

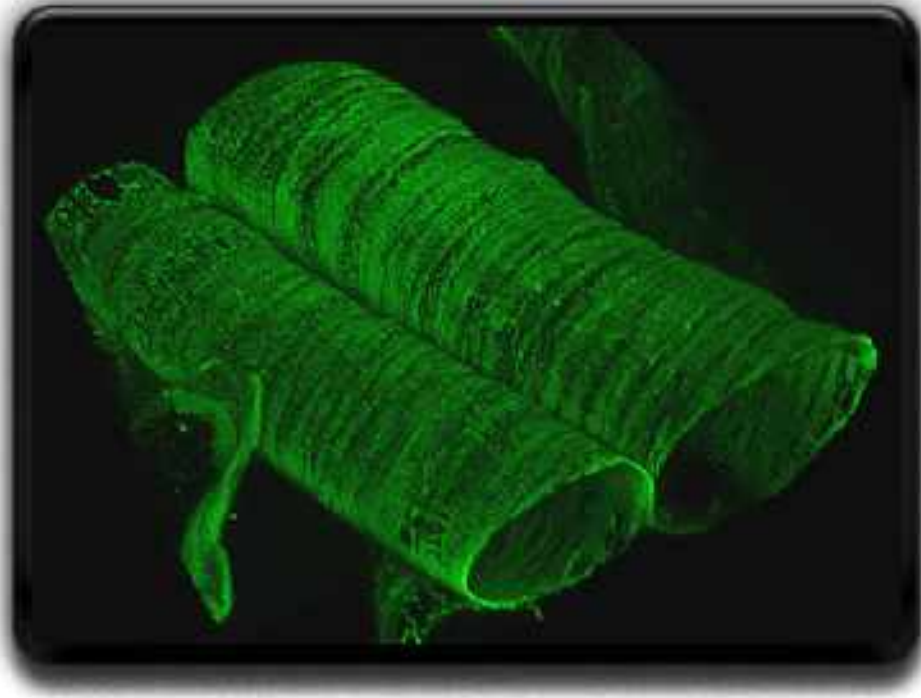
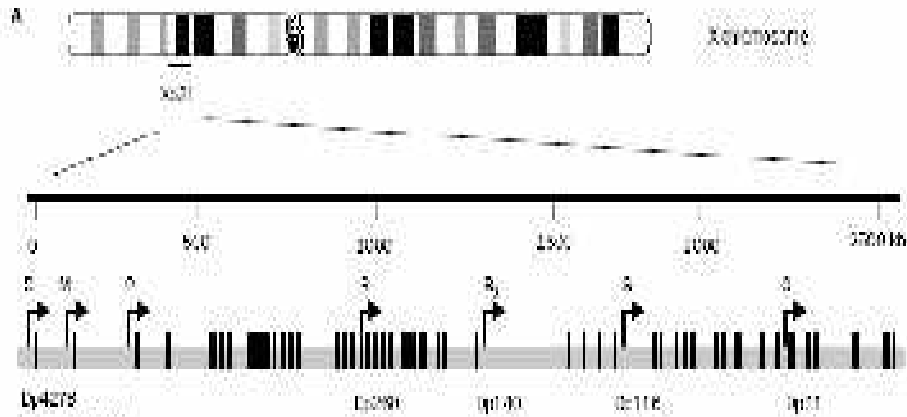
**Poliomielite e sindrome post-polio:
nuove frontiere terapeutiche. Malcesine 25/09/2010**

**“Utilizzo di cellule staminali nelle
malattie muscolari”**

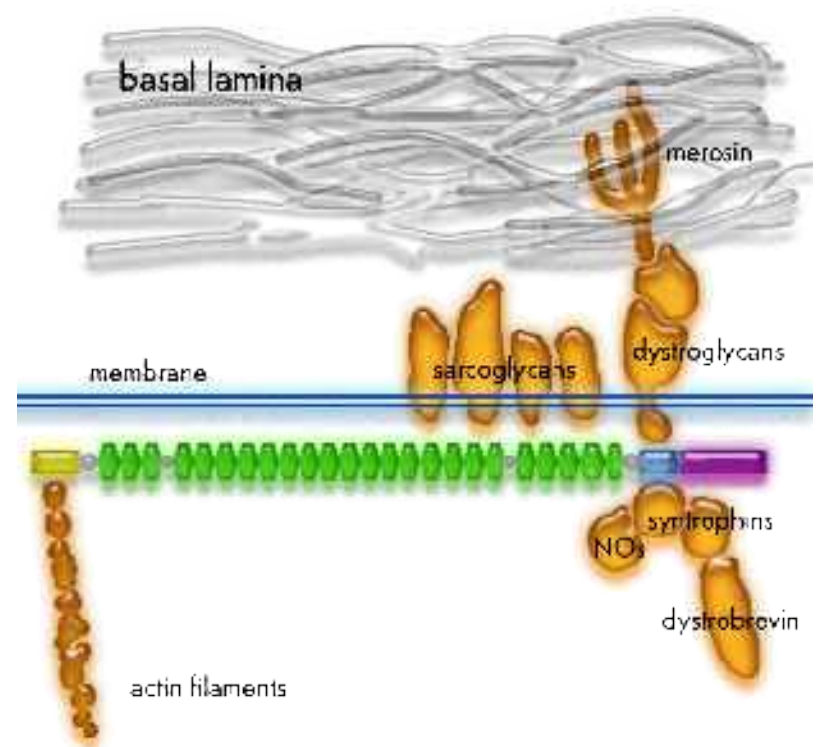
**Torrente Yvan MD, PhD
Dino Ferrari Center
Dep. Neurological Sciences
Fondazione IRCCS Policlinico of Milan,
University of Milan, Italy**



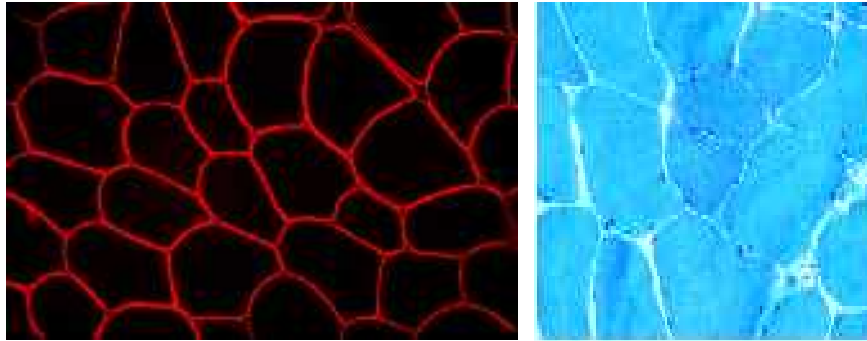
Duchenne muscular dystrophy



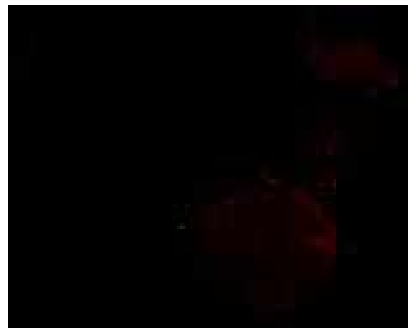
The muscle dystrophin glycoprotein complex



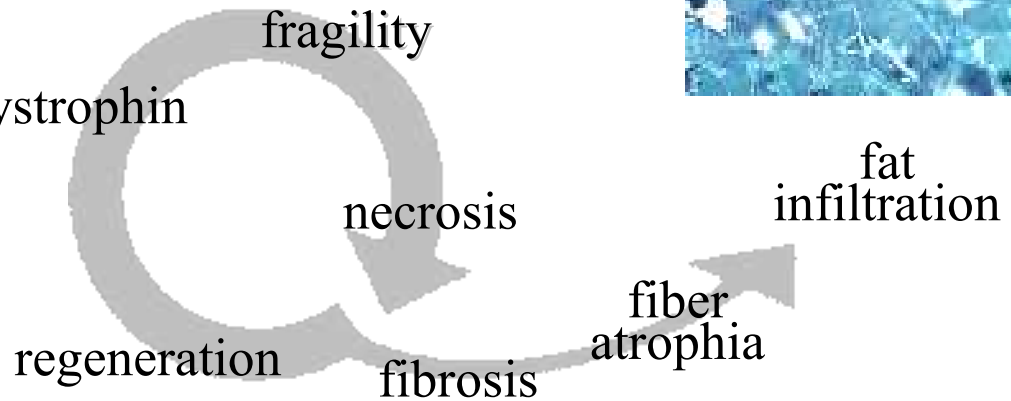
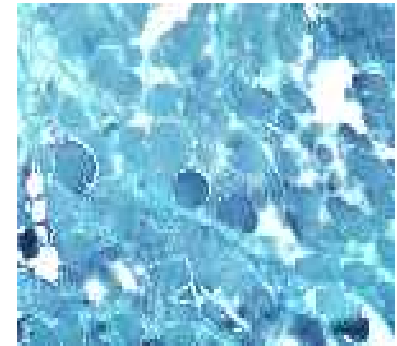
The absence of dystrophin leads to progressive fibre damage and muscle wasting



Pathophysiology of DMD



Δ dystrophin



Duchenne Muscular Dystrophy

- **Onset:** 3 to 5 yrs

- **Weakness**

 - Distribution

 - Proximal > Distal
 - Symmetric
 - Legs & Arms
 - Hand strength increases by 10 years
 - Gowers sign: Standing up with the aid of hands pushing on knees

 - Course

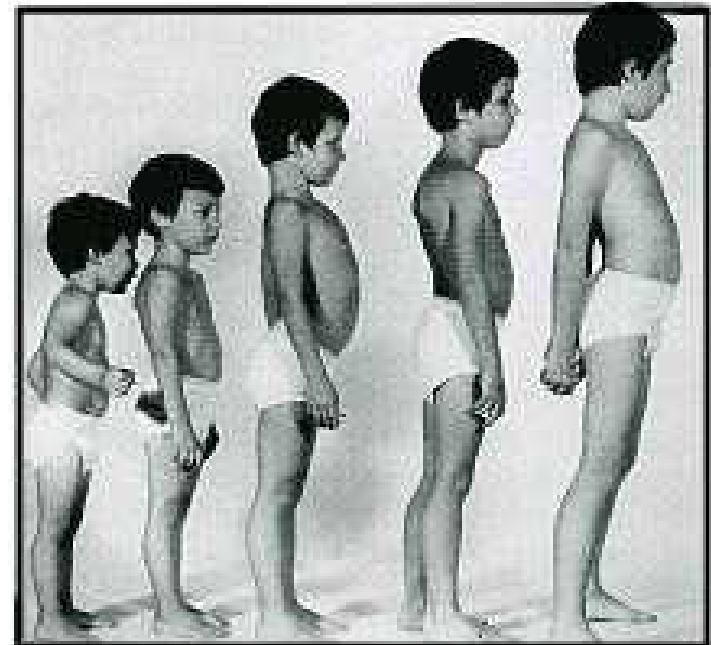
 - Reduced motor function by 2 to 3 years
 - Steady decline in strength: After 6 to 11 years

 - Failure to walk: 9 - 13 years; Later with steroid treatment

 - A small reduction in muscle force is shown by a relatively large deterioration in functional ability function



Figure 21-11 A. Duchenne's muscular dystrophy. (Reprinted from [source])



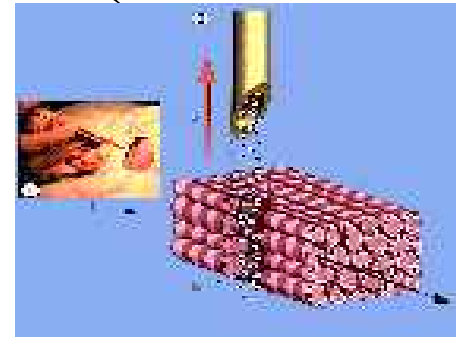
Cell-based treatment for muscular dystrophies

Types of cell

- Myoblasts
- Satellite cell
- Bone marrow/SP
- Circulating cell
- Pericytes/mesoangioblasts
- Muscle stem cell/SP

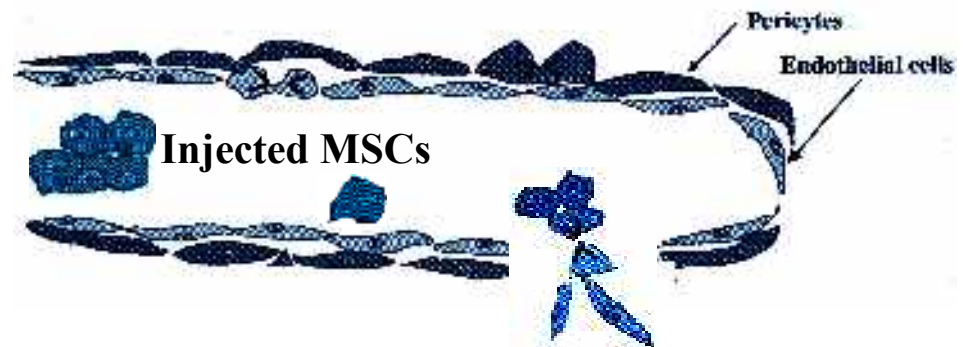
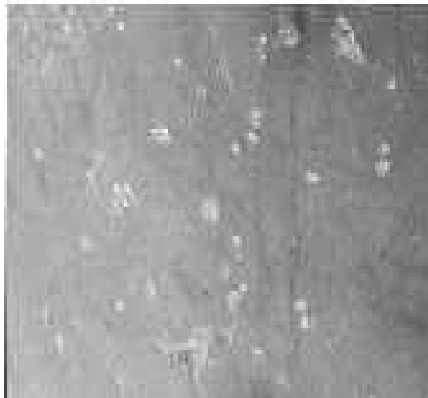
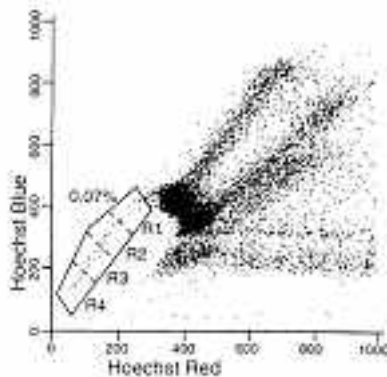
Cell delivery

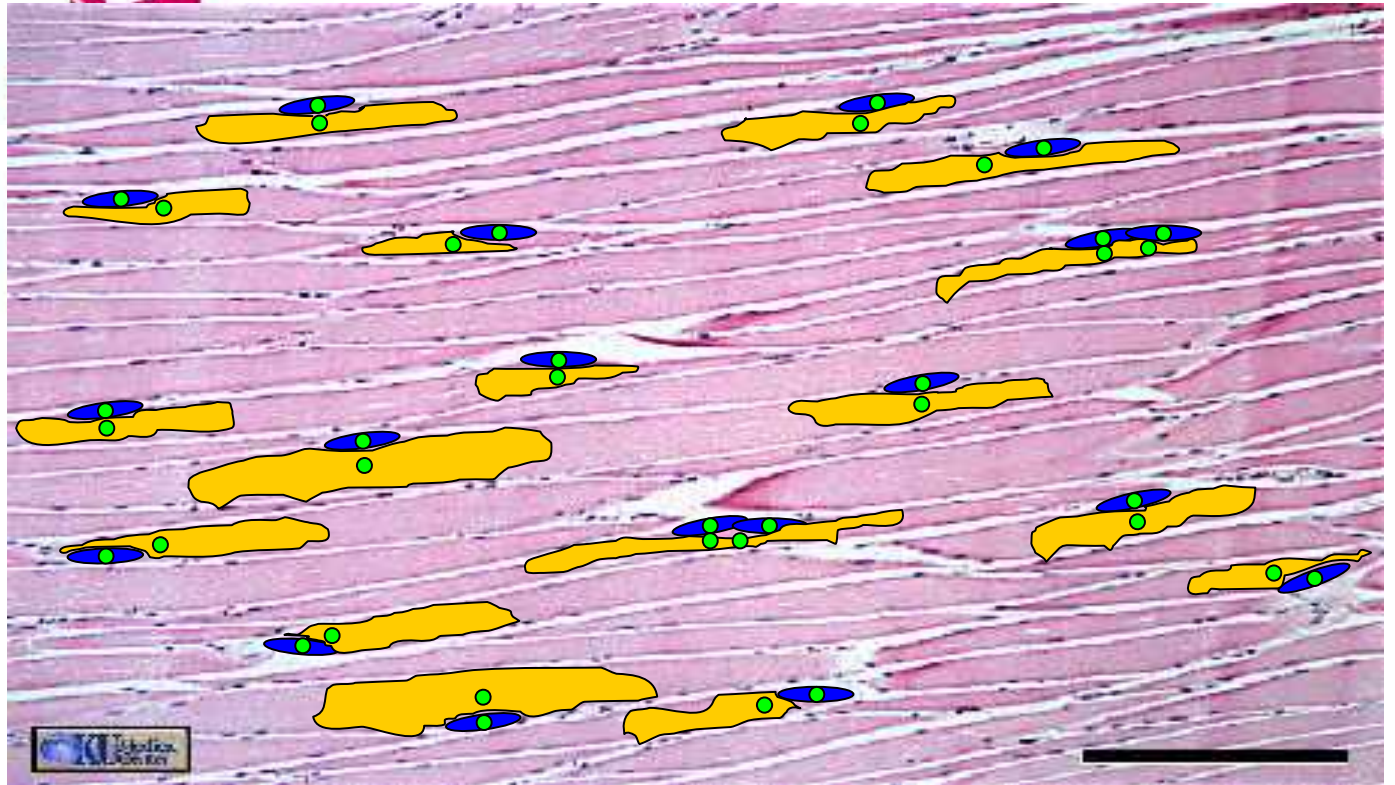
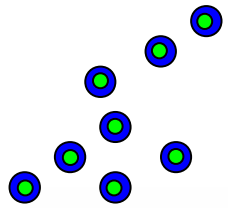
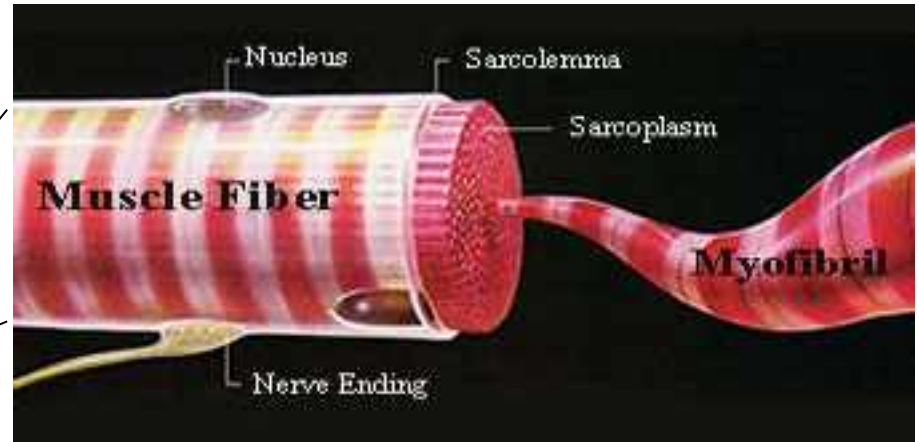
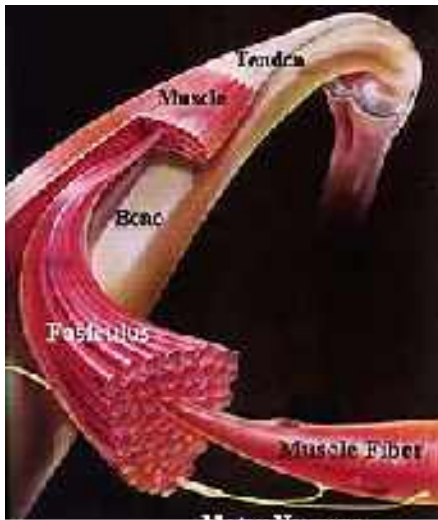
- Local (Intramuscular)



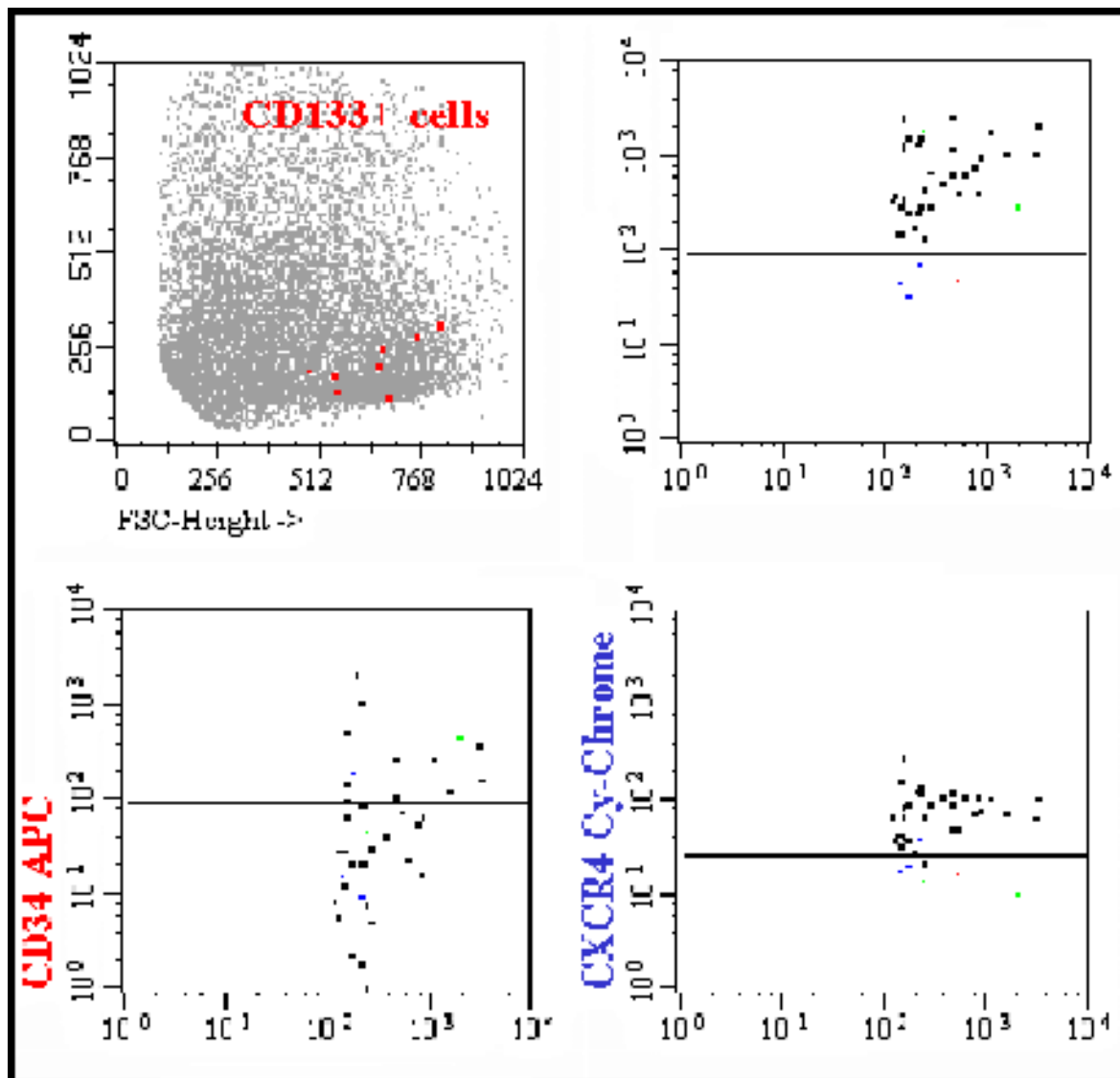
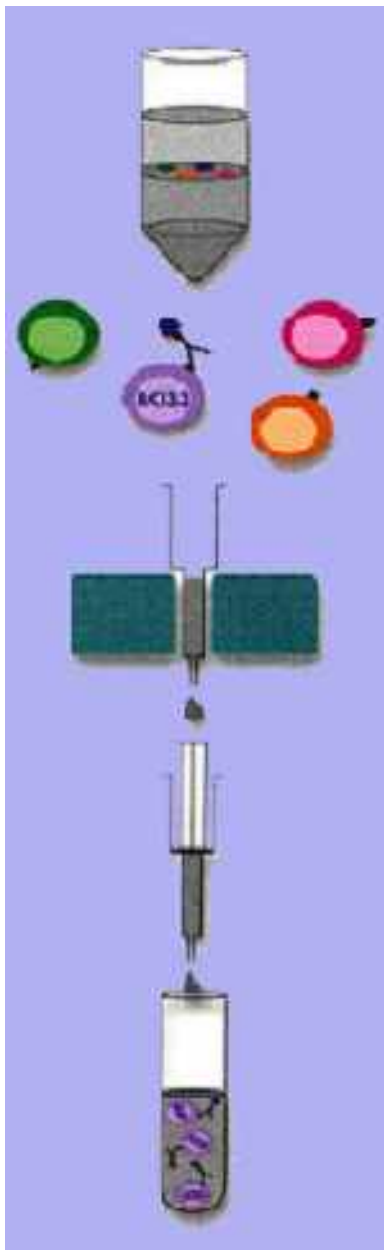
- Systemic

Intravenous vs Intra-arterial



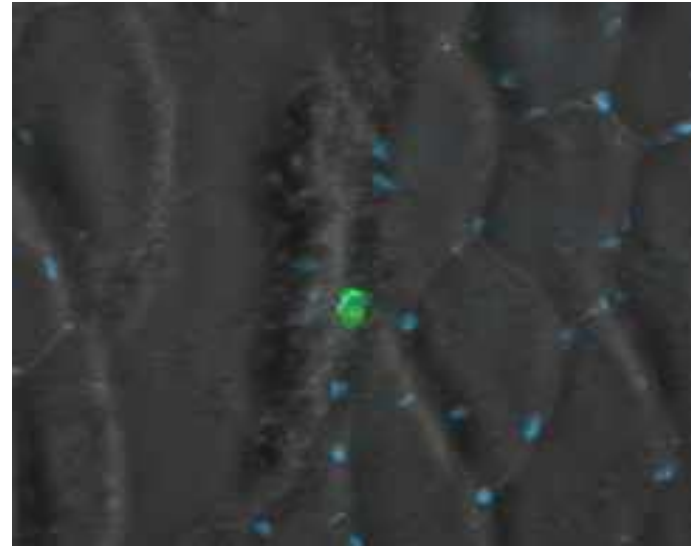
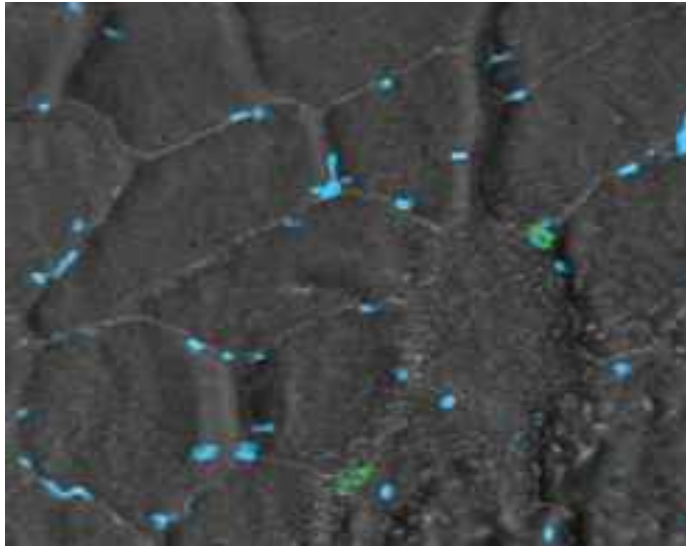


Human muscle-derived CD133+ cells

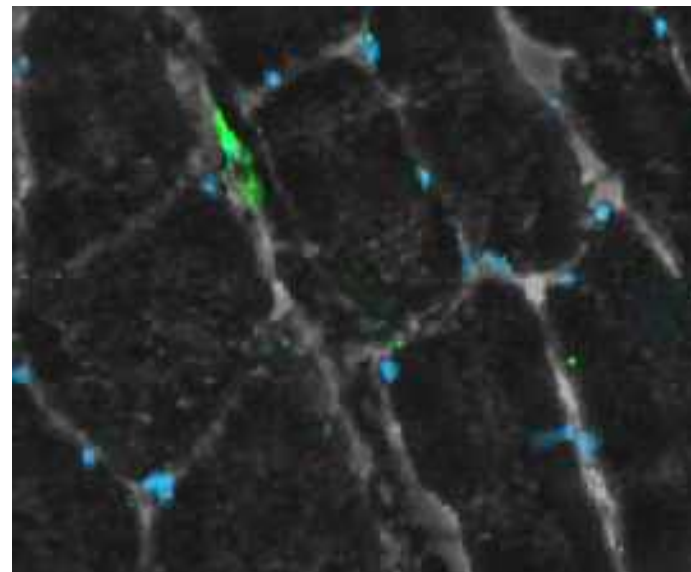
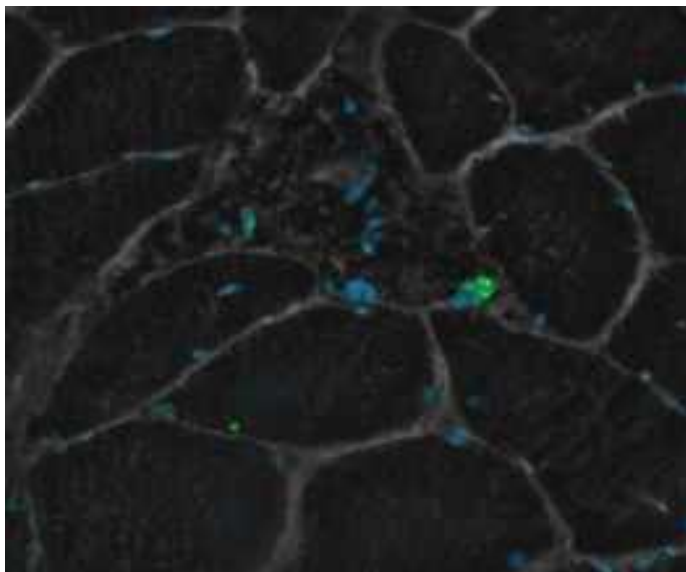


CD45⁻

Human adult muscle-derived CD133+ cells

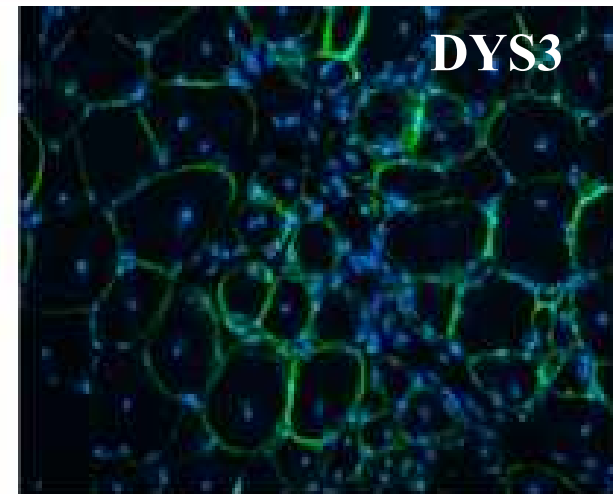
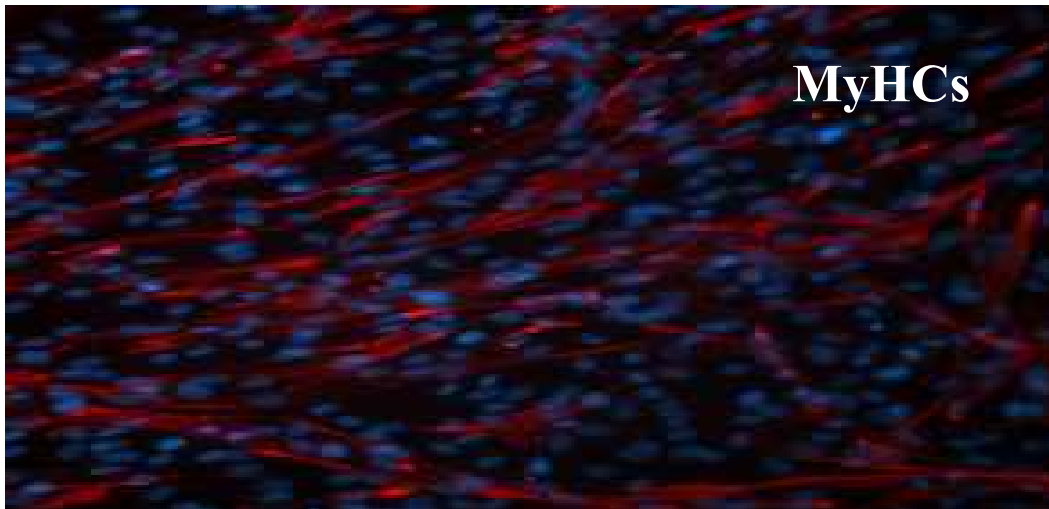
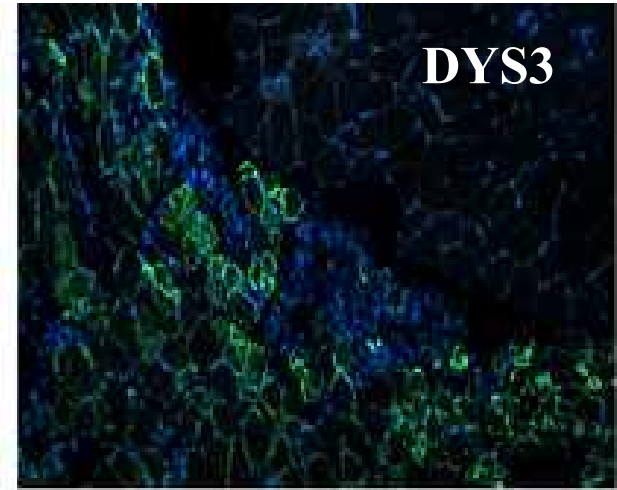
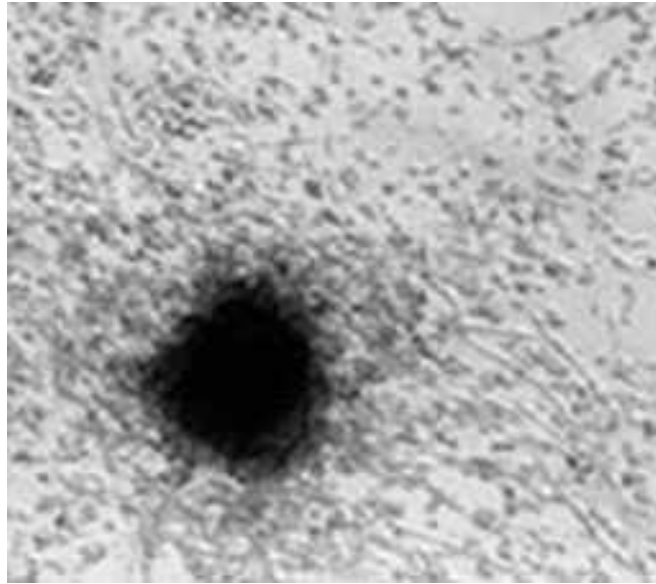


**CD 133
isoform2**



**CD133
isoform1**

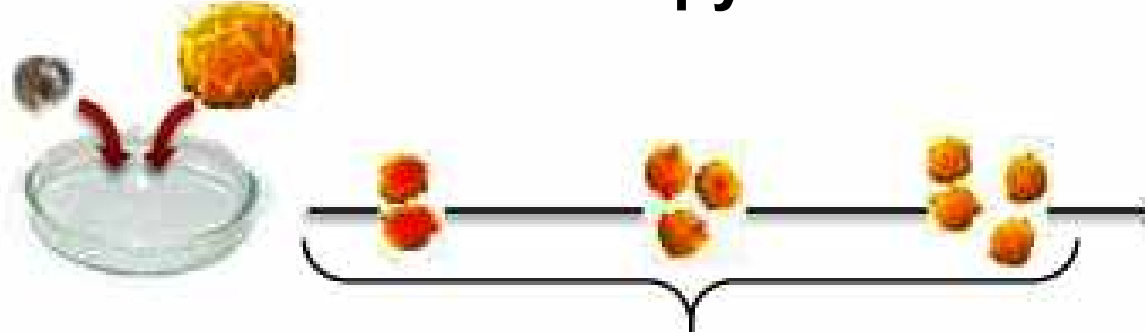
Myogenic potential of muscle-derived CD133+ stem cells



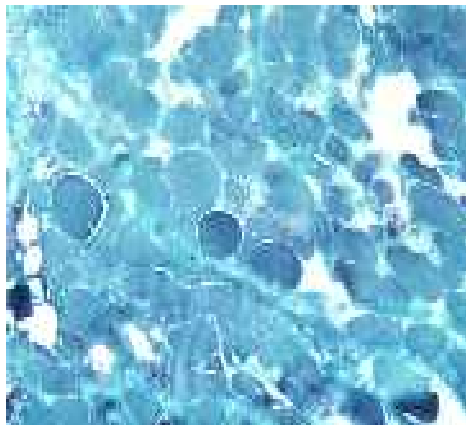
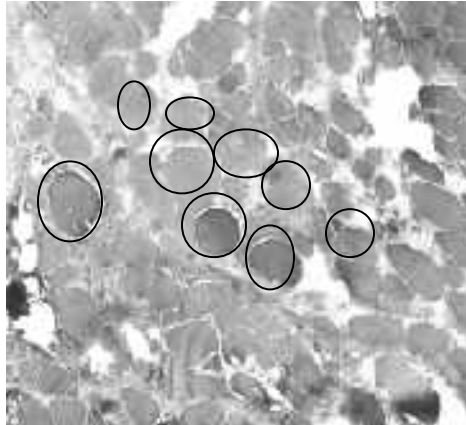
Rescue of dystrophin protein



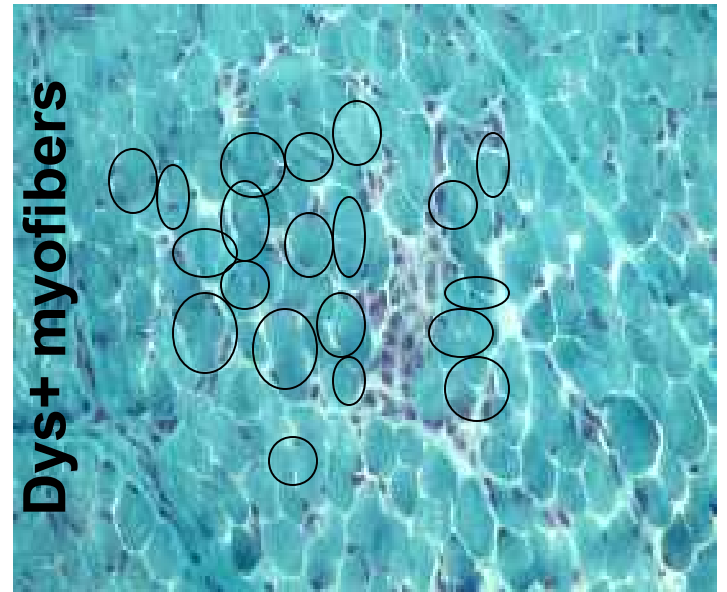
Gene and Cell therapy combination



Dys+ myofibers

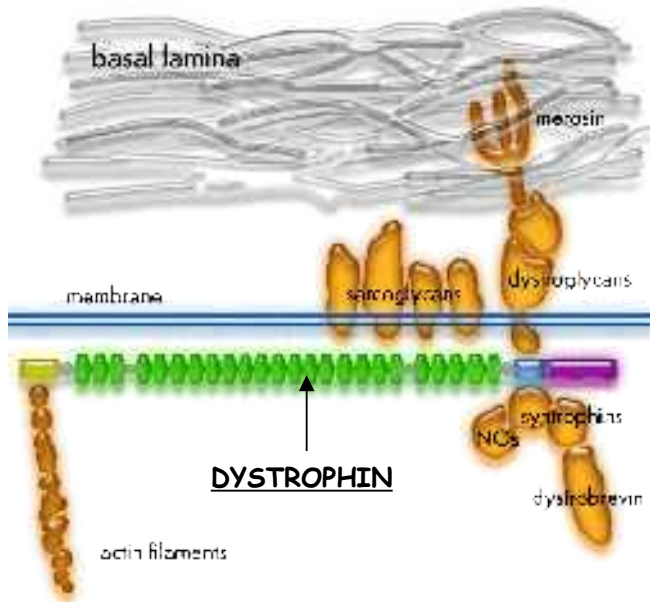


Dys+ myofibers



Duchenne Muscular Dystrophy : one protein - several genotypes

The muscle dystrophin-glycoprotein complex



DYSTROPHIN

Mutation frequencies in DMD

Deletions : >75%

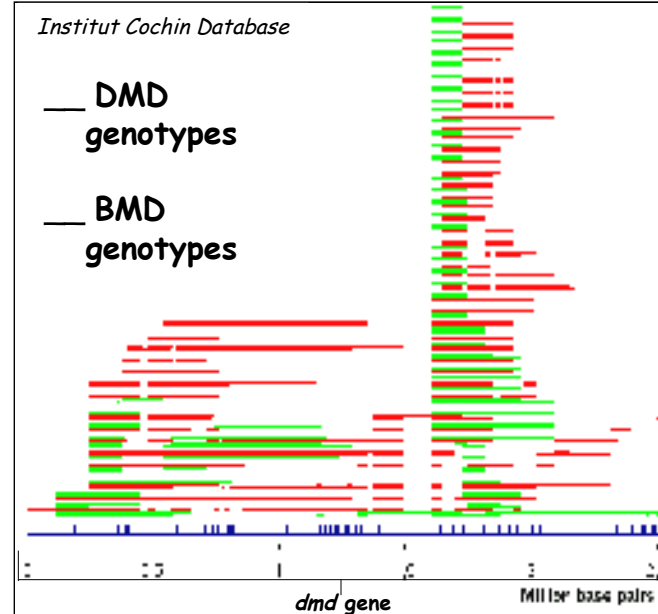
Duplications : >10%

Nonsense : >5%

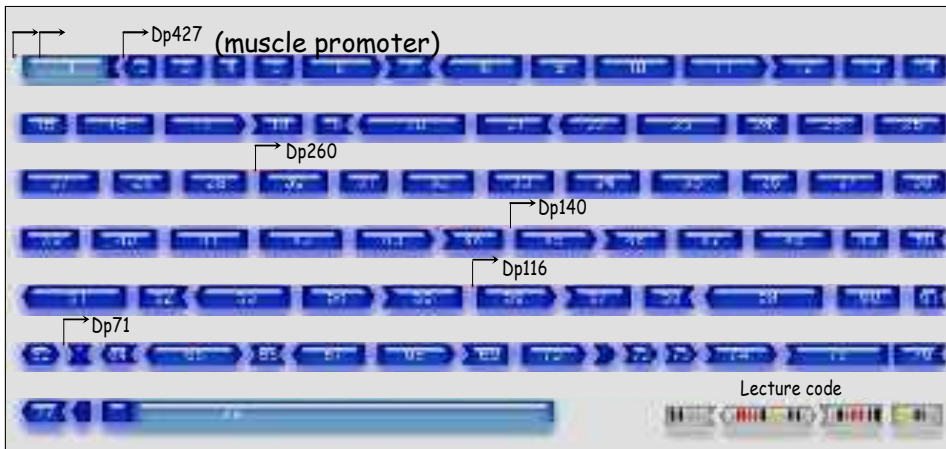
Insertions : <5%

Splice mutations : <5%

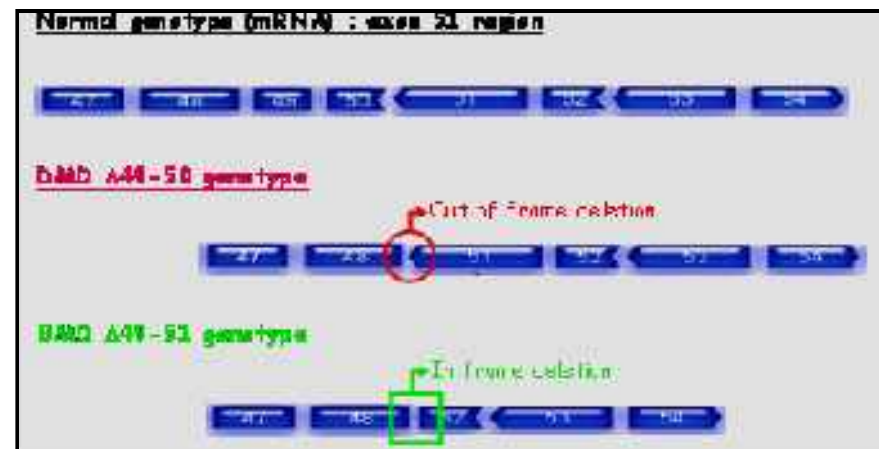
DMD/BMD deletion genotypes



Dystrophin exon phasing map

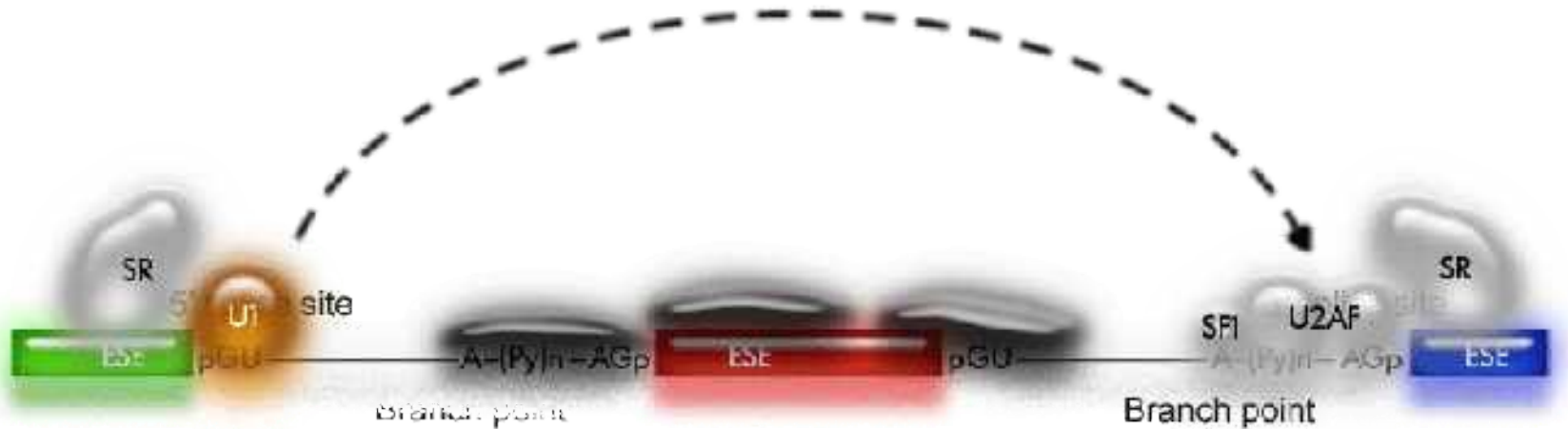


DMD vs BMD genotype characteristics

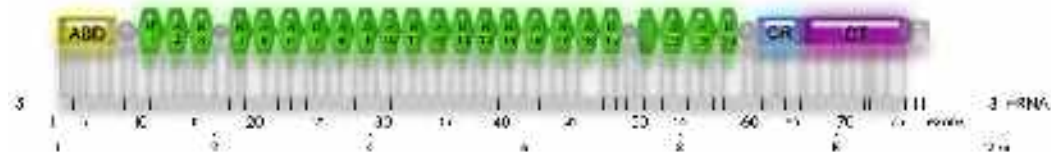
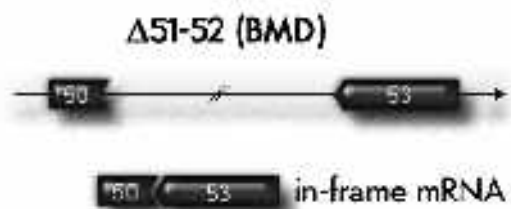


Exon-skipping

It is possible to skip one or several exon by masking important splicing sites

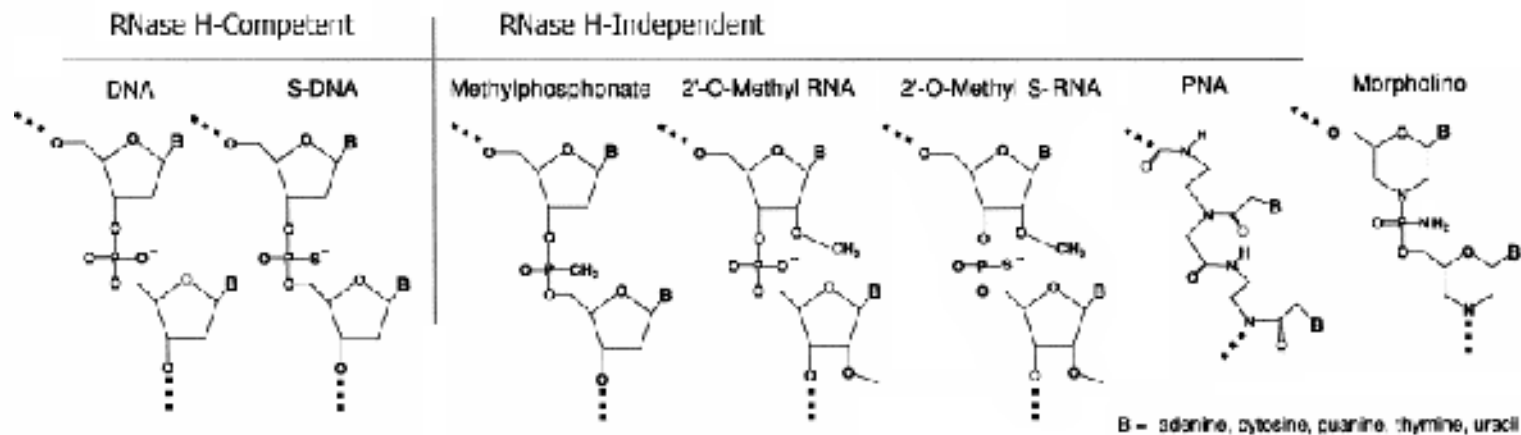


Rationale for exon skipping in DMD



Molecular tools for exon skipping

SYNTHETIC ANTISENSE OLIGONUCLEOTIDES

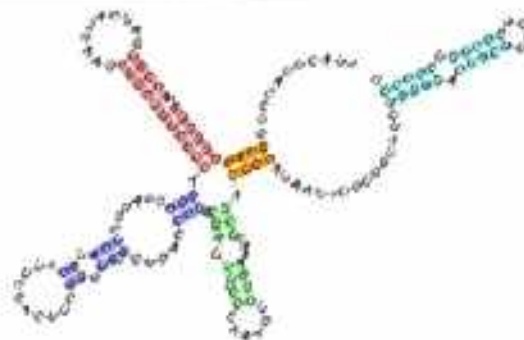


ENGINEERED SMALL NUCLEAR RNAs (U7, U1)

U7 - recognition and binding to histone mRNA



U1 - involved in the spliceosomal complex



Both involve the use of gene vectors

The exon skipping approach for DMD

General principle : application to $\Delta 49-50$ DMD deletion

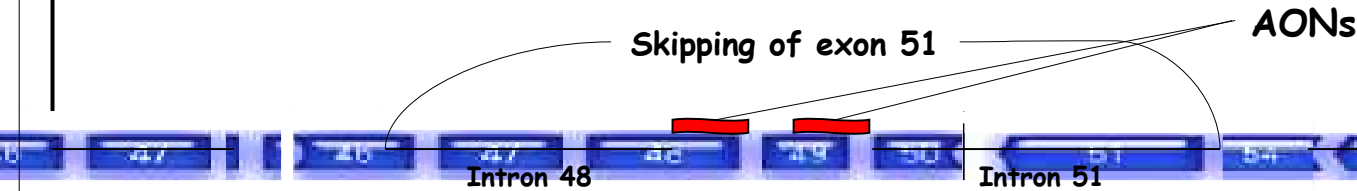
Normal genotype (mRNA) : exon 51 region



$\Delta 49-50$ genotype : DMD phenotype



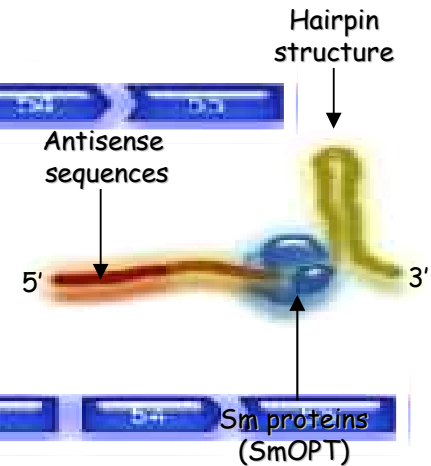
Skipping exon 51 (pre-mRNA)



Skipped $\Delta 49-50$ genotype : BMD phenotype



Structure of the modified U7snRNA carrier

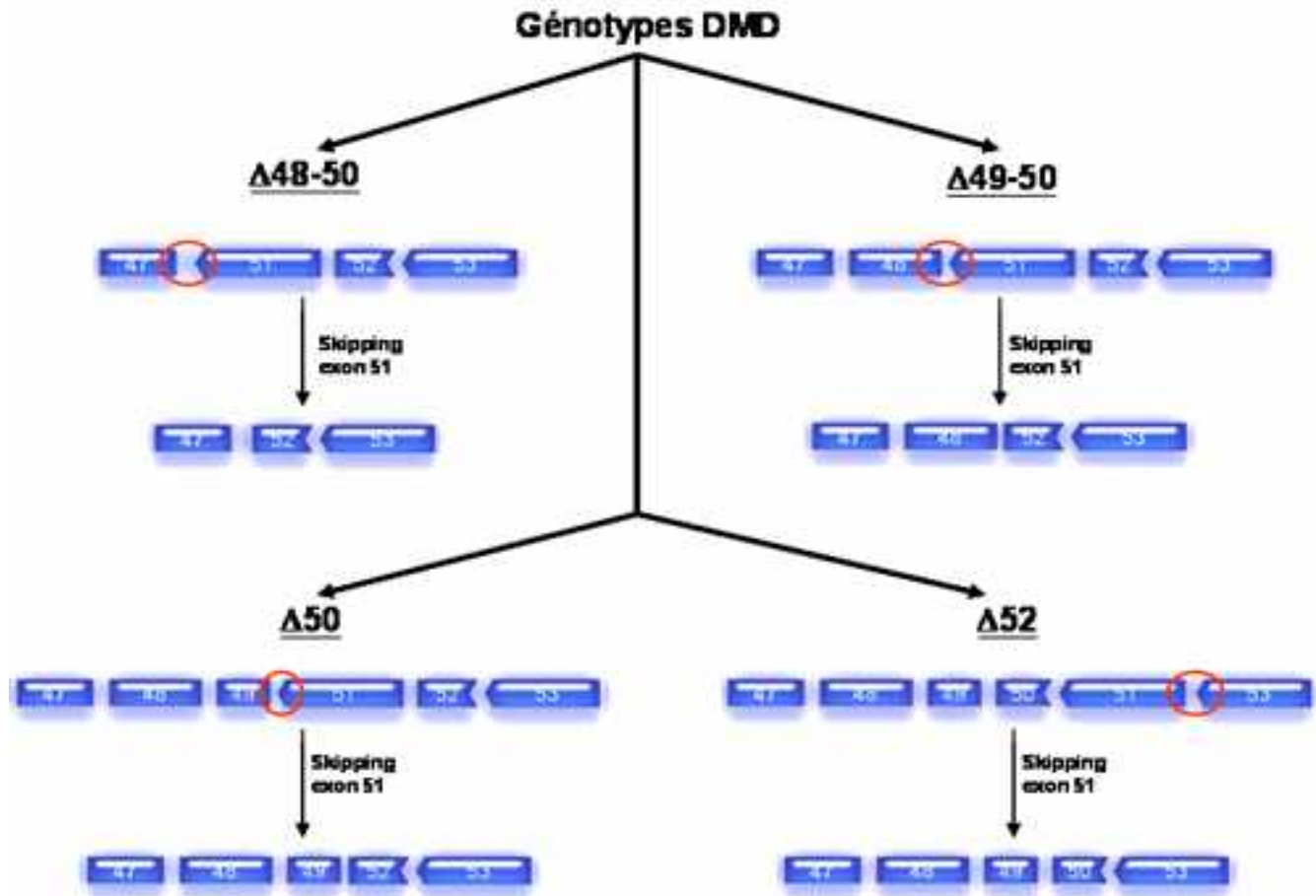


DMD genotypes for exon-skipping of CD133+ stem cells

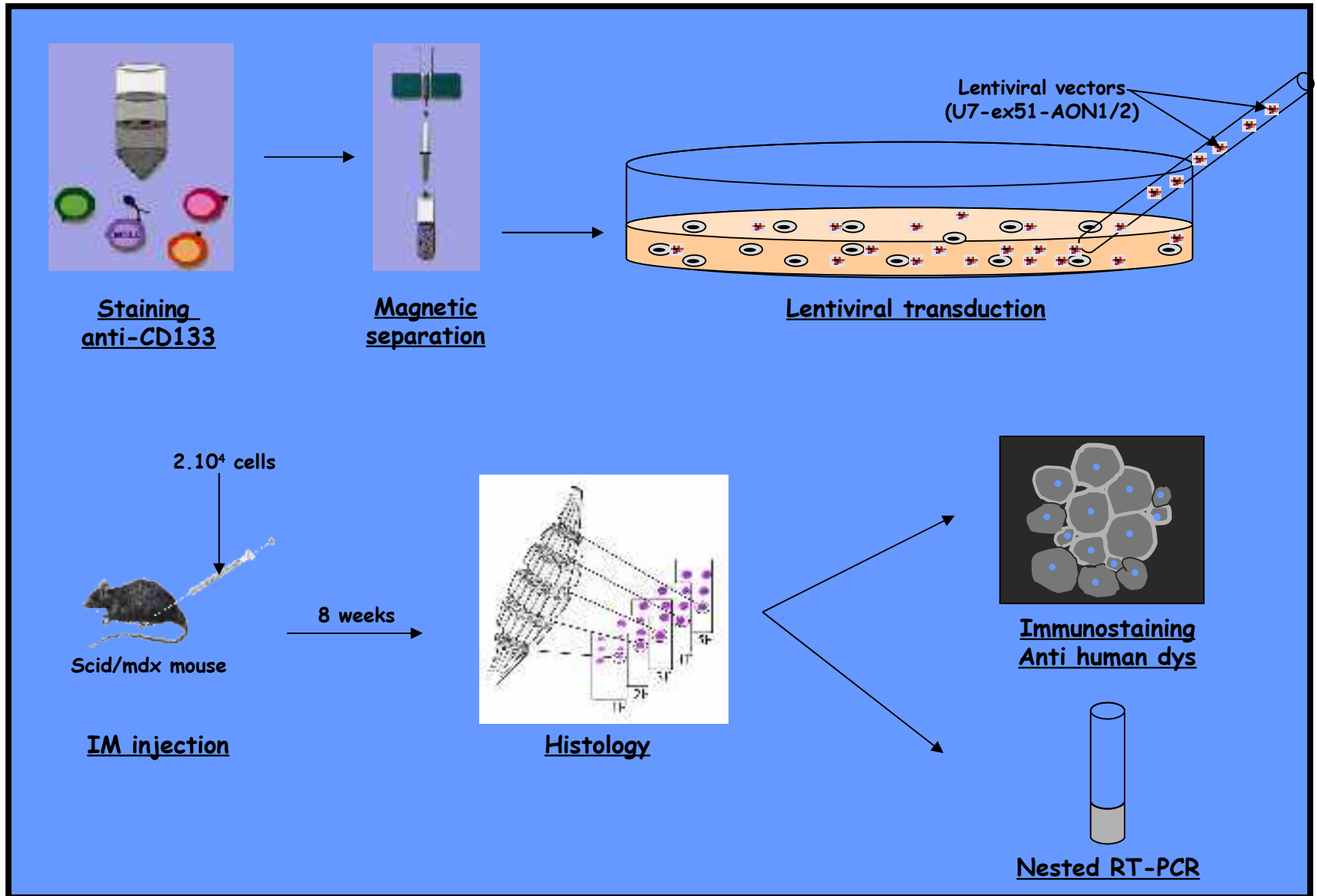
Exon phasing around exon 51 :



DMD genotypes selected :



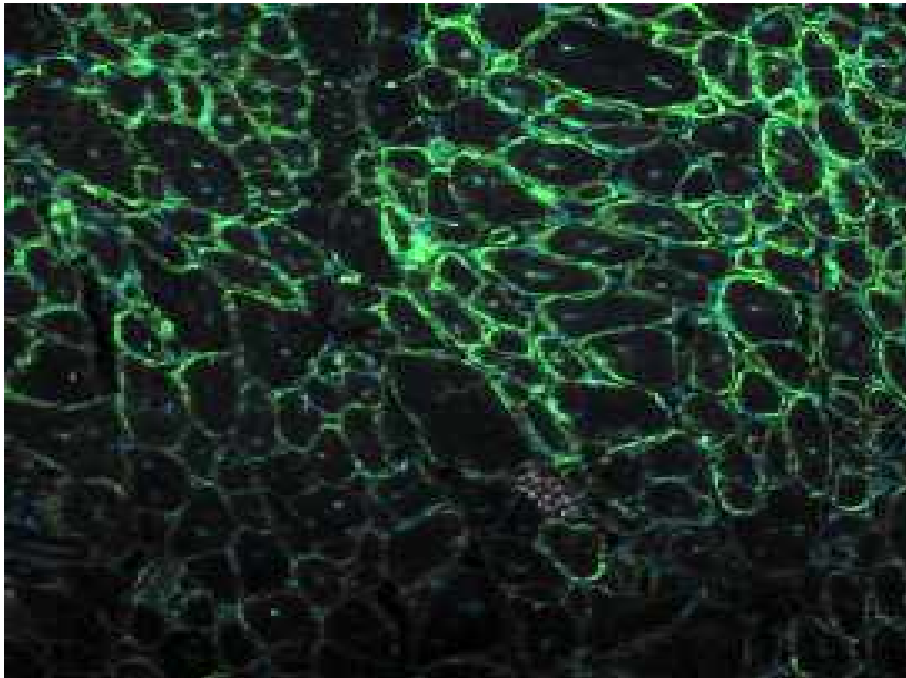
Experimental procedure : isolation, transduction and injection of CD133 cells



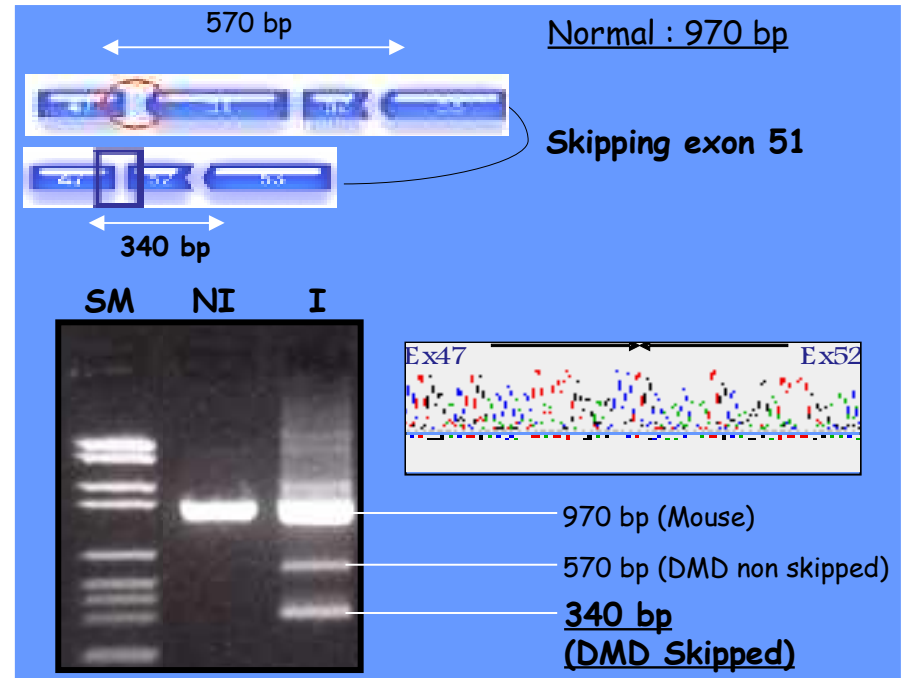
Skipped blood-derived CD133 cells *in vivo* : genotype $\Delta 48-50$



2.10^4 $\Delta 48-50$ blood-derived CD133 cells transduced with Lenti U7-ex51-AON1/2



Immunostaining anti-human dystrophin in Tibialis Anterior (TA) muscle

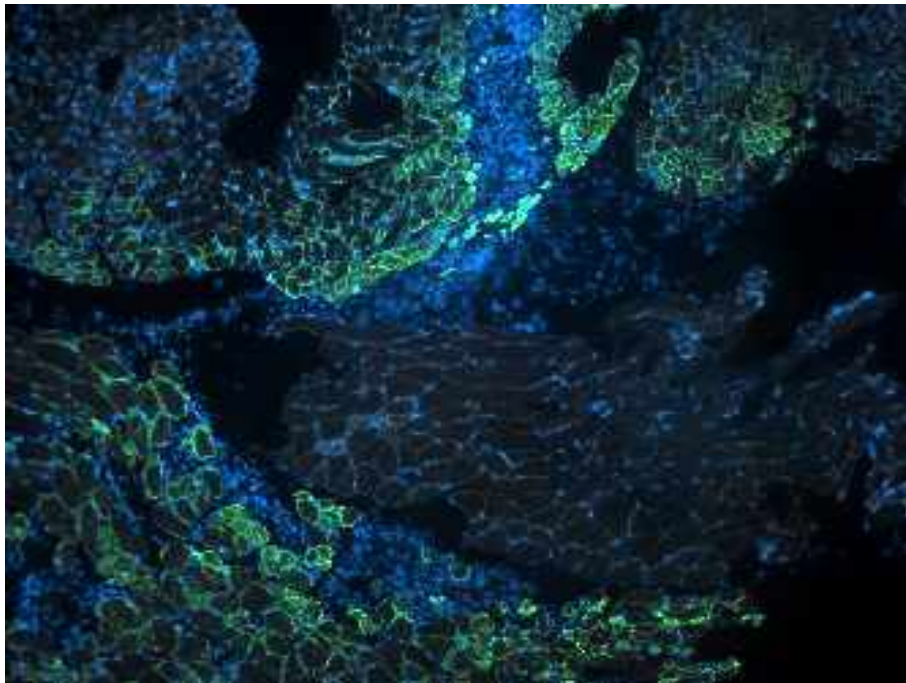


Nested RT-PCR for the analysis of the skipping of exon 51 in injected TA (I)

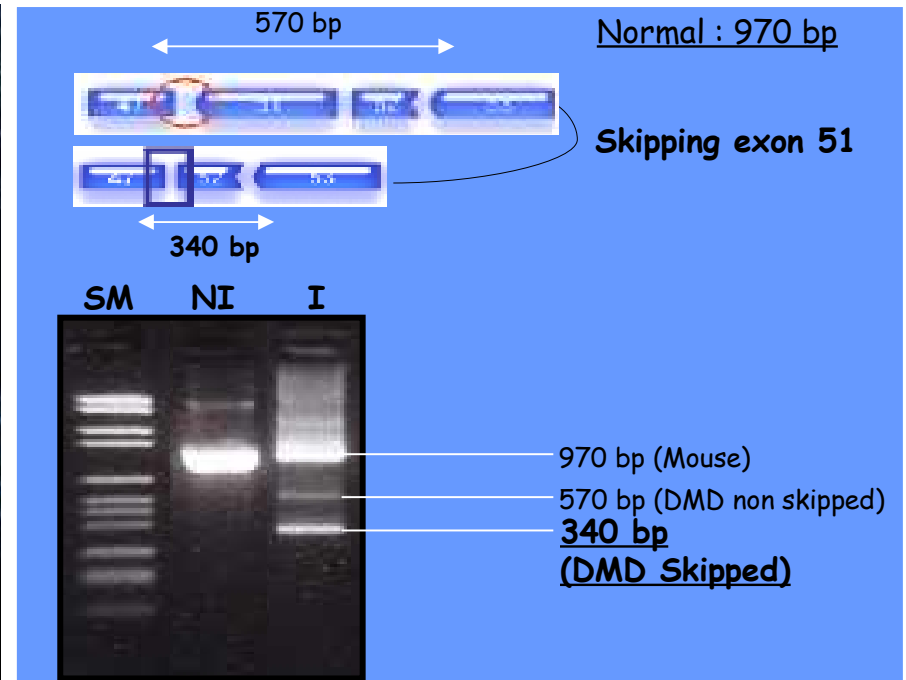
Skipped muscle-derived CD133 cells *in vivo* : genotype $\Delta 48-50$



$5 \cdot 10^4$ $\Delta 48-50$ muscle-derived CD133 cells transduced with Lenti U7-ex51-AON1/2

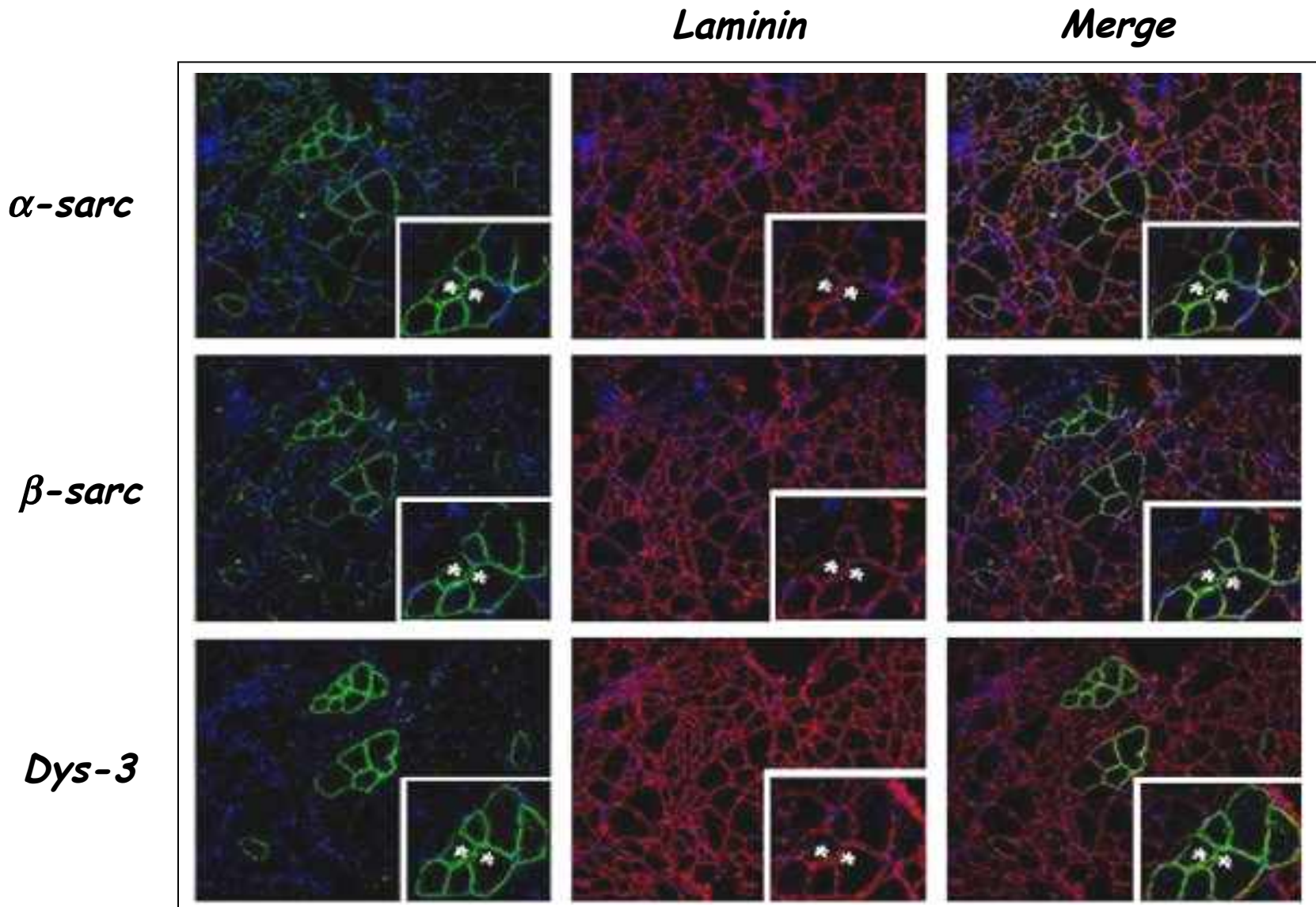


Immunostaining anti-human dystrophin in Tibialis Anterior (TA) muscle

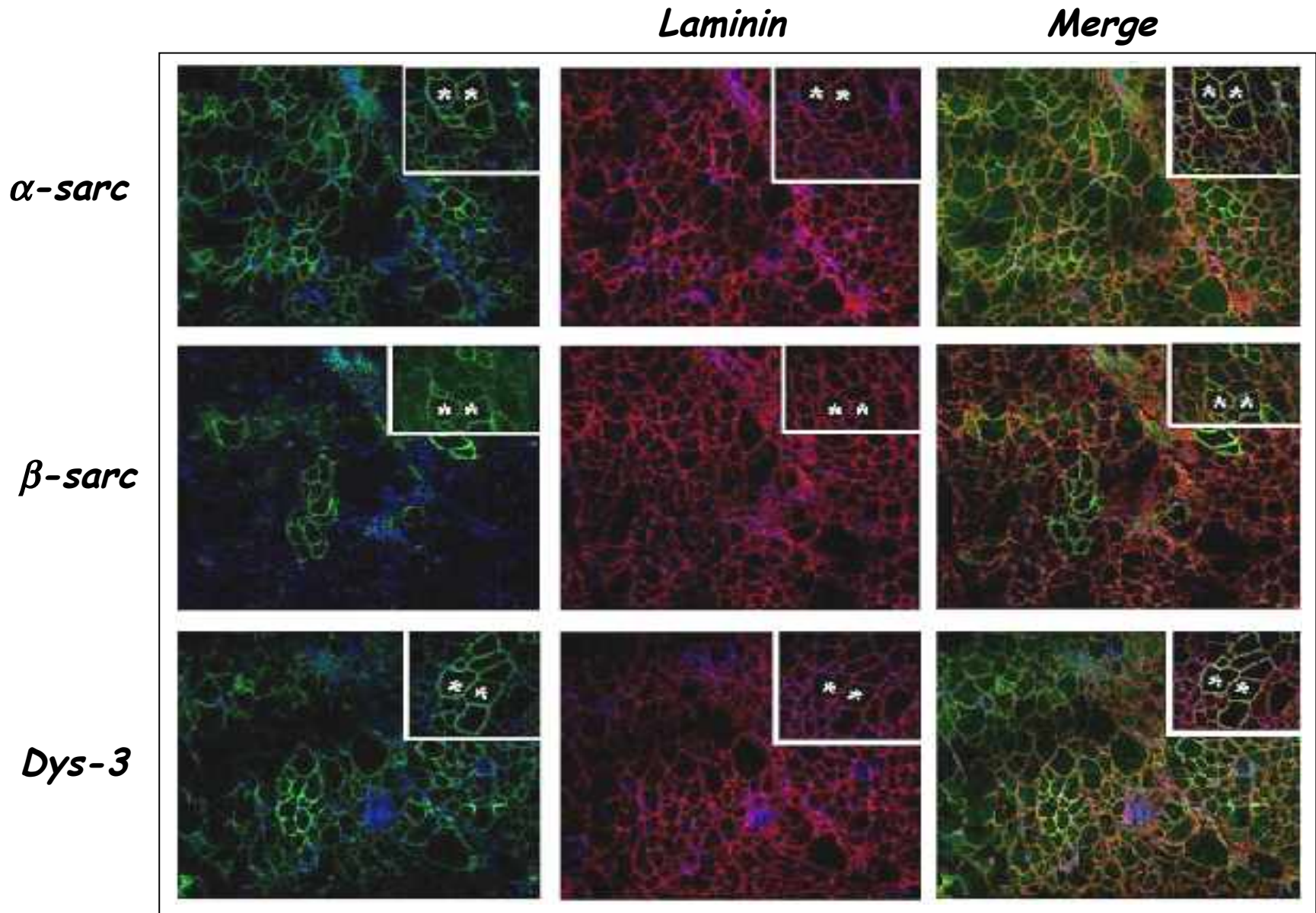


Nested RT-PCR for the analysis of the skipping of exon 51 in injected TA (I)

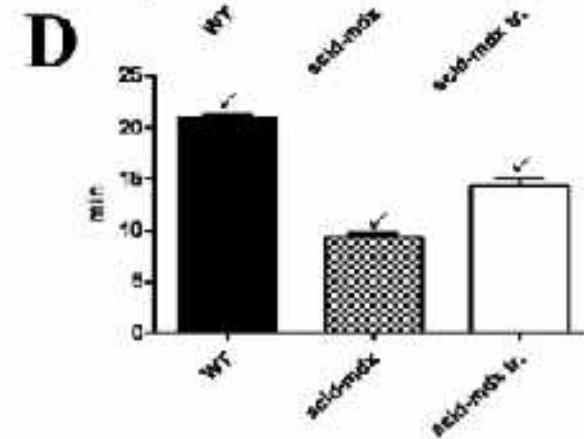
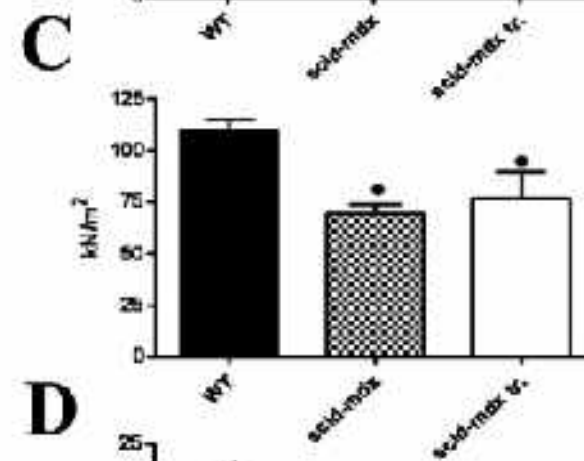
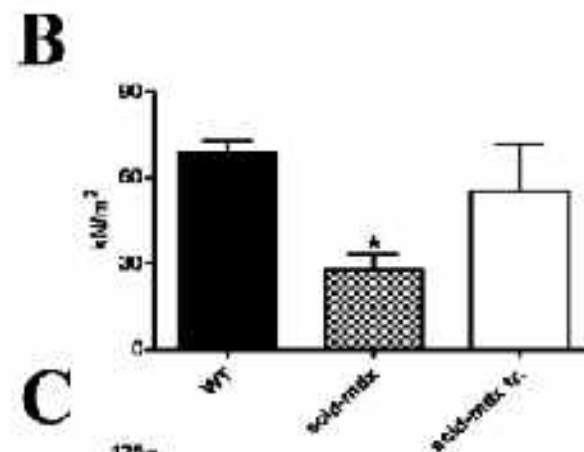
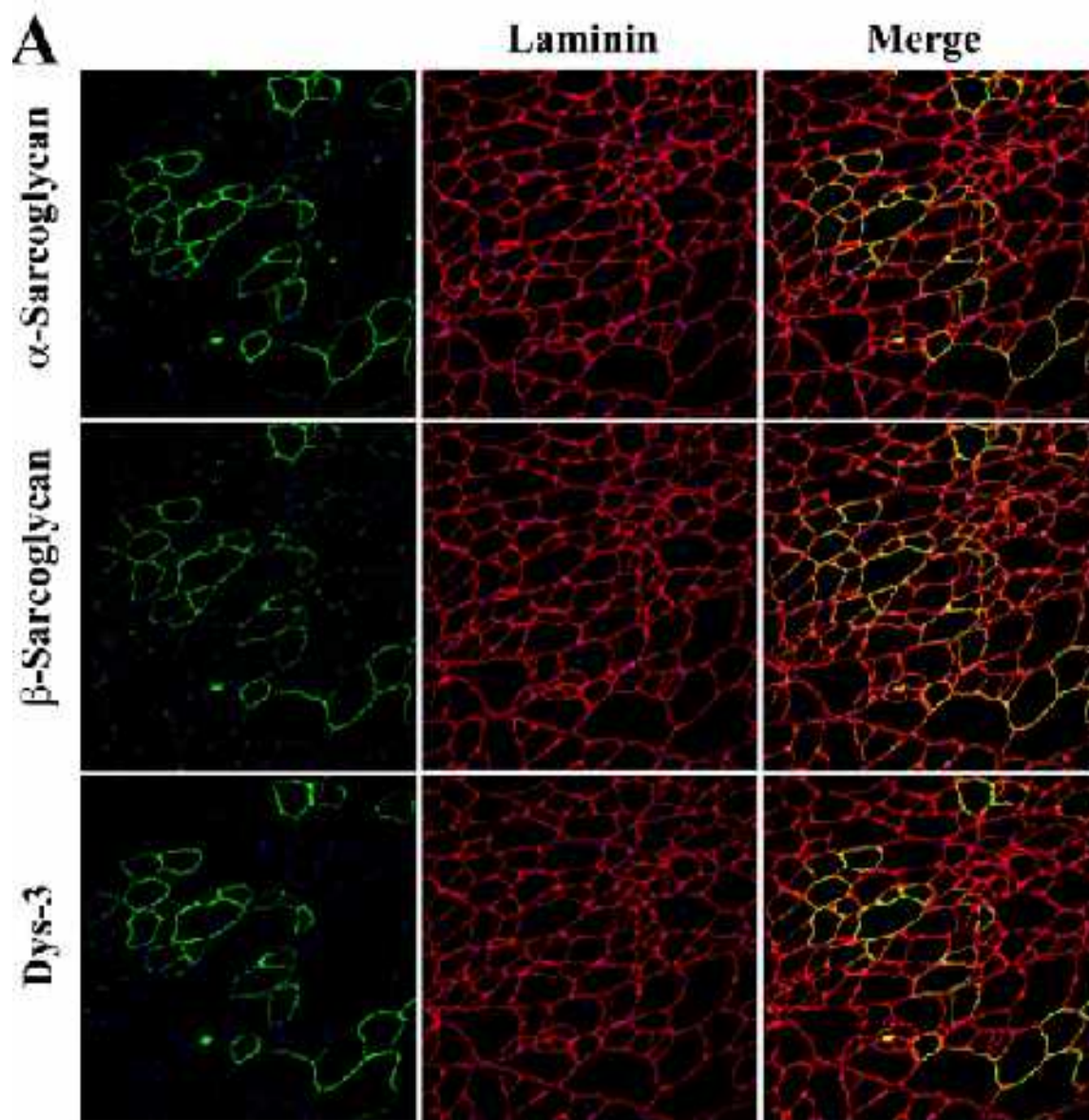
Skipped blood-derived CD133 cells *in vivo* : dystrophin complex rescue



Skipped muscle-derived CD133 cells *in vivo* : dystrophin complex rescue



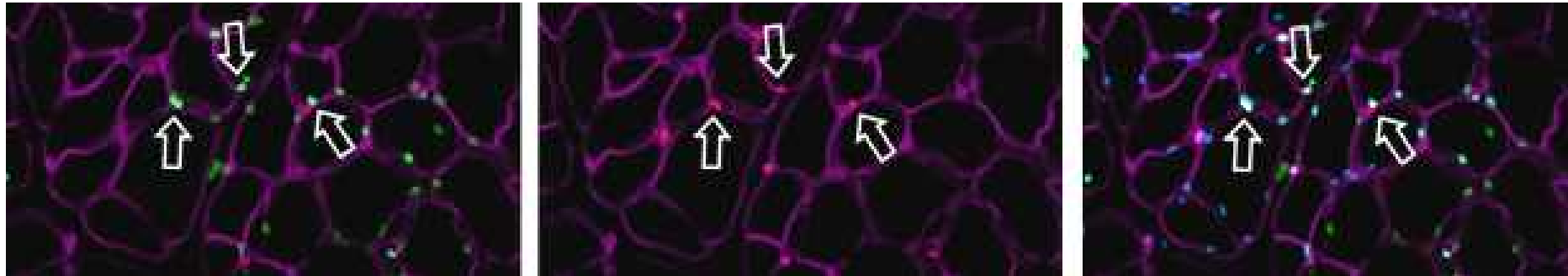
Skipped muscle-derived CD133 cells *in vivo* : dystrophin complex rescue



Skipped muscle-derived CD133 cells *in vivo* : muscle stem cell repopulation



$5 \cdot 10^4$ $\Delta 49-50$ muscle-derived CD133 cells transduced with Lenti U7-ex51-AON1/2



— Laminin
● Lamin A/C

— Laminin
● M-cadherin

MERGE

Skipped muscle-derived CD133 stem cells can localize to satellite cell positions

Patient and Clinical issues

- Need to develop approaches for all
- Different approaches for different stage of disease
- How approach rare mutation (personalized trial?)
- Limited number patients (specific mutation)
- Strategies with regulatory agencies
- The costs (15k \$/gr for morpholinos)
- Time required to move from rGMP to gGMP
- Lack agreement on outcomes measures (validation of quantitative assessment, large animals, human)

**Stem Cell Laboratory
Department of Neurological Sciences
University of Milan, Italy**



**Dr. Marzia Belicchi
Dr. Mirella Meregalli
Dr. Federica Colleoni
Dr. Razini Paola
Dr. Simona Maciotta
Dr. Silvia Erratico
Dr. Serena Barilla
Dr. Claire Navarro
Dr. Daniele Parolini
Dr. Andrea Farini
Dr. Alberto Lerario**

Dr. Yvan Torrente



Collaborations

Bosisio Parini Institute

Dr. D'Angelo G.
Dr Sironi M.
Dr Cagliani R.

Dep. Neurological Sciences of University of Milan

Prof. Moggio M.
Prof Comi G.P.
Dr. Prella A.
Dr. Lamperti
Ciscato P.
Fagiolari G.

Stem Cell Research Institute of Milan

Prof. Cossu G.
Dr. Sampaolesi M.
Dr. Tonlorenzi R.

UMR, CNRS 7000 Paris

Prof. Butler-Browne G.
Dr. Mouly V

University of Paris 7

Prof. Paulin D.
Inserm, CNRS
Dr. Garcia L.
Dr. Lévy N.

Università Laval, Sainte Foy, Canada

Prof. Tremblay J.

University of Pavia

Prof. Bottinelli R.
Dr. D'Antona G.

University of Verona

Dr Constantin G.

Genethon

Dr. Richard I.

Funds

