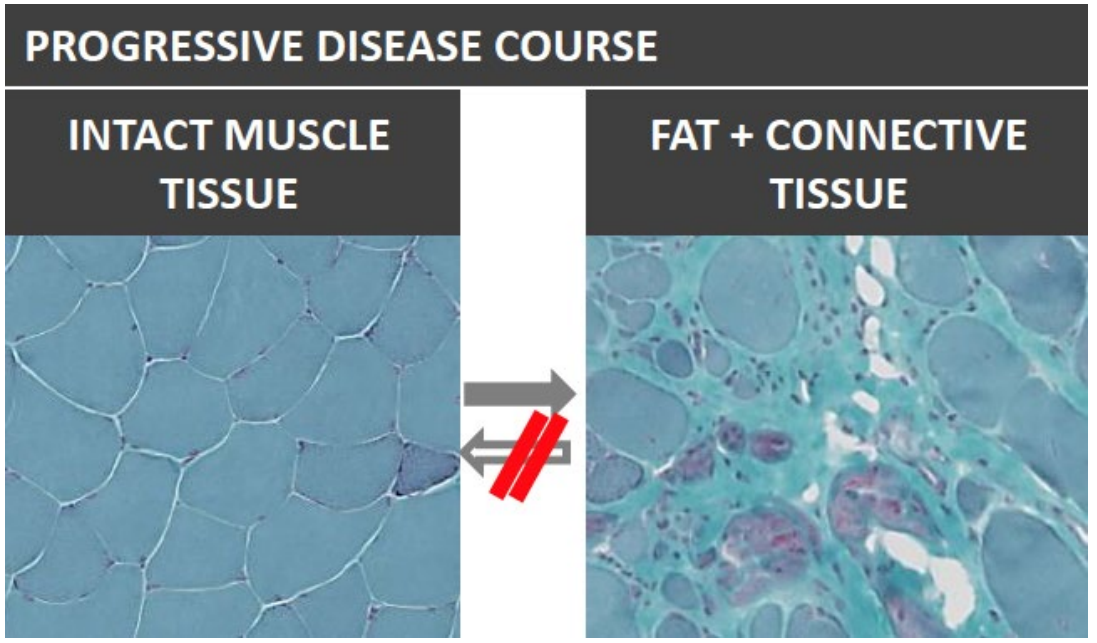
SUMUS

Muscular dystrophies

50 different **monogenic progressive** disorders

Incidence 30/100.000

No treatment



It is the right time!

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Review 10.1172/JCI142031

Stem cell therapy for muscular dystrophies

Stefano Biressi,[1,2] Antonio Filareto,[3] and Thomas A. Rando[4,5,6]

First published September 18, 2020 - More info

Muscular dystrophies are a heterogeneous group of genetic diseases, characterized by progressive degeneration of skeletal and cardiac muscle. Despite the intense investigation of different therapeutic options, a definitive treatment has not been developed for this debilitating class of pathologies. Cell-based therapies in muscular dystrophies have been pursued experimentally for the last three decades.

nature biotechnology

News in Brief | Published: 05 August 2020

High-dose AAV gene therapy deaths

Nature Biotechnology **38**, 910(2020) | Cite this article

4915 Accesses | **22** Altmetric | Metrics

The US Food and Drug Administration placed Audentes Therapeutics' phase 2 gene therapy trial for a rare neuromuscular disease on hold following the deaths of two patients receiving the higher dose of the investigational treatment AT132. Both deaths were caused by progressive liver dysfunction followed by sepsis in patients who had pre-existing liver disease. The deaths add to emerging safety concerns surrounding the use of AAV vectors. The AT132 therapy treats X-linked myotubular myopathy, a life-threatening condition characterized by profound muscle weakness

It is the right time!

The Nobel Prize in Chemistry 2020

Development of a method for genome editing

Emmanuelle Charpentier

Max Planck Unit for the Science
of Pathogens, Berlin, Germany

Jennifer A. Doudna

Howard Hughes Medical Center
University of California, Berkeley, USA

Published February 1, 1961

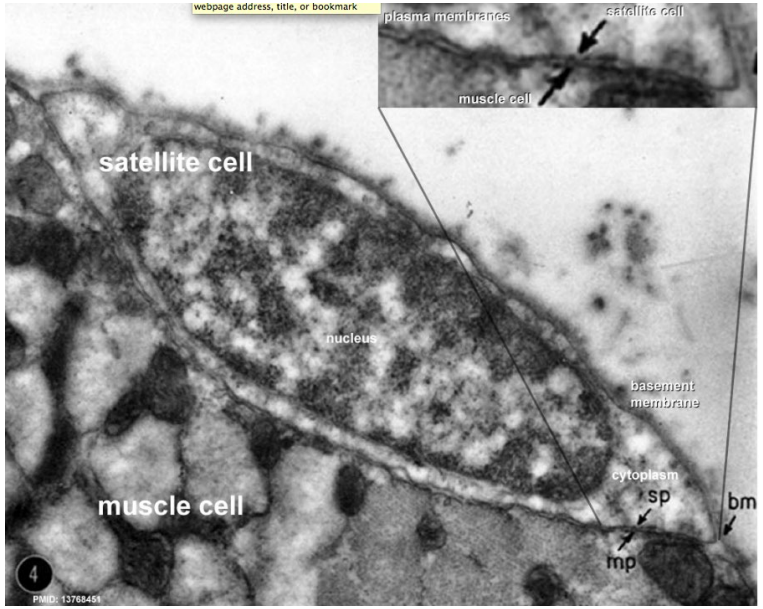
SATELLITE CELL OF SKELETAL MUSCLE FIBERS

ALEXANDER MAURO. From The Rockefeller Institute

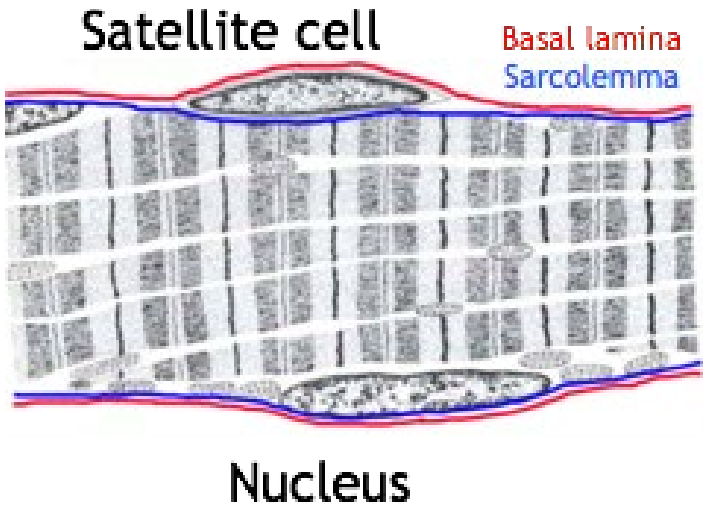
In the course of an electron microscopic study of the peripheral region of the skeletal muscle fiber of the frog, the presence of certain cells, intimately associated with the muscle fiber, have been observed which we have chosen to call *satellite cells*. Since these cells have not been reported previously and indeed might be of interest to students of muscle histology and furthermore, as we shall suggest, might be pertinent to the vexing problem of skeletal muscle regeneration, a brief communication describing this finding is warranted prior to a more detailed study.

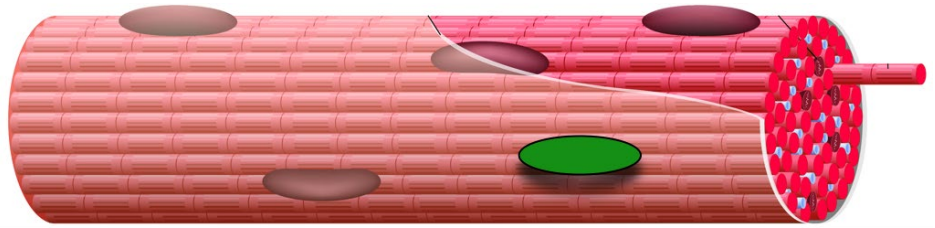
is that the peripheral muscle nuclei proper occur much more frequently than the satellite cells.

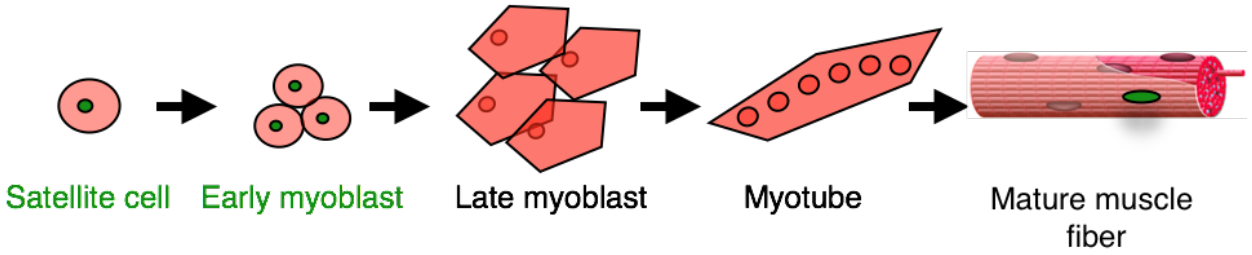
It is interesting that upon alerting other investigators to these findings, similar cells have been found in electron micrographs of two other muscles of the frog, namely sartorius (2) and ileofibularis (3), and of the sartorius and tongue muscle of the white rat (4). (Though the direct evidence is restricted to these two vertebrates, it seems reasonable to hazard a guess that skeletal muscle fibers of vertebrates in general contain satellite cells.)



J Biophys Biochem Cytol. 1961 Feb;9:493-5.


Satellite cell
Basal lamina
Sarcolemma
Nucleus




Satellite cell
Early myoblast
Late myoblast
Myotube
Mature muscle fiber

Nature, 337, 1989

Conversion of mdx myofibres from dystrophin-negative to -positive by injection of normal myoblasts

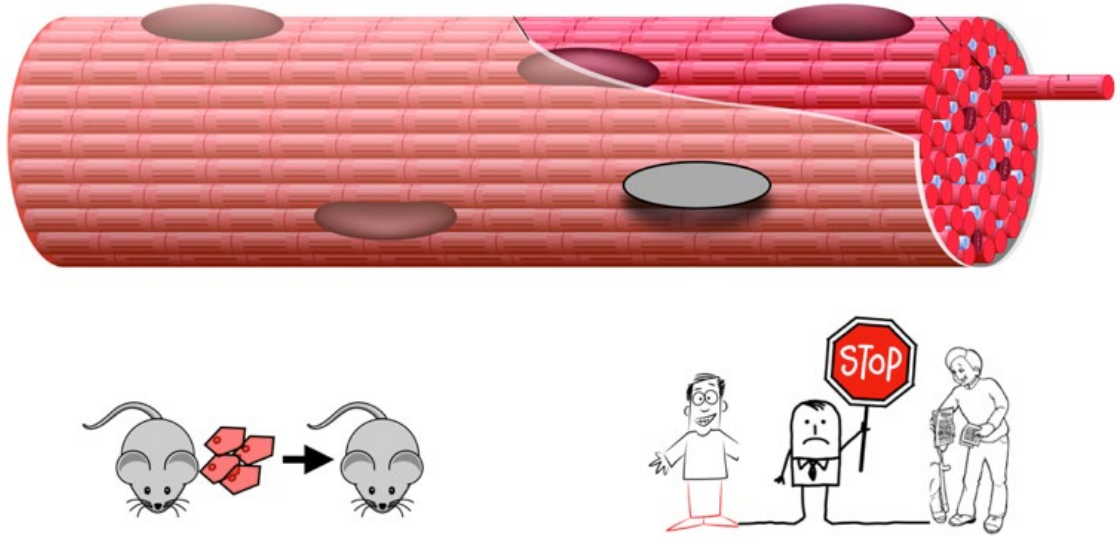
T. A. Partridge*, J. E. Morgan*, G. R. Coulton*, E. P. Hoffman† & L. M. Kunkel†

* Department of Histopathology, Charing Cross and Westminster Medical School, Fulham Palace Road, London W6 8RF, UK
† Division of Genetics, Childrens Hospital, Pediatrics, Harvard Medical School and Howard Hughes Medical Institute, Boston, Massachusetts 02115, USA

An important corollary to the recent advances in our understanding

Failure of transplantation of myoblasts for therapeutic purposes

1995



832 THE NEW ENGLAND JOURNAL OF MEDICINE Sept. 28, 1995

MYOBLAST TRANSFER IN THE TREATMENT OF DUCHENNE'S MUSCULAR DYSTROPHY

JERRY R. MENDELL, M.D., JOHN T. KISSEL, M.D., ANTHONY A. AMATO, M.D., WENDY KING, B.S., L.P.T., LINDA SIGNORE, R.N., THOMAS W. PRIOR, PH.D., ZARIFE SAHENK, M.D., SANDRA BENSON, B.A., PATRICIA E. MCANDREW, PH.D., ROBERT RICE, PH.D., HAIKADY NAGARAJA, PH.D., RALPH STEPHENS, PH.D., LAURA LANTRY, M.S., GLEN E. MORRIS, PH.D., AND ARTHUR H.M. BURGHES, PH.D.

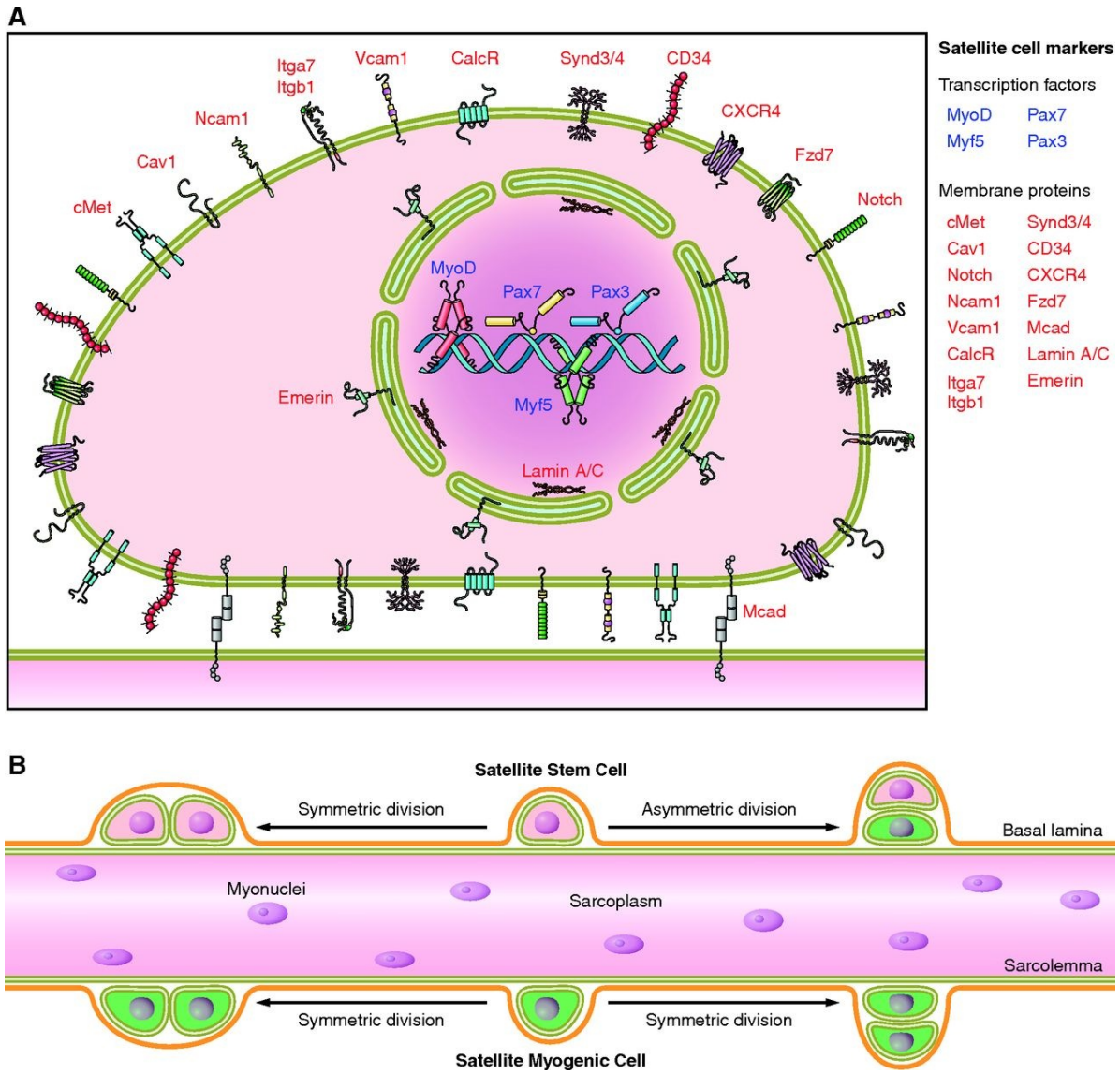
Cells with possible myogenic potential

Cell	Characteristics	Systemic delivery	+	-
Satellite cell	Under basal lamina, selfrenewal, Pax7+, CD56+, MyoD-	No	Regeneration and SC pool	No *in vivo* trials with human SCs, low numbers
MuSC	Integrin α7+, CD34+	?	Regeneration and SC pool	As Sat cells
SM Precursors	ß1-integrin+, CXCR4+, CD45-, Sca1-, Mac1-	?	++ Regenerative potential, SC replenishing	As Sat cells
Myoblasts	After Sat cell activation, MyoD+, Desmin+, Myf5+	No	Easily isolated and expanded, many human trials	Not efficient in regeneration. Limited in vitro expansion
Mesangioblasts	Blood vessel wall, Flk1+, CD34+, Sca1+, vWF-	Yes	Easy to expand, human trial in progress	In vitro myogenesis requires myoblasts
Pericytes/ MDSCs	Periphery of blood vessel, NG2, proteoglycan, ALP, PDGFRβ, CD56-	Yes	Easy to expand	Variable, limited regenerative potential
SP cells	Sca1+, ABCG2+transporter, CD45-, CD43-, Pax7-, c-kit-	?	Can be isolated from different tissues	Must be cocultured with myoblasts
CD133+	Blood or muscle tissue	Yes	Muscle regeneration better than with myoblasts	Efficient myogenesis needs myoblasts or Wnt7a+ cells
Embryonic stem cells	Derived from blastocyst	Yes	Pluripotency	Ethics, immune response, tumorigenic
IPS (Nobel prize 2012)	Can be obtained from many tissues. Oct3/4+, Sox-, c-Myc+, klf4+, Nanong+	Yes	Pluripotency	Genetic manipulation tumorigenicity, risk of viral infection
MSCs	Many tissues, CD34-, CD45-, CD73+, CD90+, CD105+, CD117+	Yes	Readily available, autologous	Delivery unclear, limited long-term therapeutic contribution
PW1-interstitial cells	Muscle interstitial cells. PW1+	?	Contribute to muscle regeneration, SC and interstitial population	No information on human cells

2011: No satellite cells ➽ No muscle regeneration!

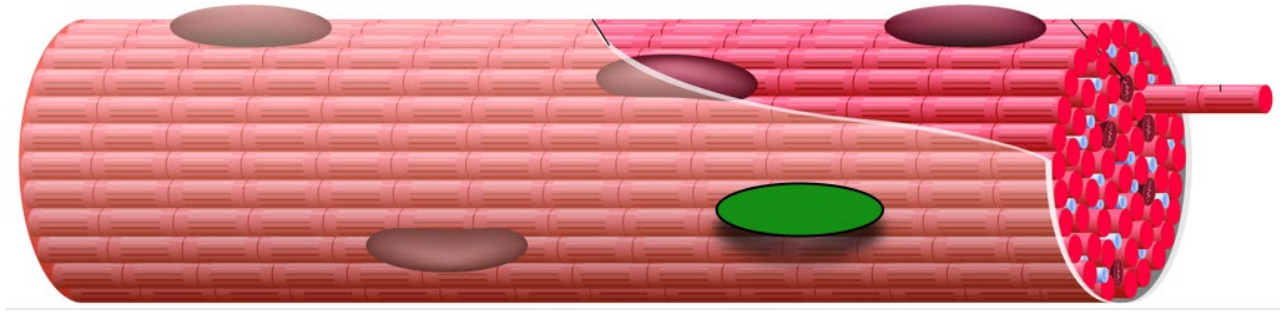
- Lepper C, Partridge TA, Fan CM. An absolute requirement for Pax7-positive satellite cells in acute injury-induced skeletal muscle regeneration. Development 138: 3639-3646; 2011
- Sambasivan R, Yao R, Kissenpfennig A, Van Wittenberghe L, Paldi A, Gayraud-Morel B, Guenou H, Malissen B, Tajbakhsh S, Galy A. Pax7-expressing satellite cells are indispensable for adult skeletal muscle regeneration. Development. 138: 3647-3656; 2011
- Relaix F, Zammit PS. Satellite cells are essential for skeletal muscle regeneration: the cell on the edge returns centre stage. Development 139: 2845-2856; 2012

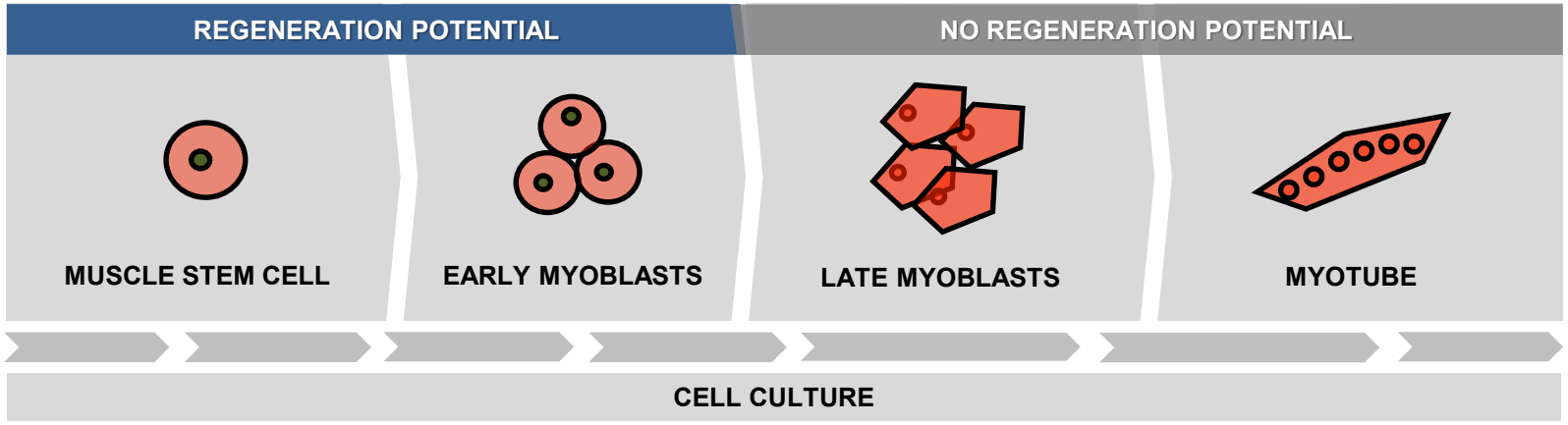
Characterization of satellite cells



Yin H et al. Physiol Rev 2013;93:23-67

The muscle stem cell: satellite cell

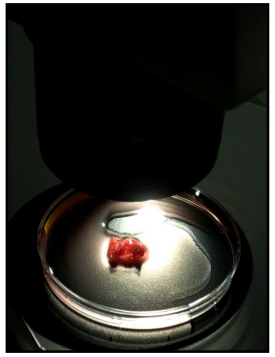




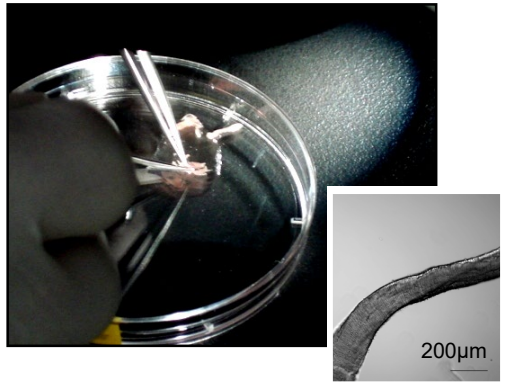
Muscle regeneration depends on satellite cells

Pax7+ satellite cells enrich within the human myofiber fragment

- Human skeletal muscle biopsy specimen
- 5^3mm

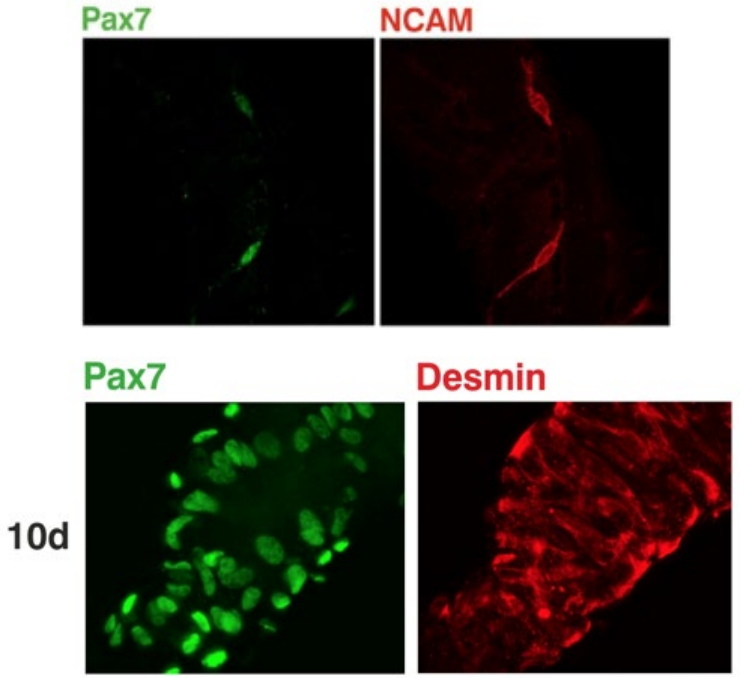


- Manual dissection into fiber fragments
- **No** enzymatic digest

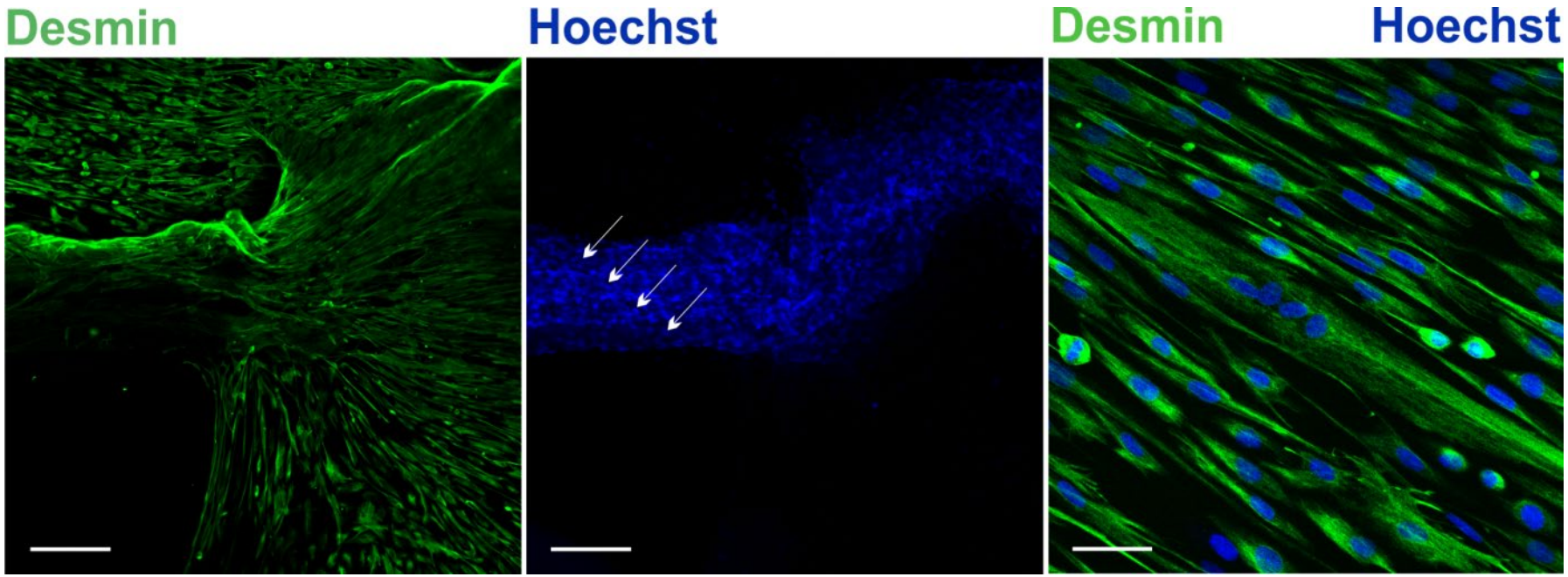


Human muscle fiber fragment 7 days in culture

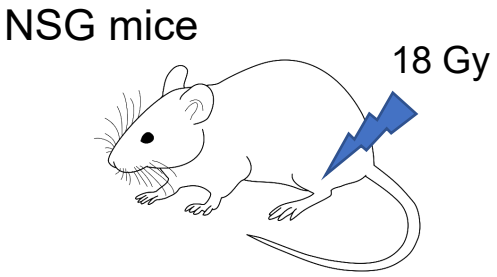
Enrichment of PAX7 positive cells within the fragment



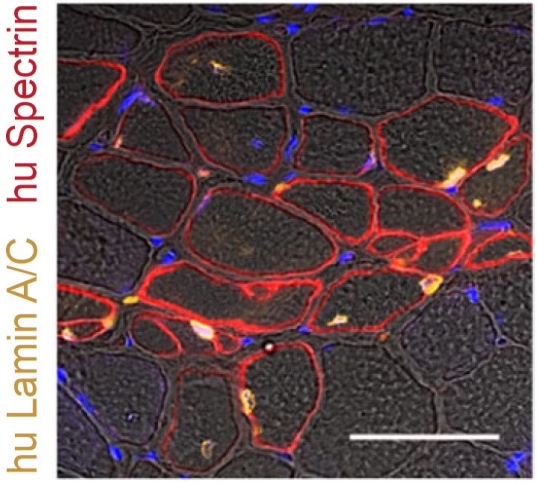
Myoblasts form colonies outside
the fiber fragments and fuse to myotubes

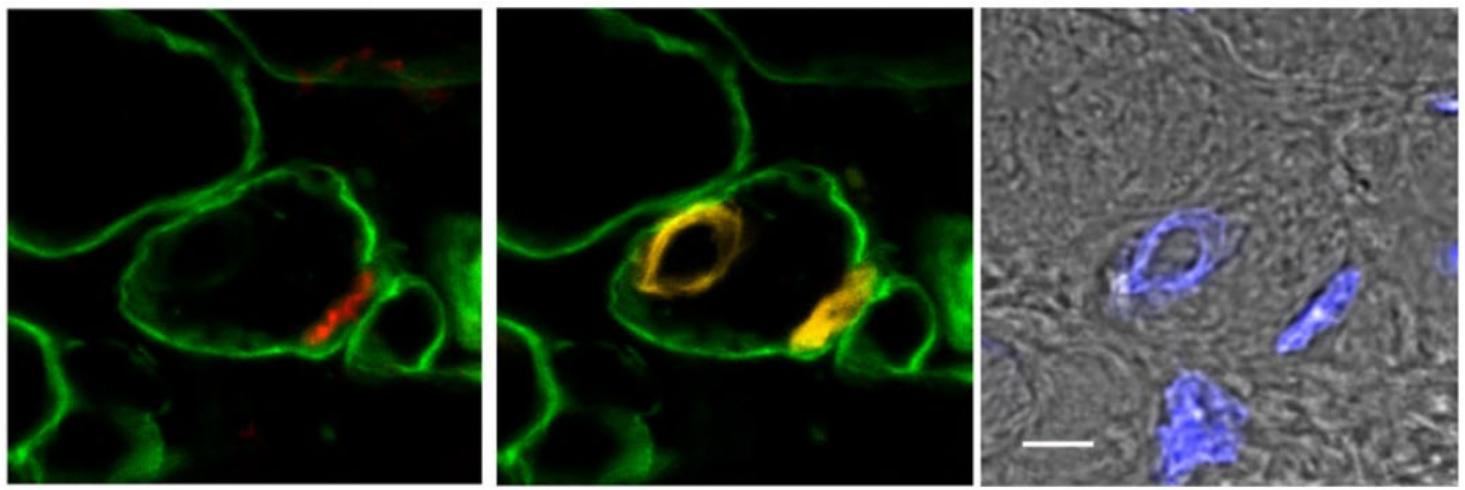


Proof: *Ex vivo* expanded human satellite cells regenerate muscle *in vivo*

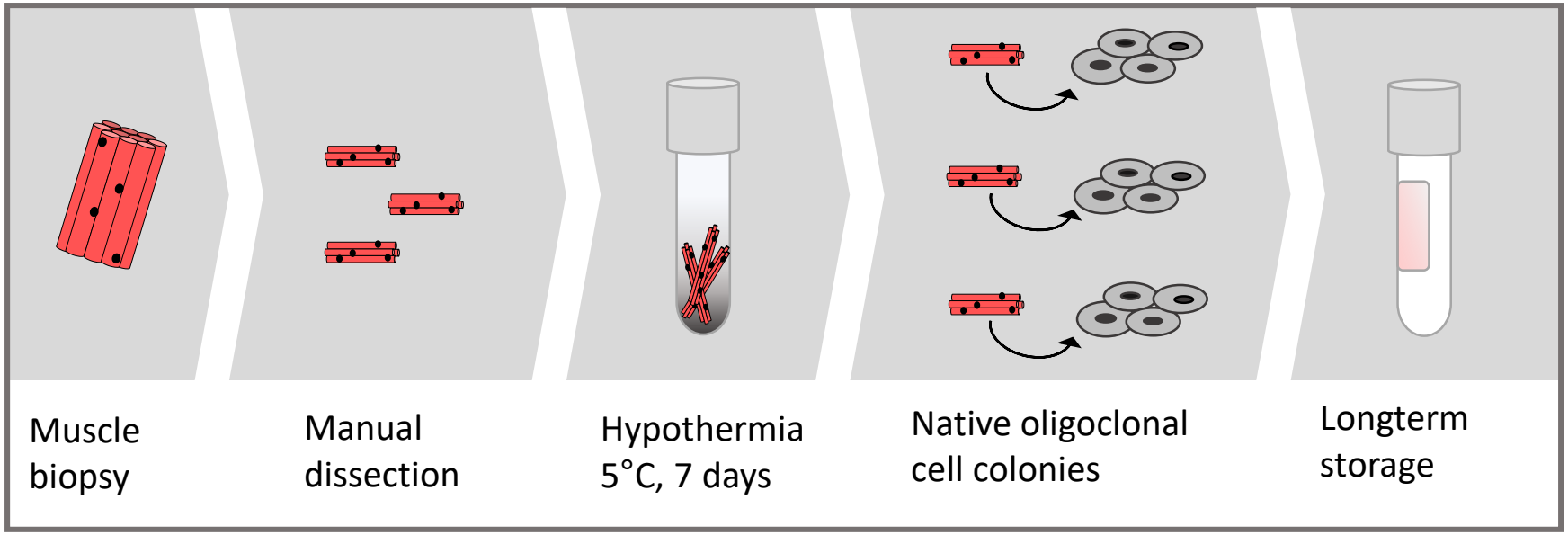


Marg et al., *Nat Commun*, 2019





Our innovation: new isolation and cultivation technique for human muscle stem cells



- 100% myogenic cells
- High regenerative potential

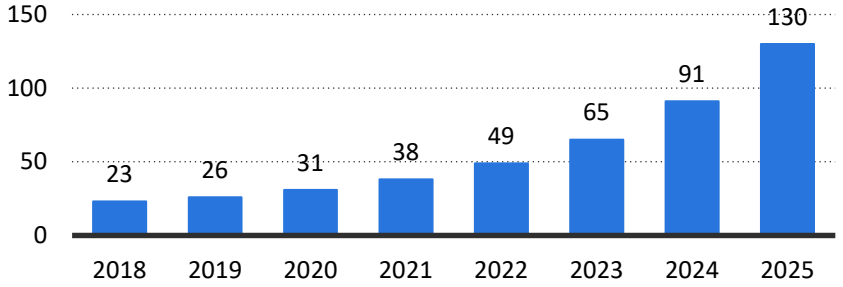
- IP: Charité/MDC (DE10 2014 216872), 2015 PCT (WO 2016/030371), since 2017 national phase EU, US, JPN
- Marg et al., *J Clin Invest,* 2014
- Marg et al., *Nature Communications*, 2019

Advanced Therapy Medicinal Products (ATMP): young and growing market

ATMPS IN THE EU MARKET

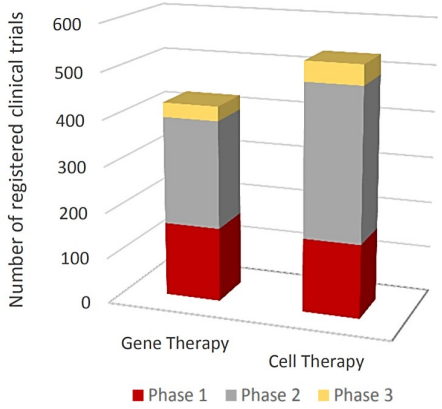
ATMP classification	Product	Market approval		Orphan design.
		EMA	FDA	
GMTP	Glybera®	2012-2017*		X
	Strimvelis®	2016		X
	Kymriah®	2018	2018	X
	Yescarta®	2018	2017	X
	Imlygic®	2015	2015	X/-
	Luxturna®	2018	2017	X
	Zynteglo®	2019		X
TEP (autologous)	Holoclar®	2015		X
	MACI®	2013-2014*	2016	
	ChondroCelect®	2009-2016*		
	Spherox®	2017		
sCTMP	Zalmoxis®	2016		X
	Provenge®	2013-2015*	2010	
	Alosifel®	2018		X

FORECAST OF WORLDWIDE REVENUE FROM REGENERATIVE MEDICINE NEXT YEARS (IN BILLION €)



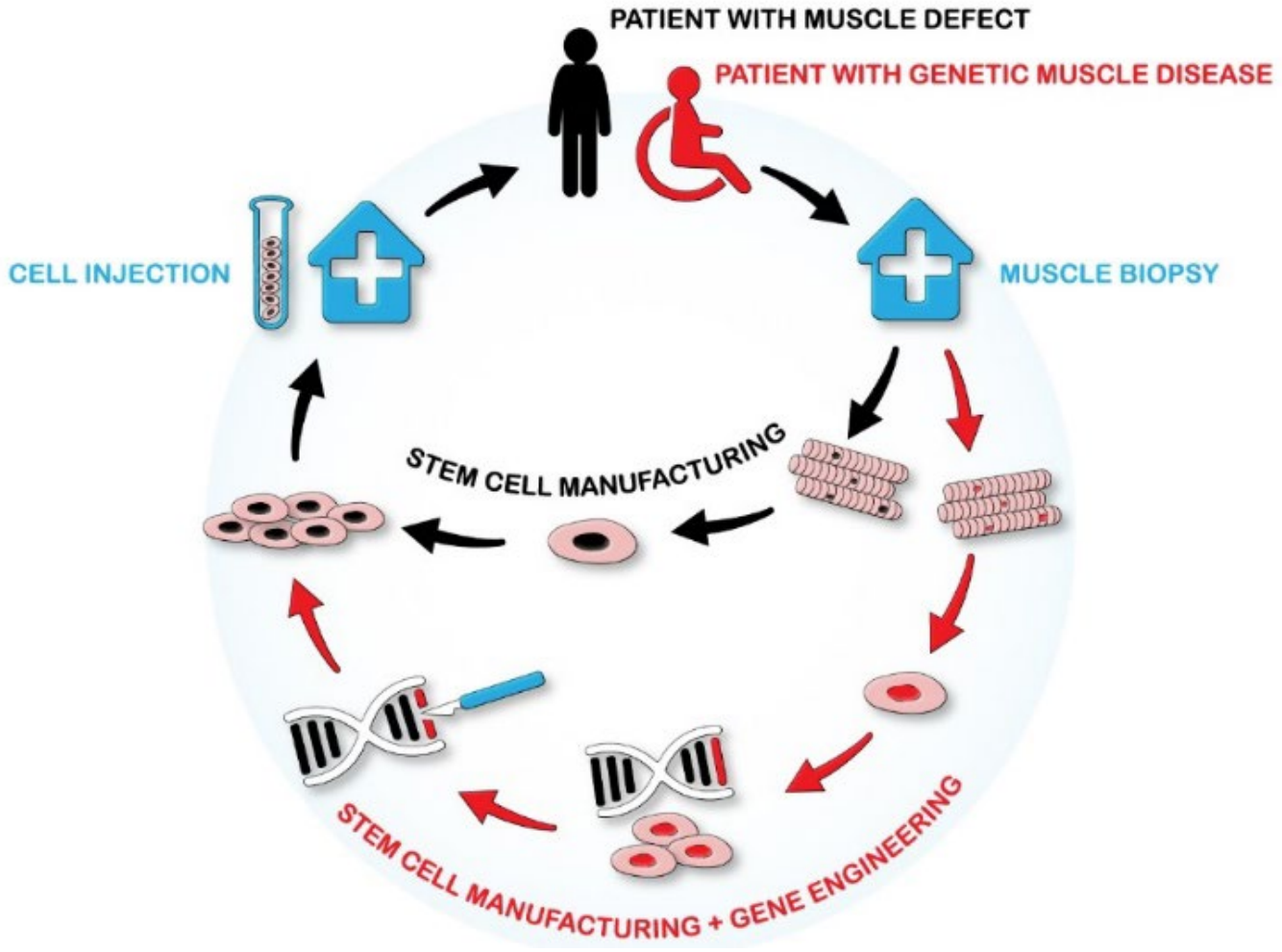
Roland Berger: Focus regenerative medicine. "The next generation of therapeutic products is set to shake up the pharmaceutical world." Page 5 https://www.rolandberger.com/publications/publication_pdf/roland_berger_regenerative_medicine.pdf/

>1000 ATMPS CURRENTLY IN CLINICAL STUDIES



- * **First approved ATMPs** were **not successful** on the EU market: Difficulties in national pricing negotiations, competing products
- **Newer ATMPs** are primarily targeting **rare diseases** (orphan designation).

Our concept for developing treatments for muscular dystrophy



1st in human clinical trial using primary human muscle stem cells as ATMP

Indication: Epispadias

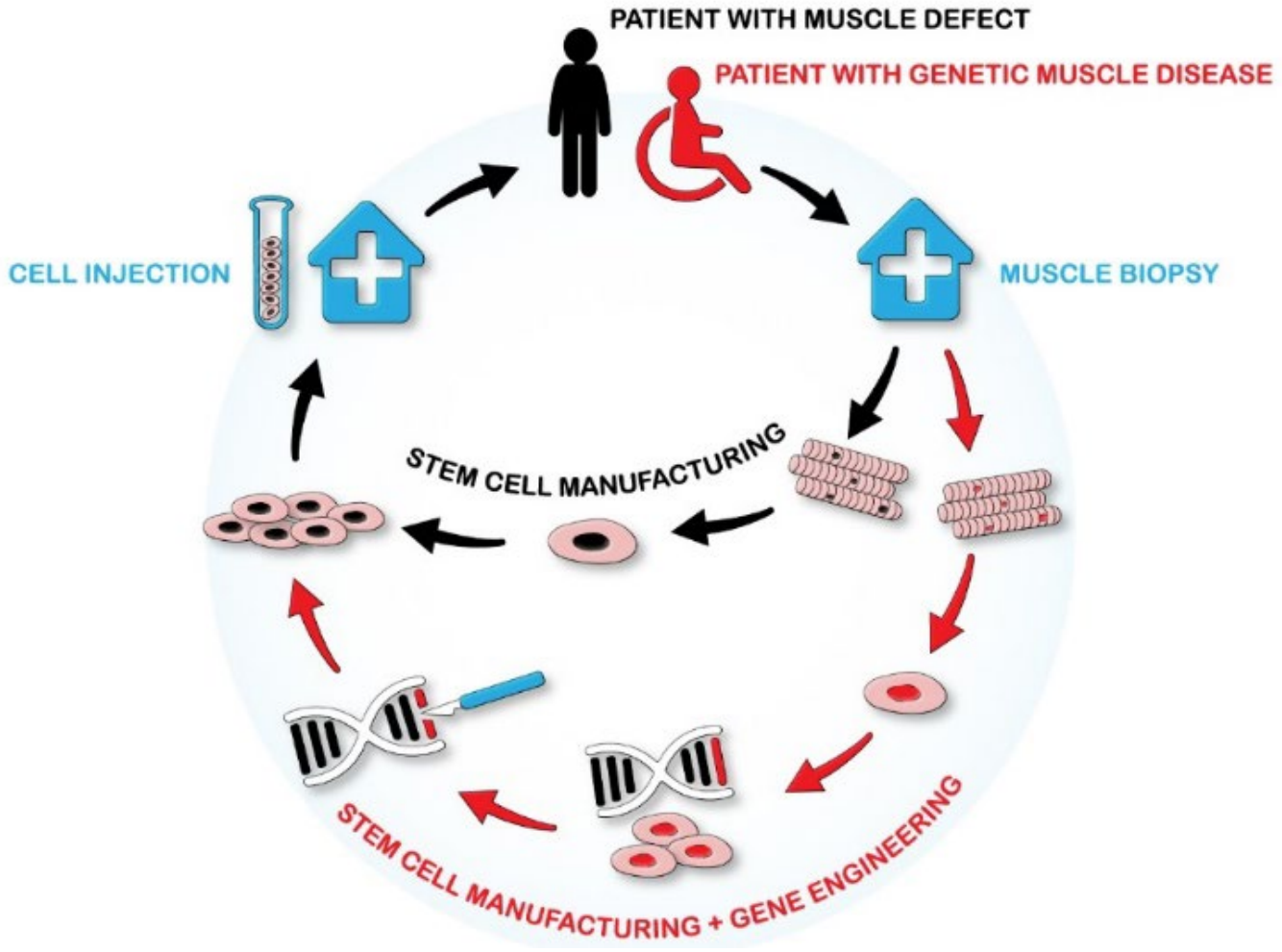
POC: yes, uncontrolled design
Kajbafzadeh, 2008, 2011

Preclinic: Supported by BIH/SPARK

Trial: Financed by BMBF (from 5.2021)

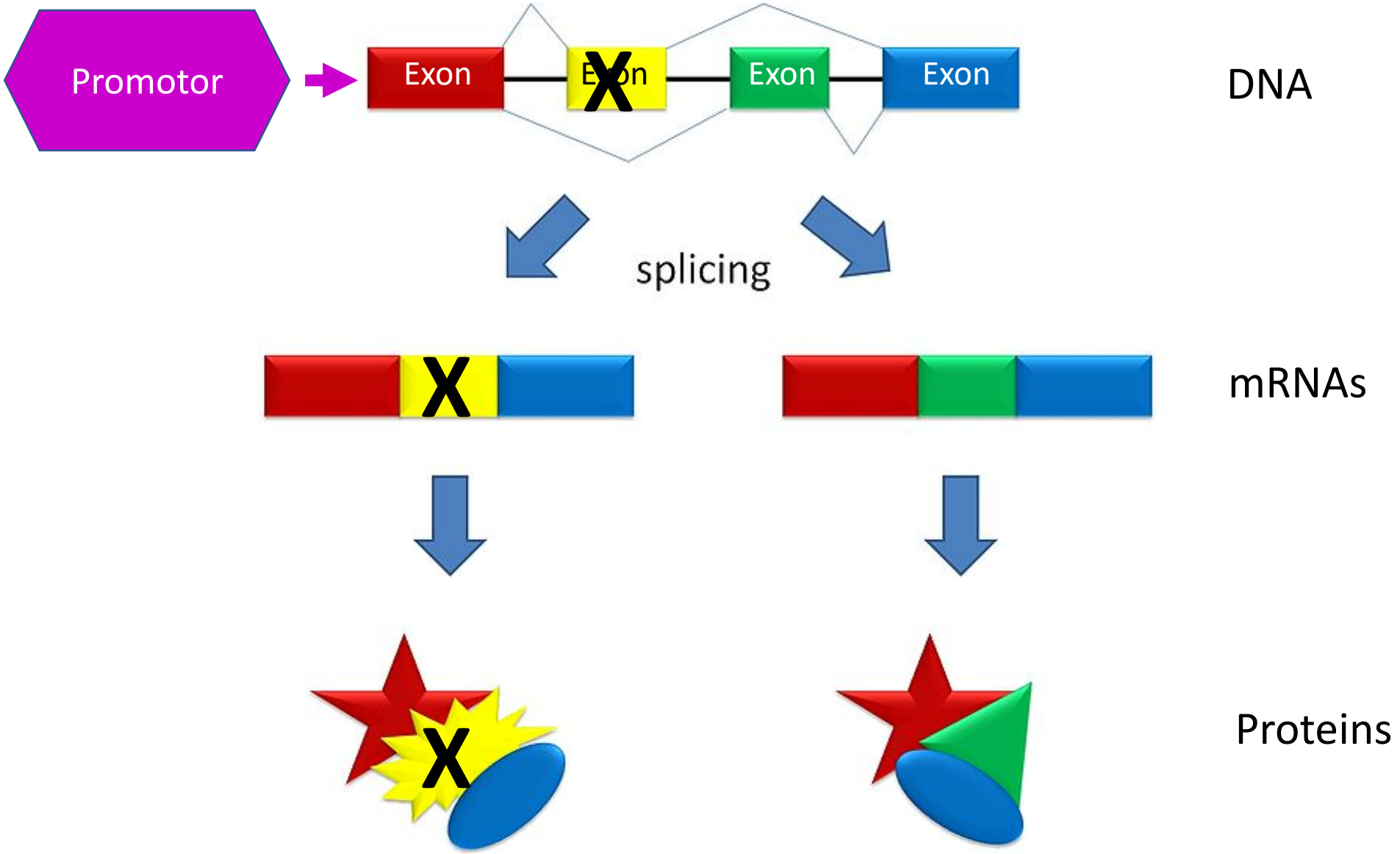
Timeframe: 2022-2025

Our concept for developing treatments for muscular dystrophy

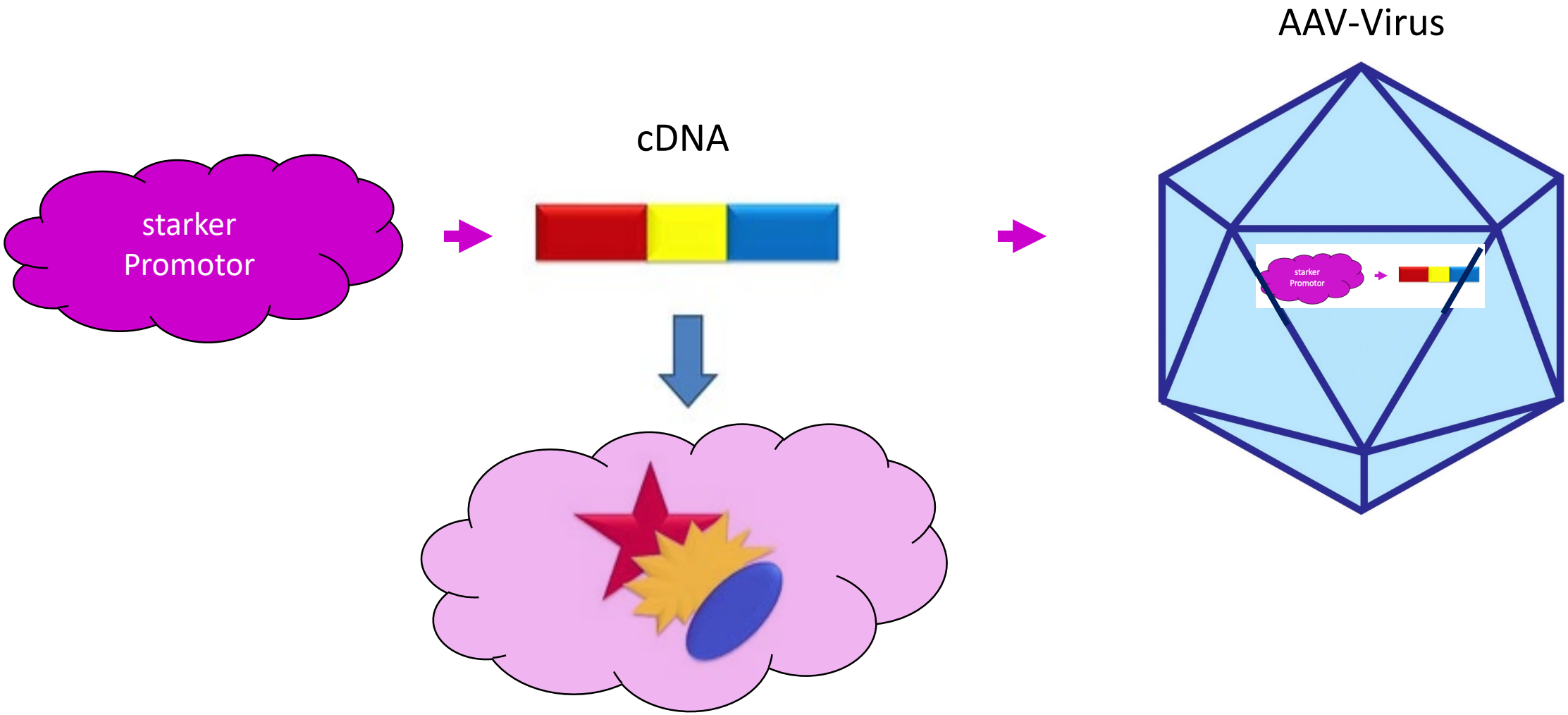


„Gene therapy“ I: Exon-Skipping and Stopcodon-readthrough

X = Mutation



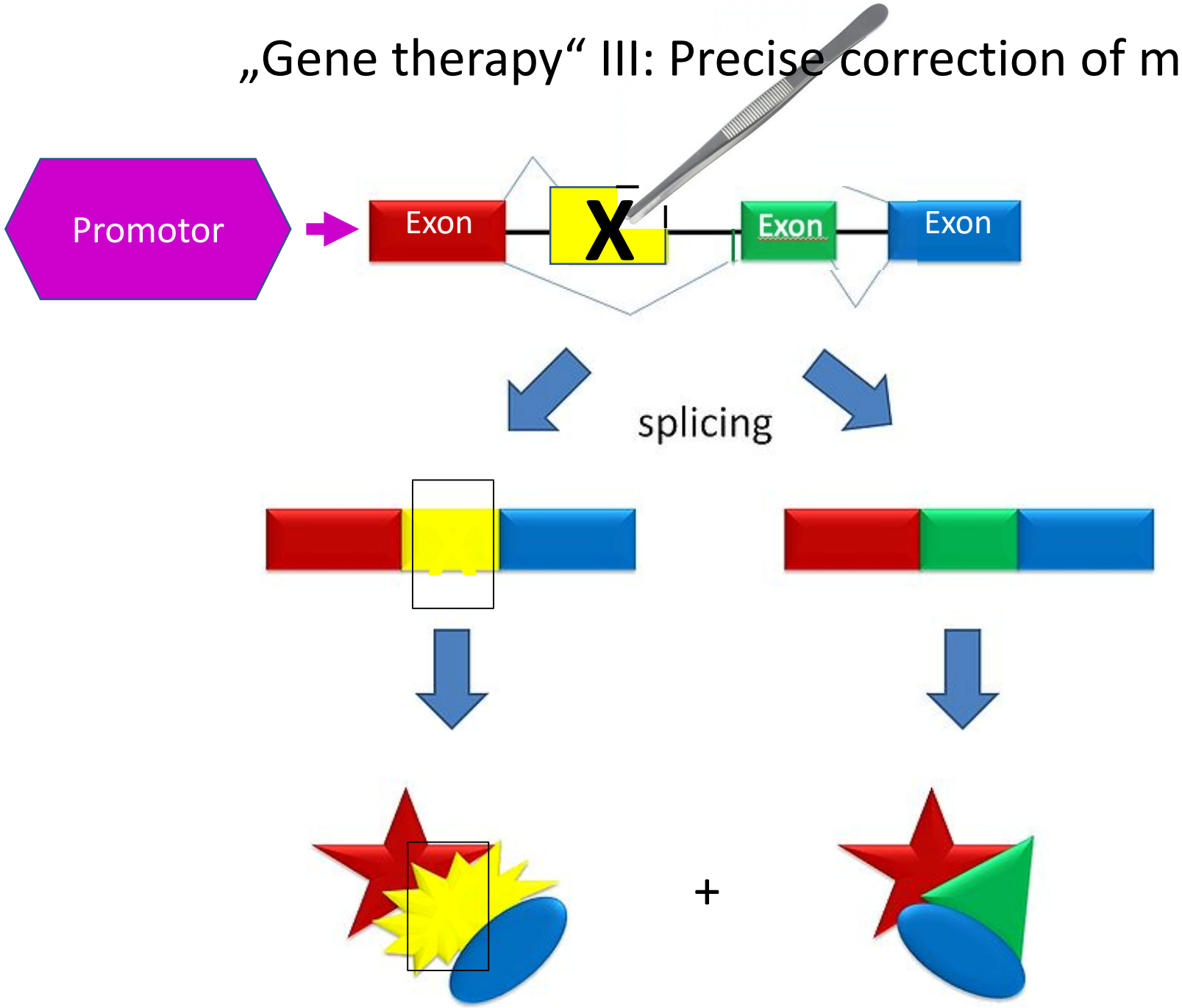
„Gene therapy“ II: Additional cDNA copy



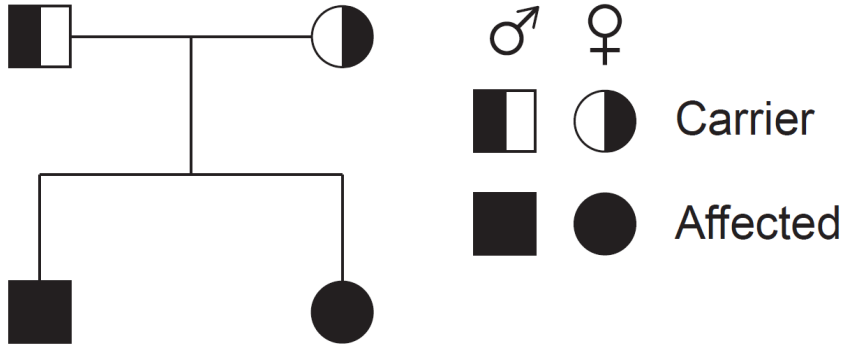
„Gene therapy“ II: Additional cDNA copy

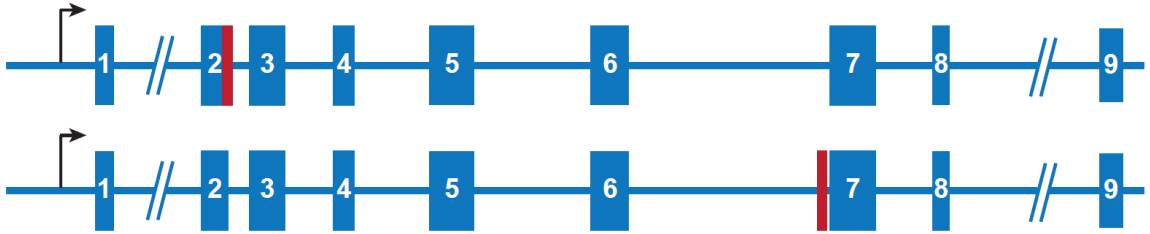
Sarepta Therapeutics: Muscular dystrophy trial for SGCB mutations

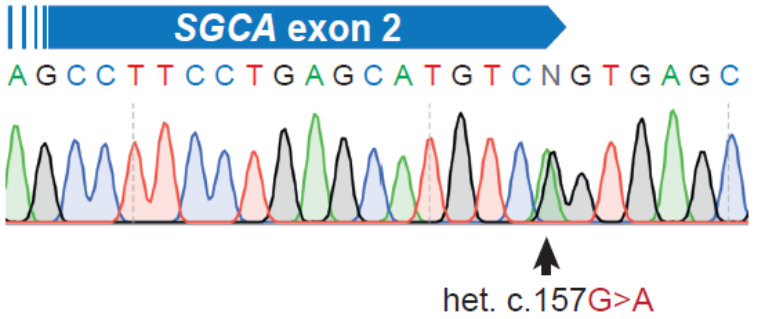
„Gene therapy“ III: Precise correction of mutation

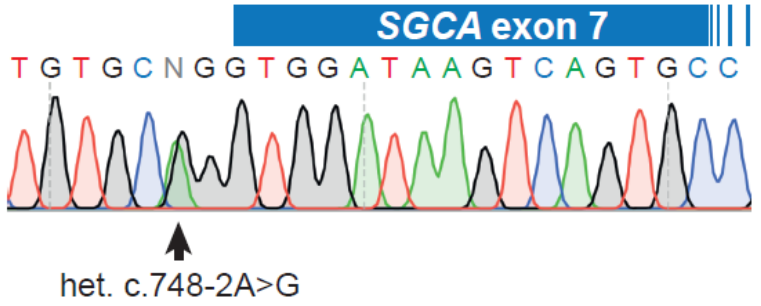


Family with sarcoglycanopathy









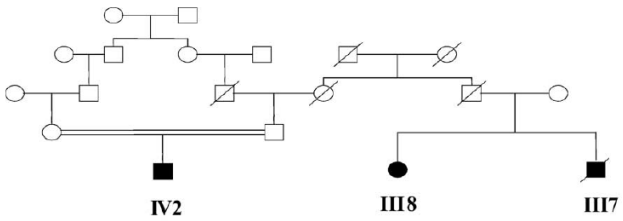
Age at onset in both: 7-8

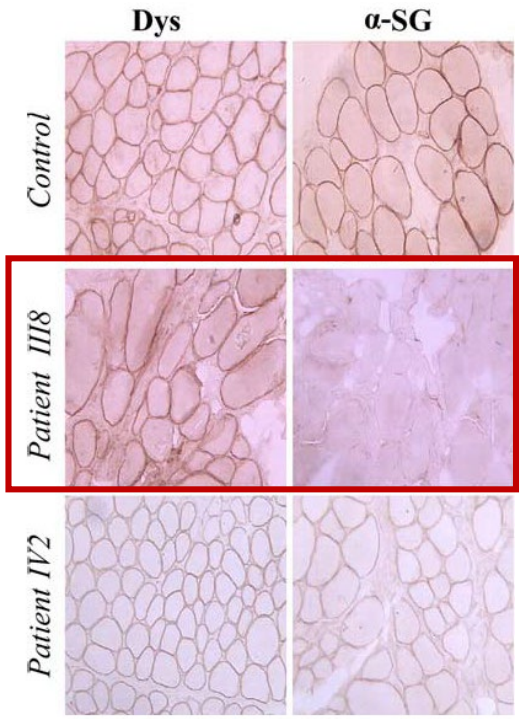
Wheelchair bound: 13-15 years of age

No cardiac involvement so far

Both mutations were previously reported

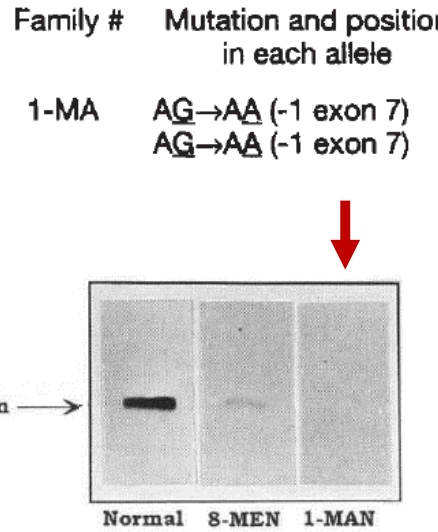
Fendri et al., 2006 *Neuromusc Disorders*

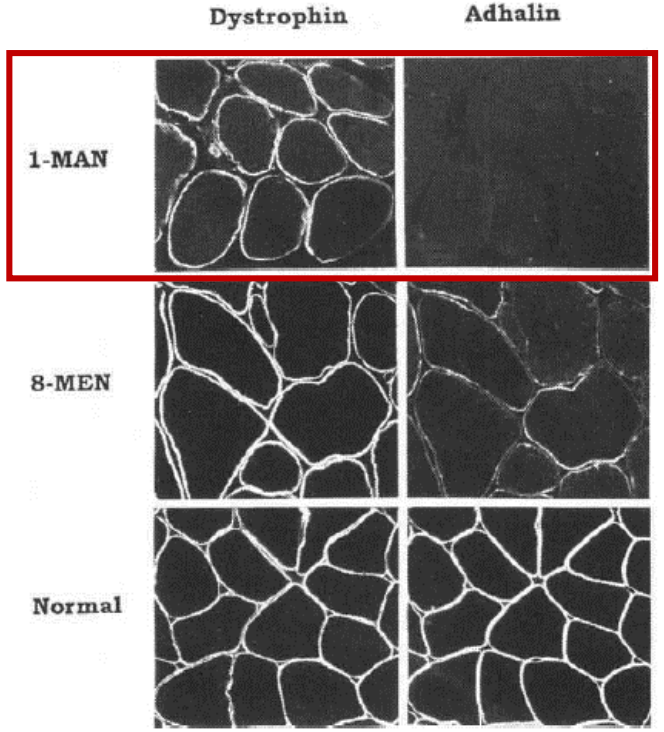




p.Ala53Thr, *missense*

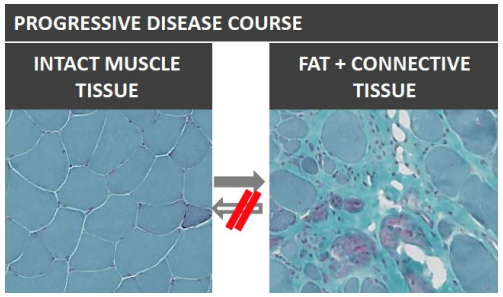
Piccolo et al., 1995 *Nat Genet*

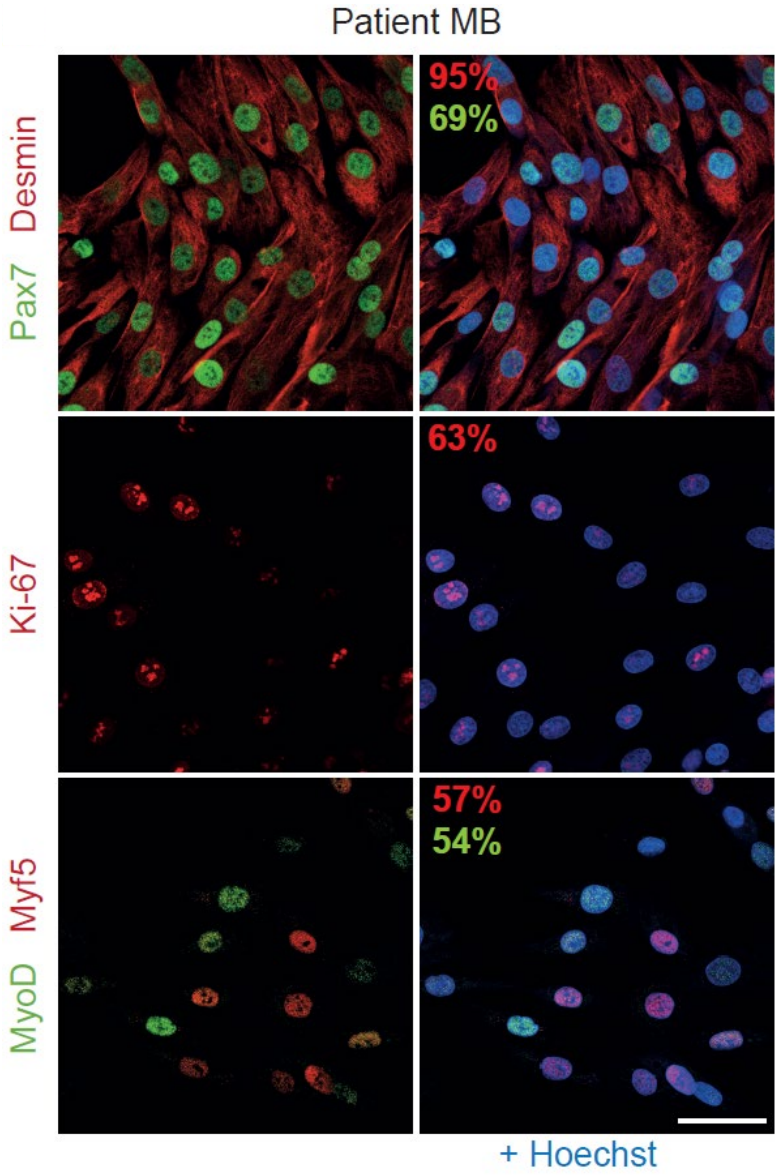


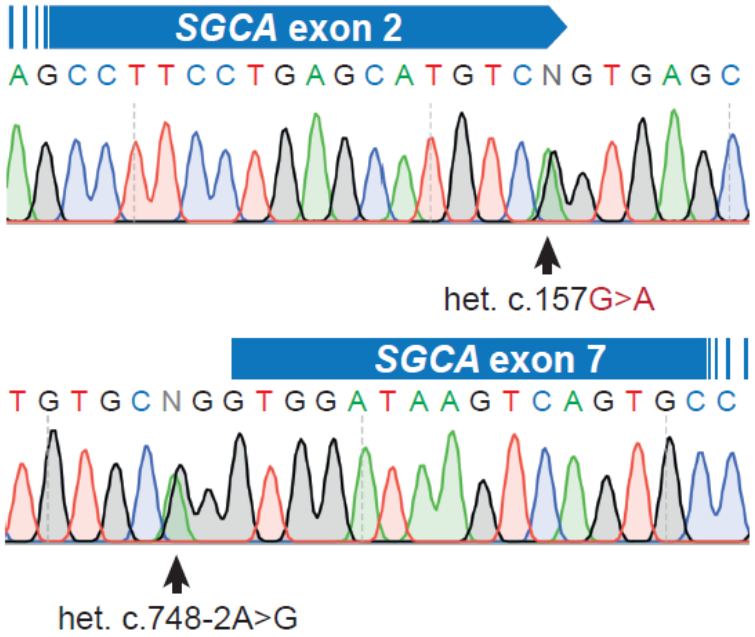


Aberrant splicing

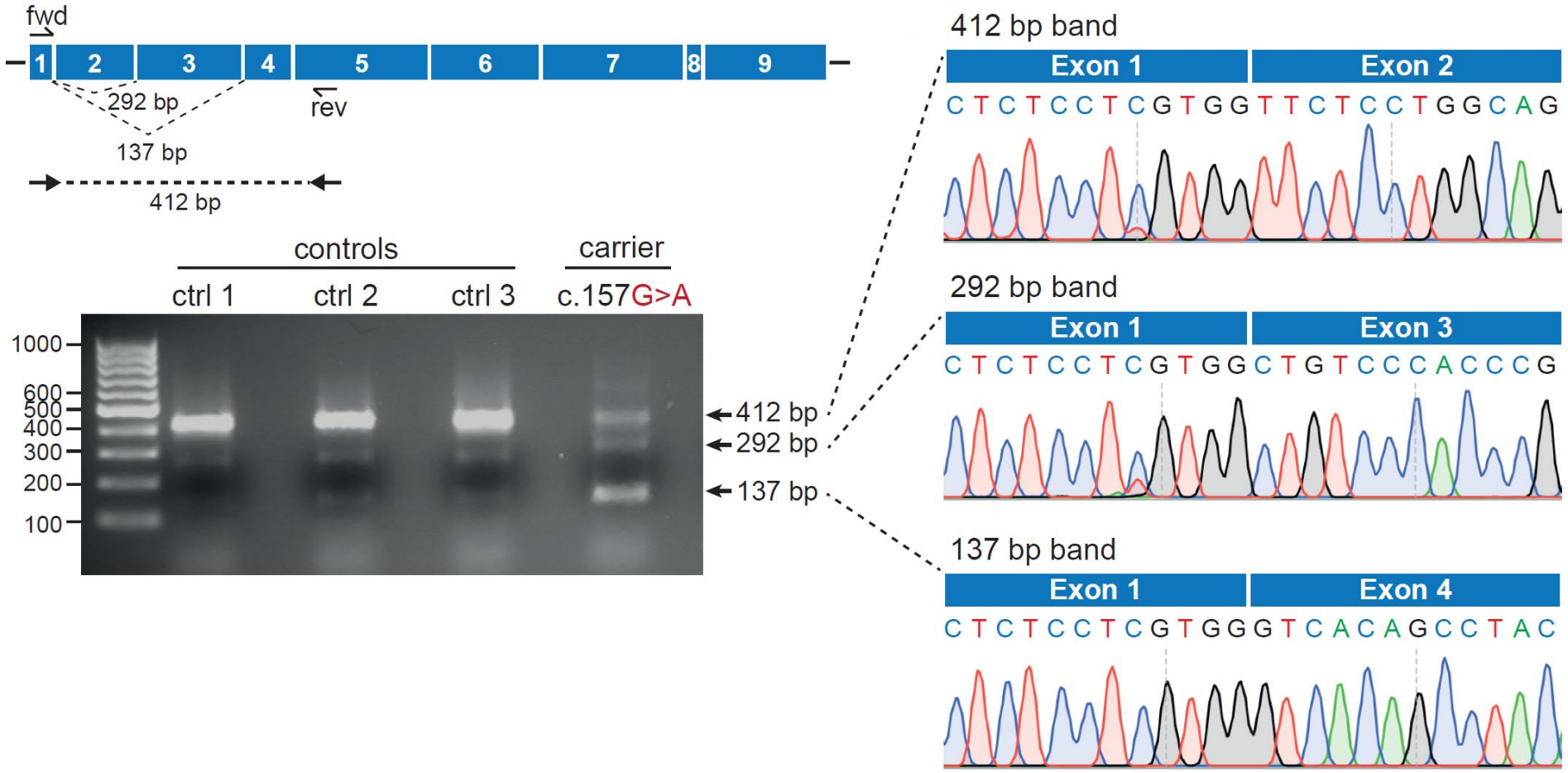
95% pure myoblasts were obtained from the LGMD2D patient



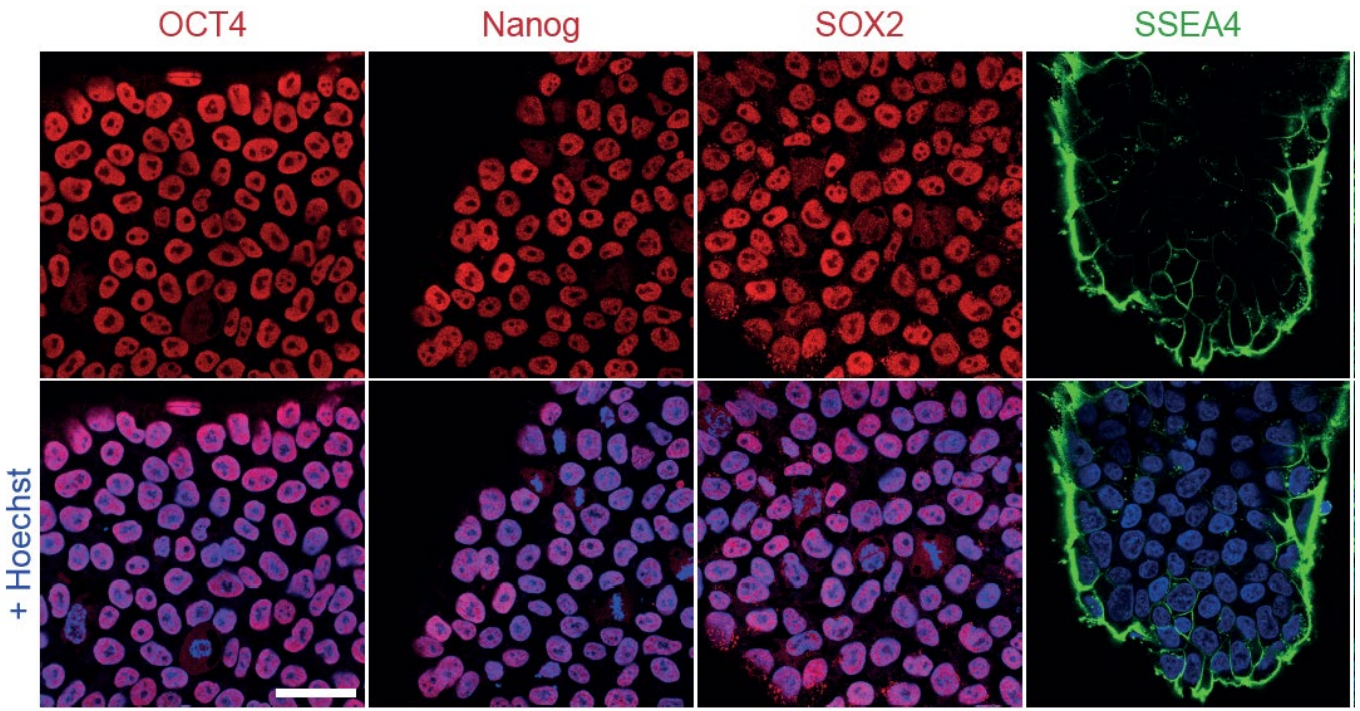


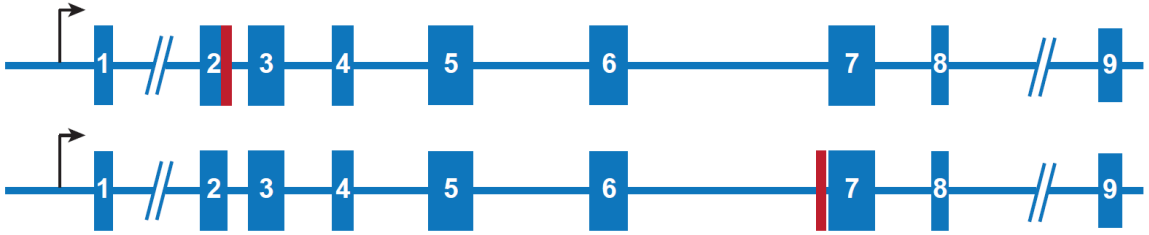


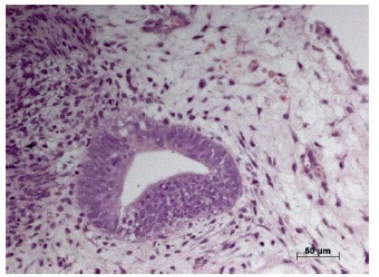
SGCA c.157G>A induces co-skipping of exons 2 and 3



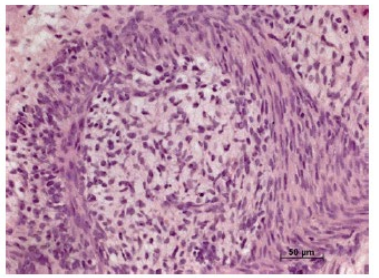
Generation and characterization of patient iPSC



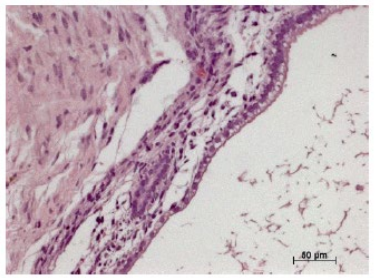




Ectoderm
Beginning rosette formation, beginning stratification

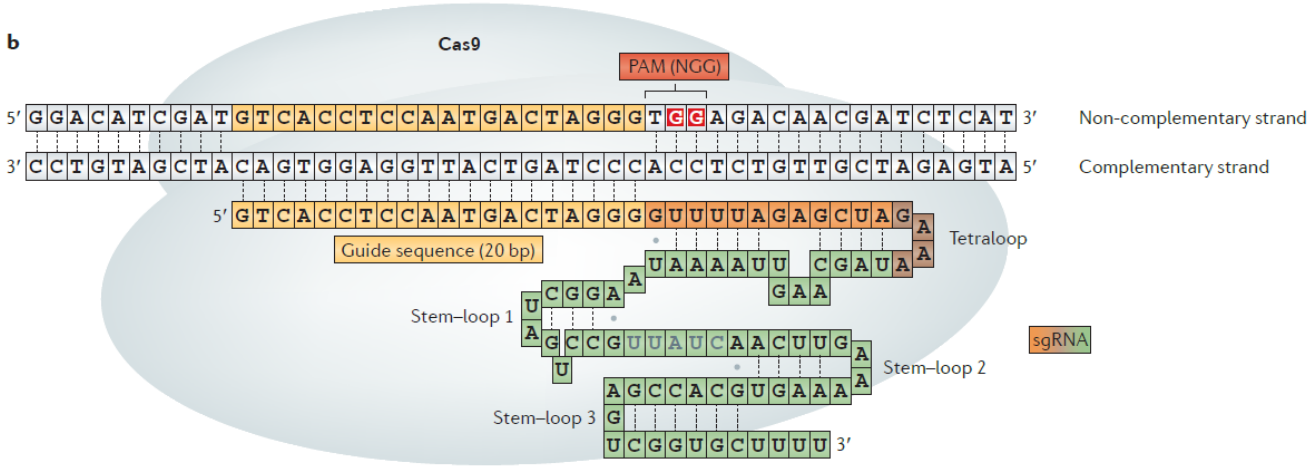


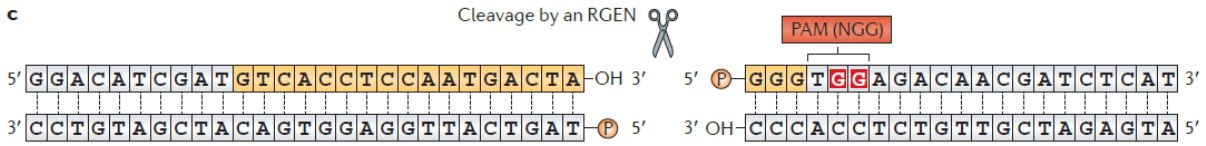
Mesoderm
Immature mesenchyme

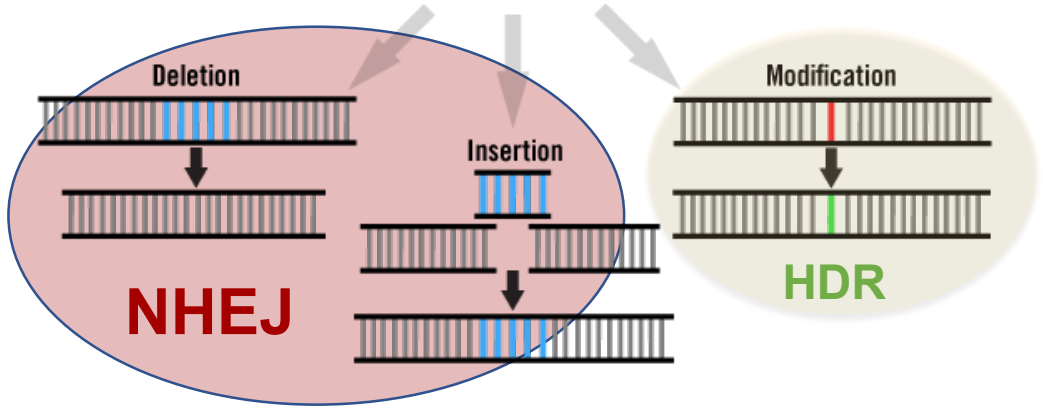


Endoderm
Cuboidal vacuolated epithelial cells, lining cyst-like space

Genome editing with CRISPR/Cas systems

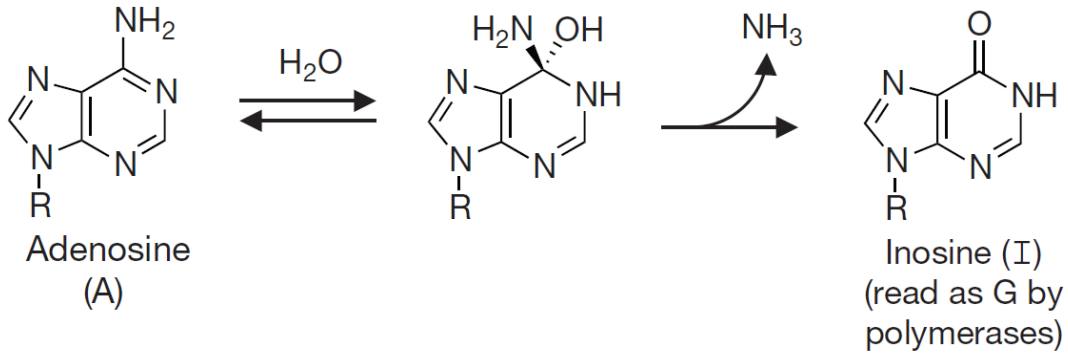




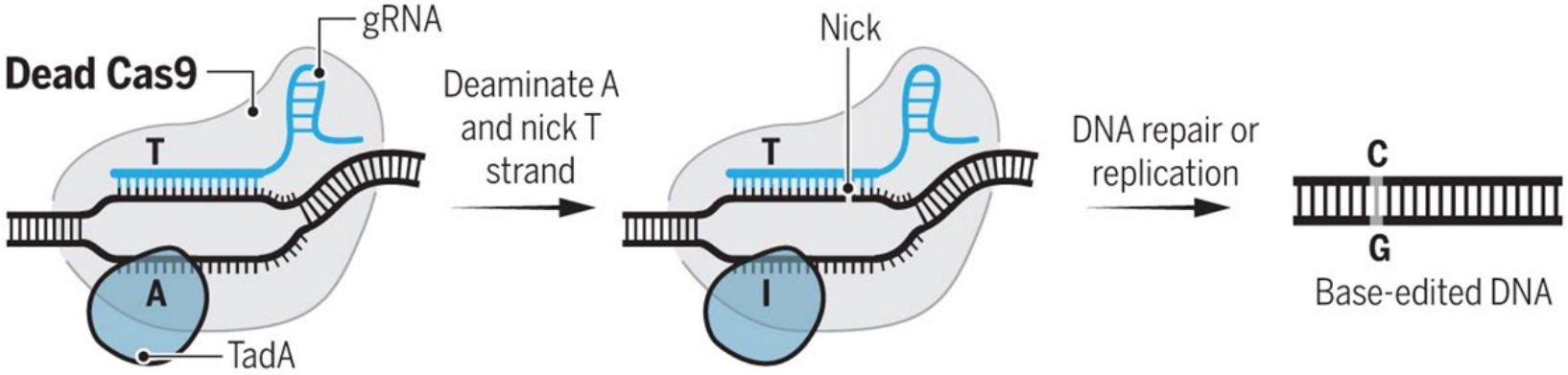


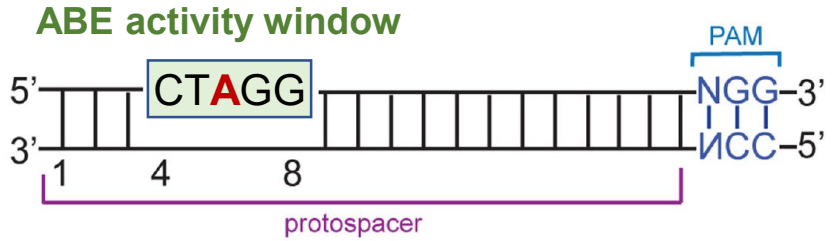
Kim et al., 2014 *Nat Rev Gen*

Adenine Base Editing (ABE) for precise A>G nucleotide conversions

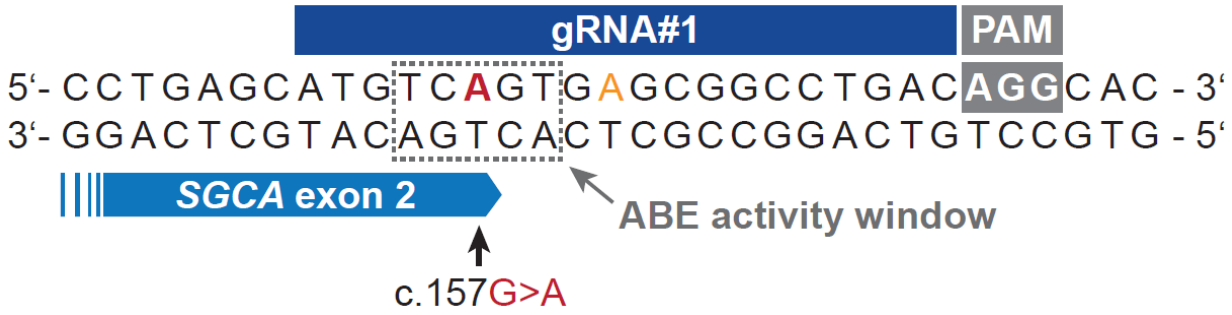


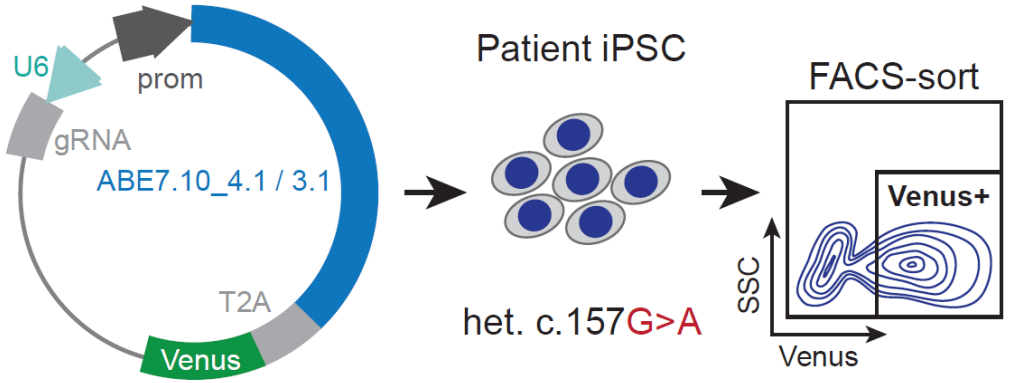
Gaudelli et al., 2017 *Nature*



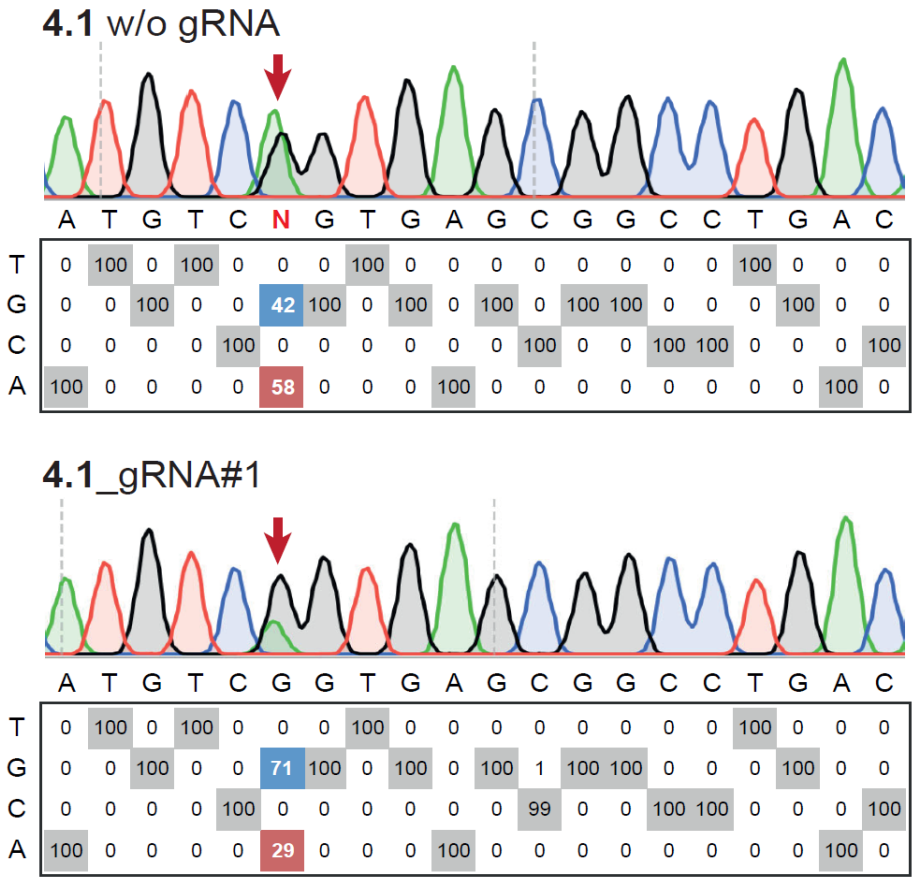


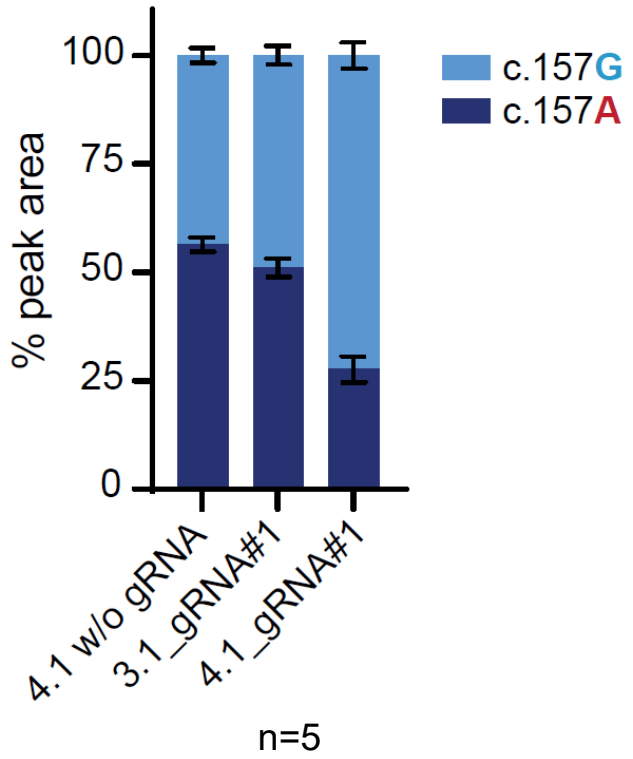
SGCA c.157G>A mutation is an ideal candidate for ABE



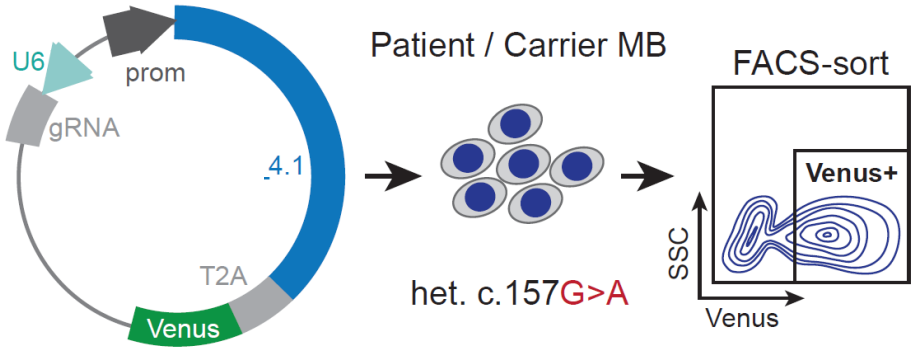


ABE repairs the *SGCA* c.157G>A mutation in patient iPSC

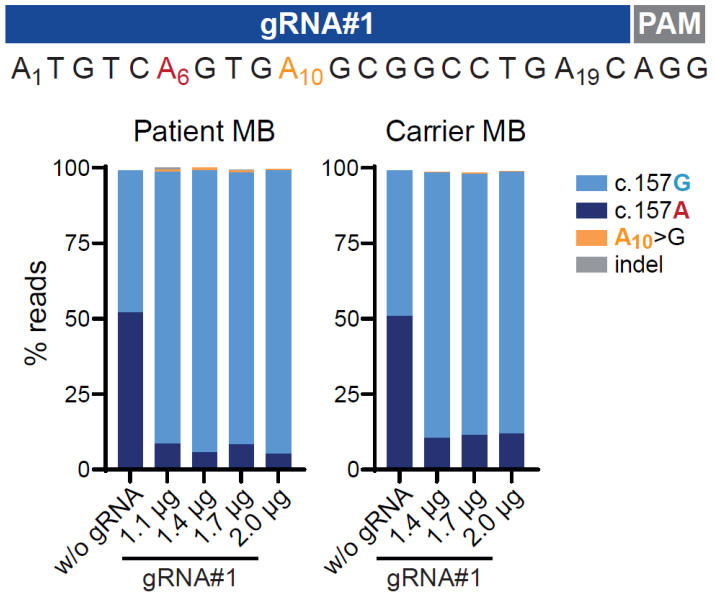




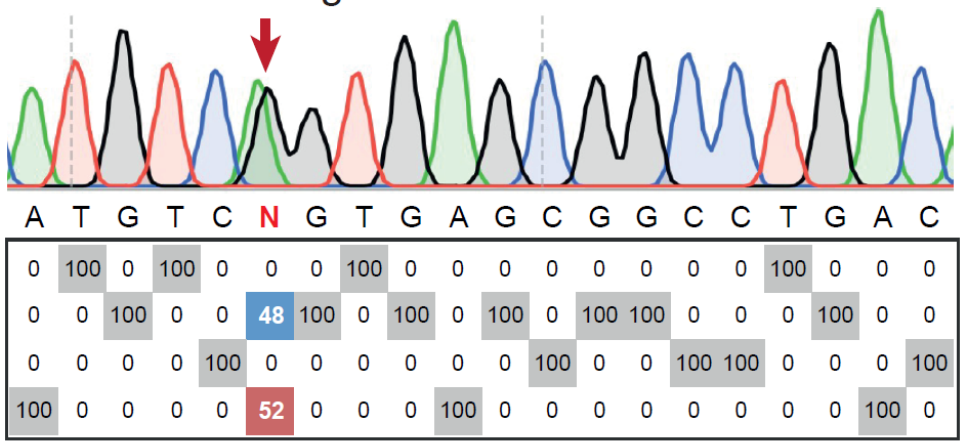
Highly efficient repair of *SGCA* c.157G>A in PHSats (primary human myoblasts)



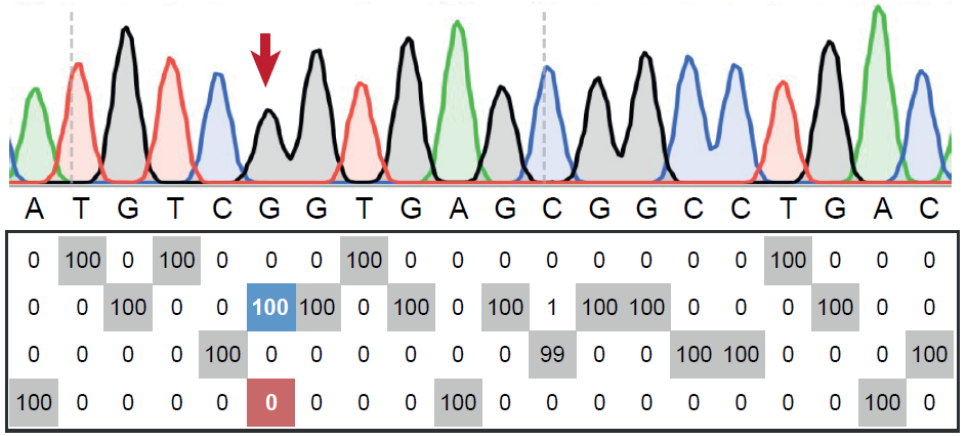
No editing of predicted off-target sites



Patient MB - w/o gRNA

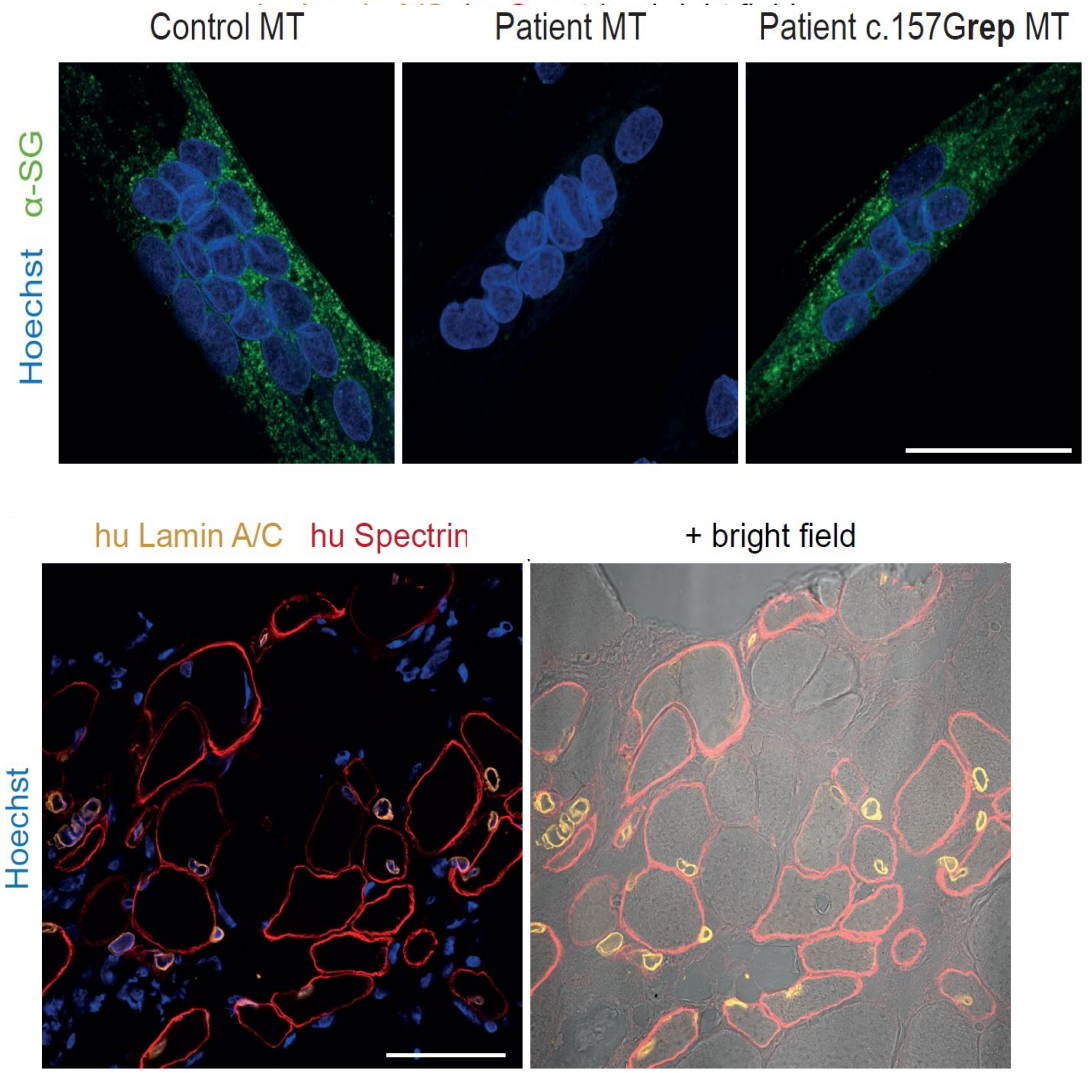


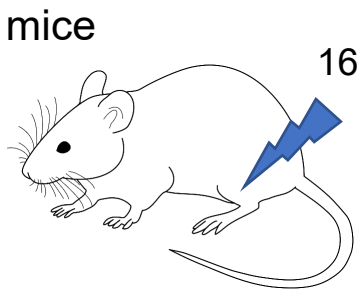
Patient MB - gRNA#1

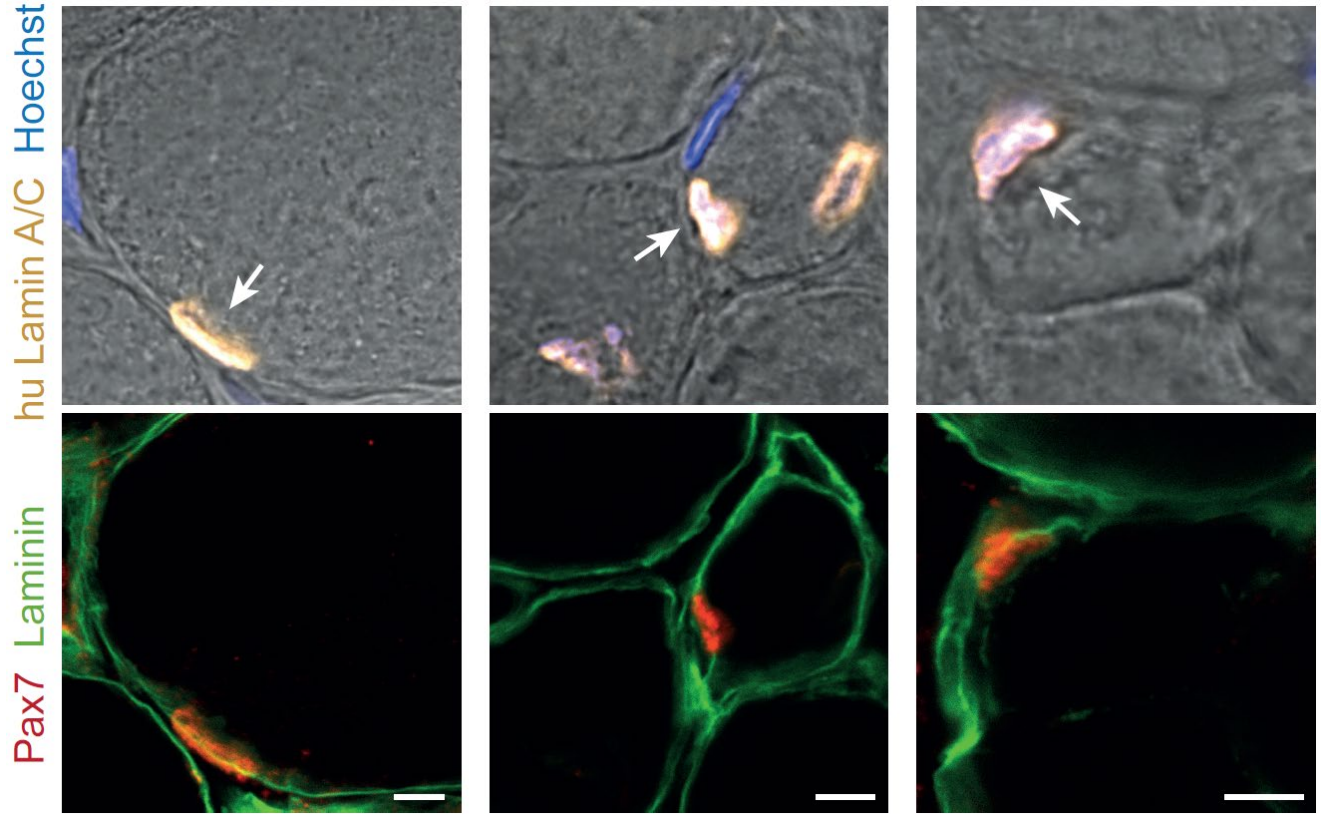


Escobar 2021 *J Clin Inv Ins*

SGCA c.157G**rep** express sarcoglycan, give rise to human myofibers *in vivo* and make new muscle stem cells

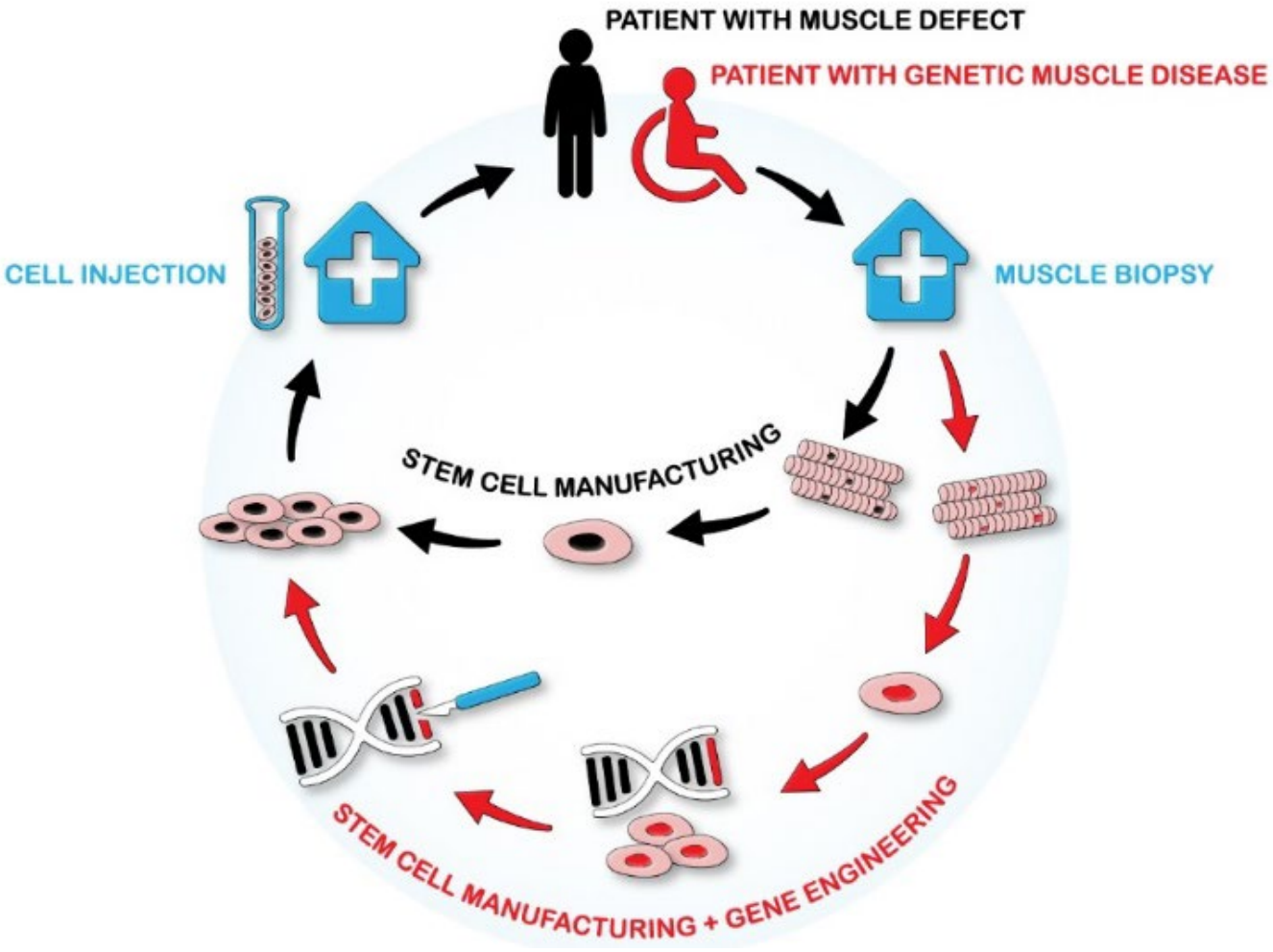




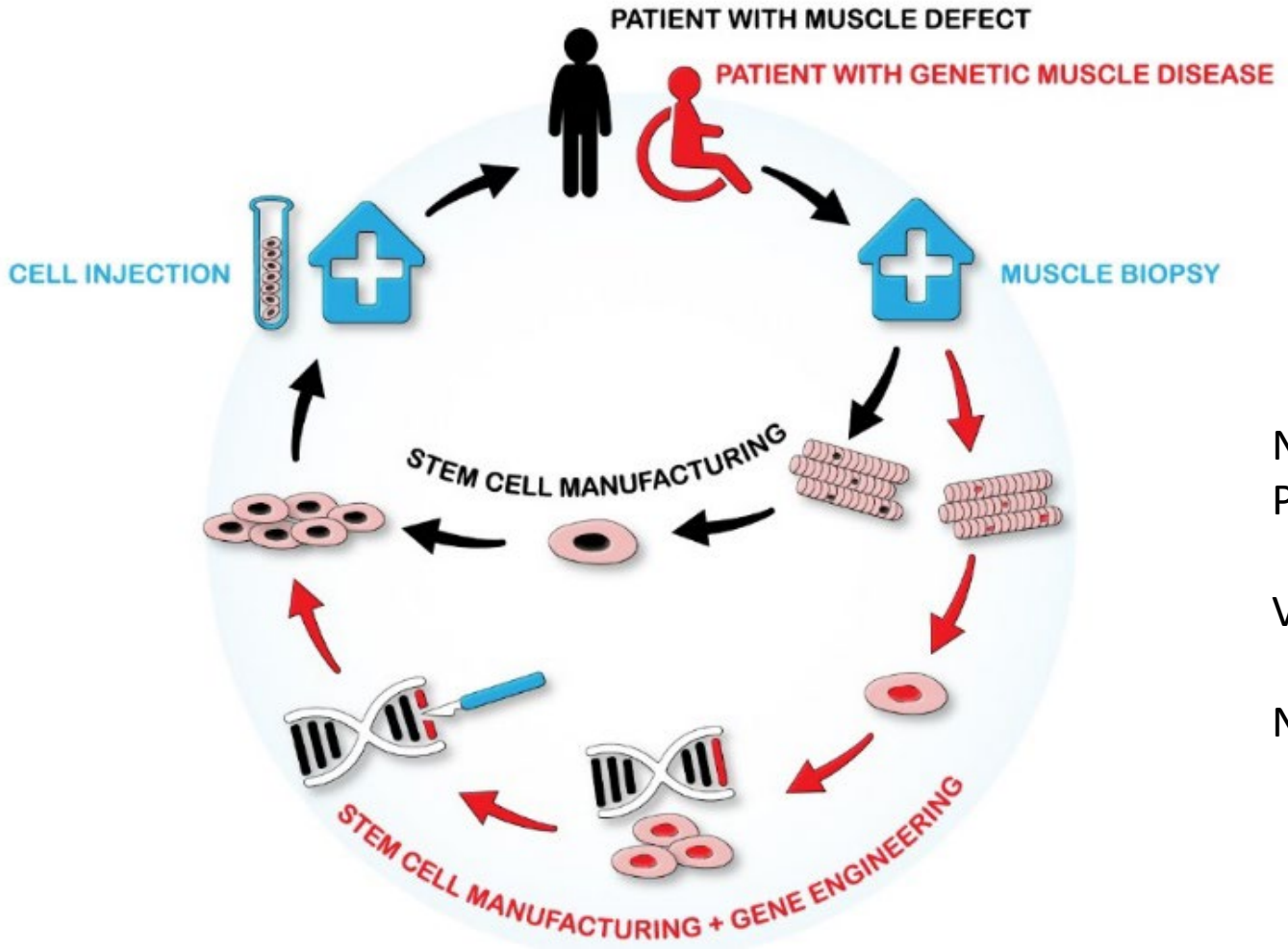


Escobar 2021 *J Clin Inv Ins*

Are we there?


PATIENT WITH MUSCLE DEFECT
PATIENT WITH GENETIC MUSCLE DISEASE
MUSCLE BIOPSY
STEM CELL MANUFACTURING
STEM CELL MANUFACTURING + GENE ENGINEERING
CELL INJECTION

Are we there?

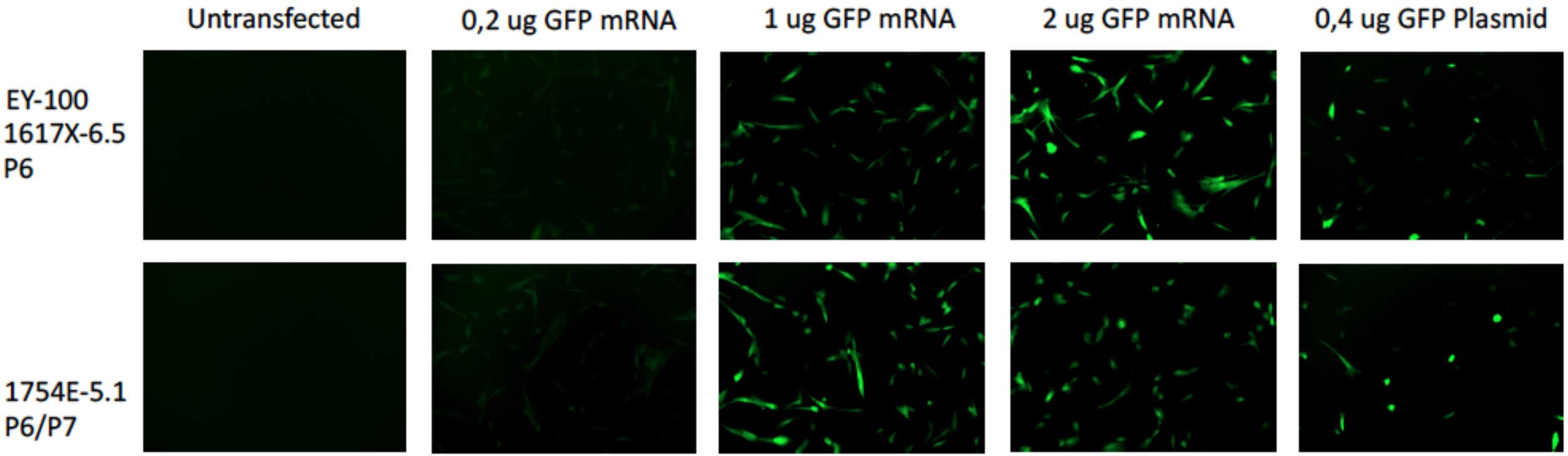


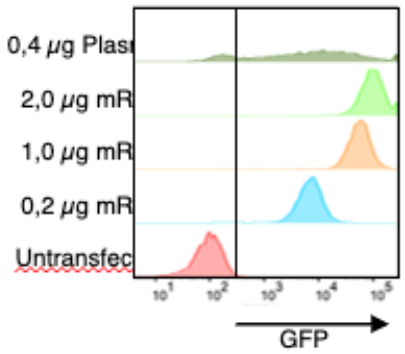
No:
Plasmid based

Venus reporter

No GMP

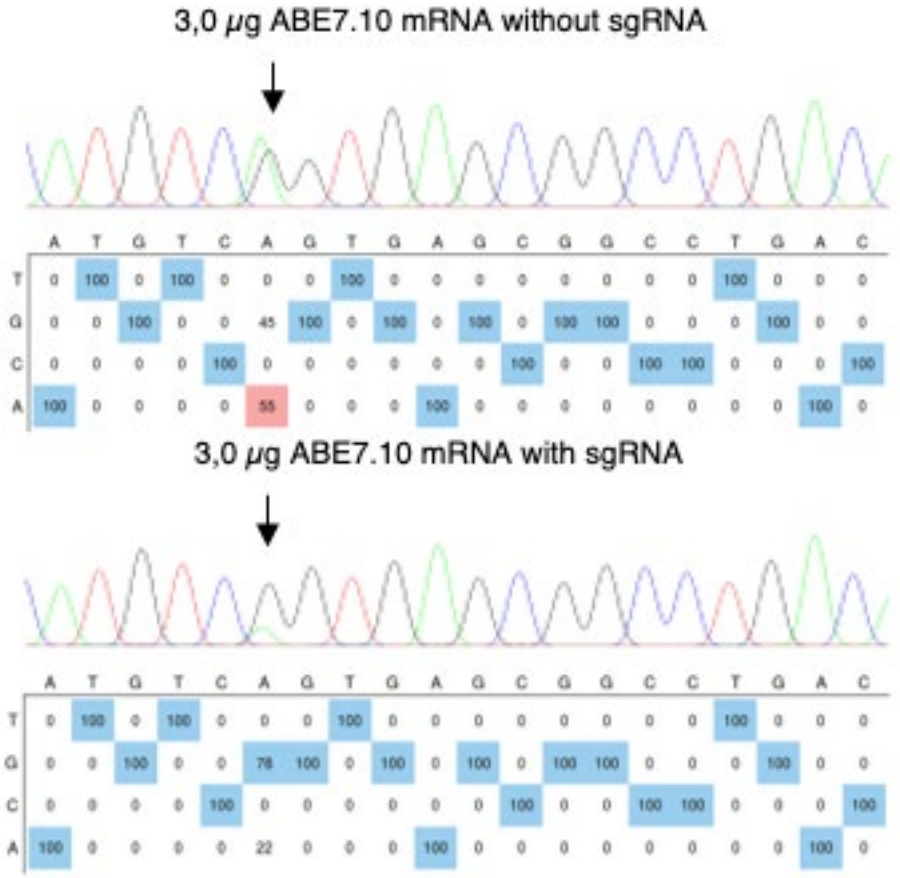
Transfection of primary muscle human stem cells with mRNA-GFP





Stadelmann, unpublished

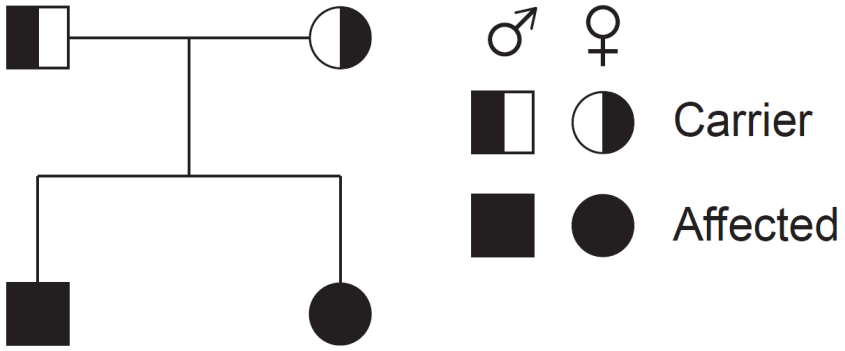
Transfection of primary muscle human stem cells with ABE-mRNA plus sgRNA



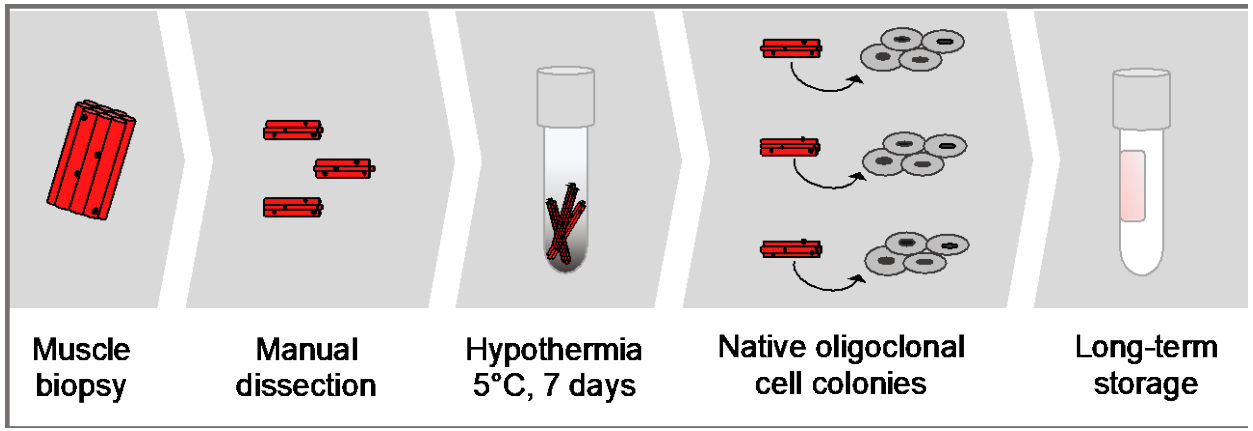
Stadelmann, unpublished

Summary:
Translational Workflow for gene corrected primary human muscle stem cells

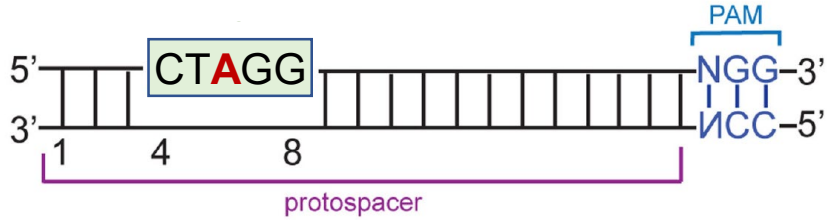
Patients



Generation of primary human muscle stem cells



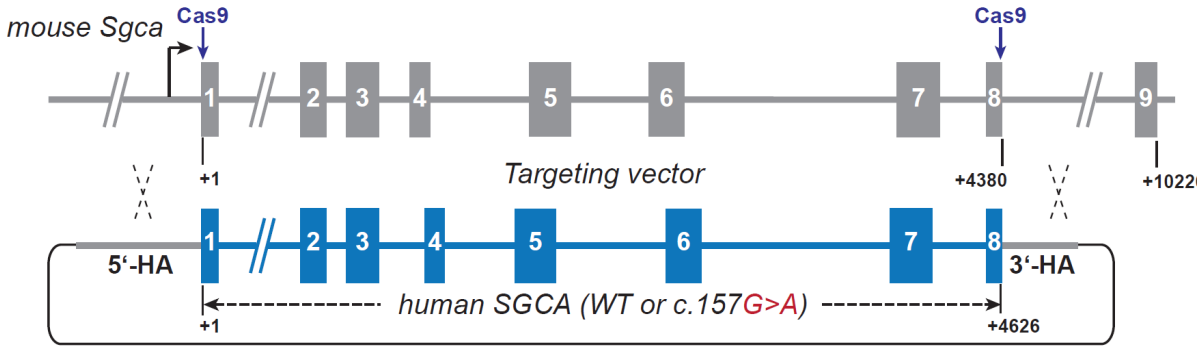
CRISPR/Cas9-derived genetic correction

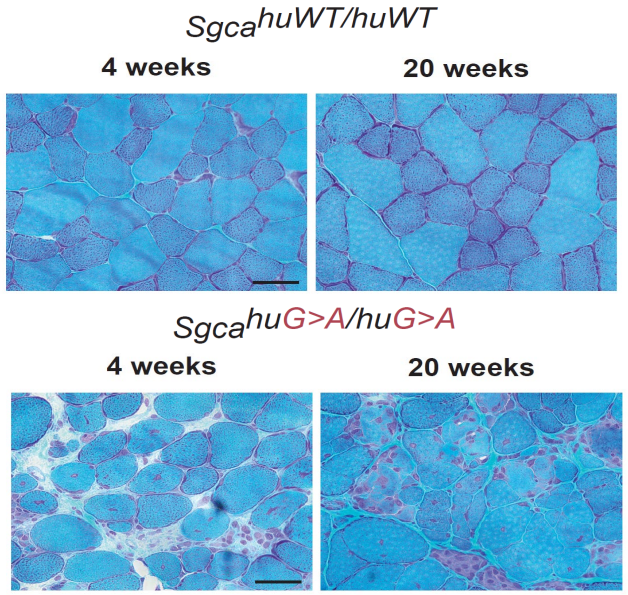


Translational impact

- Quantification of correction
- Off-target analysis
- Viability and functionality of corrected cells
- *In vivo* regenerative capacity

What is next: *in vivo* editing





Coop: Ralf Kühn, MDC

Muscle Research Unit

https://www.mdc-berlin.de/spuler

Patient care

2500 patients in Charité muscle outpatient clinic

Diagnosis and follow-up

Supportive care

Clinical trials

Translational Research

Human muscle stem cells

ATMP– new therapies

Muscular dystrophy

Gene editing

UNTERSTÜTZT DURCH DEN IMPULS- UND VERNETZUNGSFONDS VON HELMHOLTZ

Bundesministerium für Bildung und Forschung

DFG Deutsche Forschungsgemeinschaft

SGK STIFTUNG GISELA KREBS

SPARK BIH

BERLIN INSTITUTE OF HEALTH

Else Kröner Fresenius Stiftung

SUMUS

Thank you