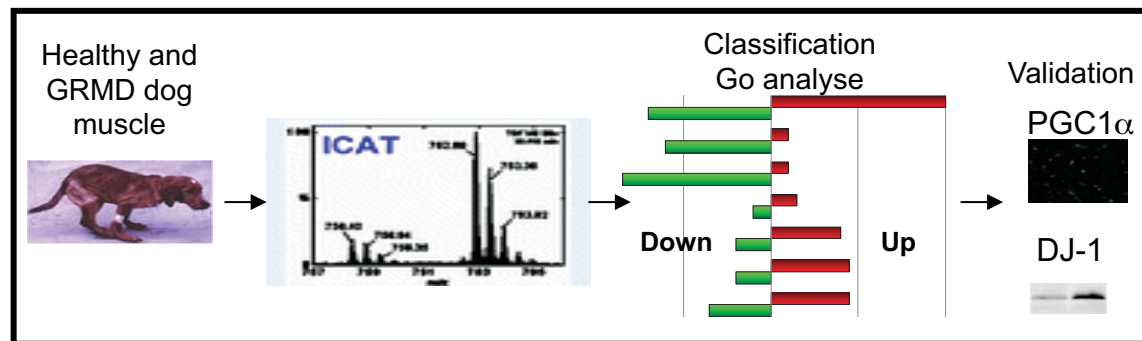


## Quantitative proteomic analysis of dystrophic dog muscle and strategy for the biochemical evaluation of experimental cell therapy



Laëtitia Guével



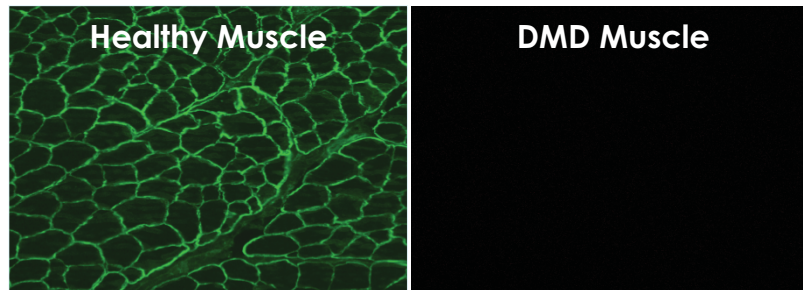
UNIVERSITÉ DE NANTES

# Duchenne Muscular Dystrophy

- A genetic muscular disease
  - X-linked recessive
  - Frequency: **1/3500 male births**
- **Mutation** on the dystrophin gene

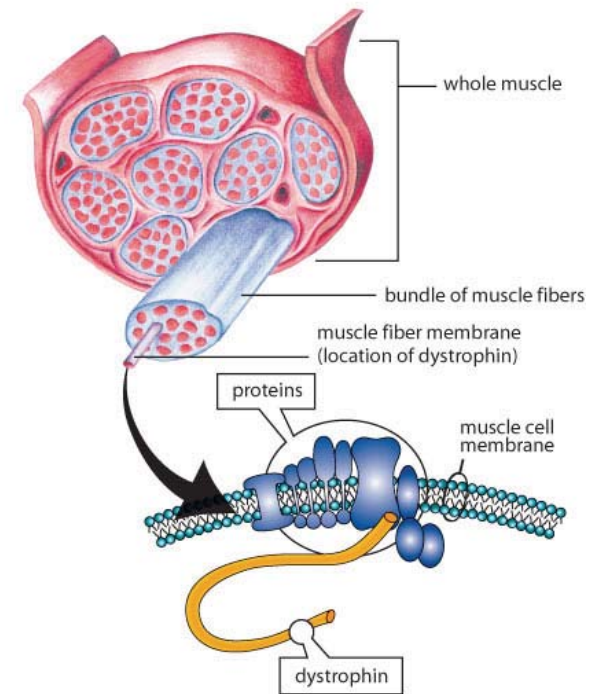
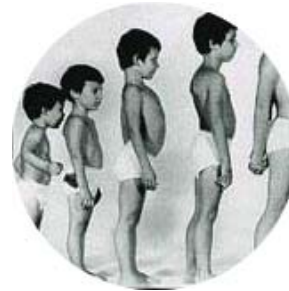


**Lack of dystrophin protein**



**DMD phenotype**

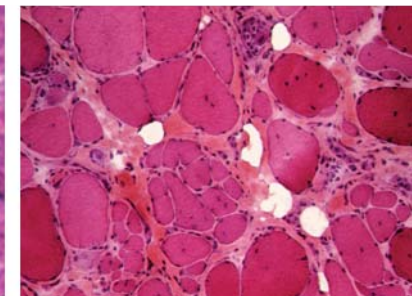
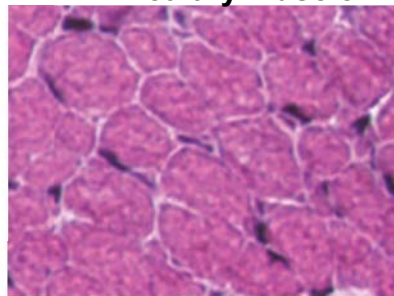
Necrosis of muscle fibers  
Progressive muscle weakness



Healthy Muscle

DMD Muscle

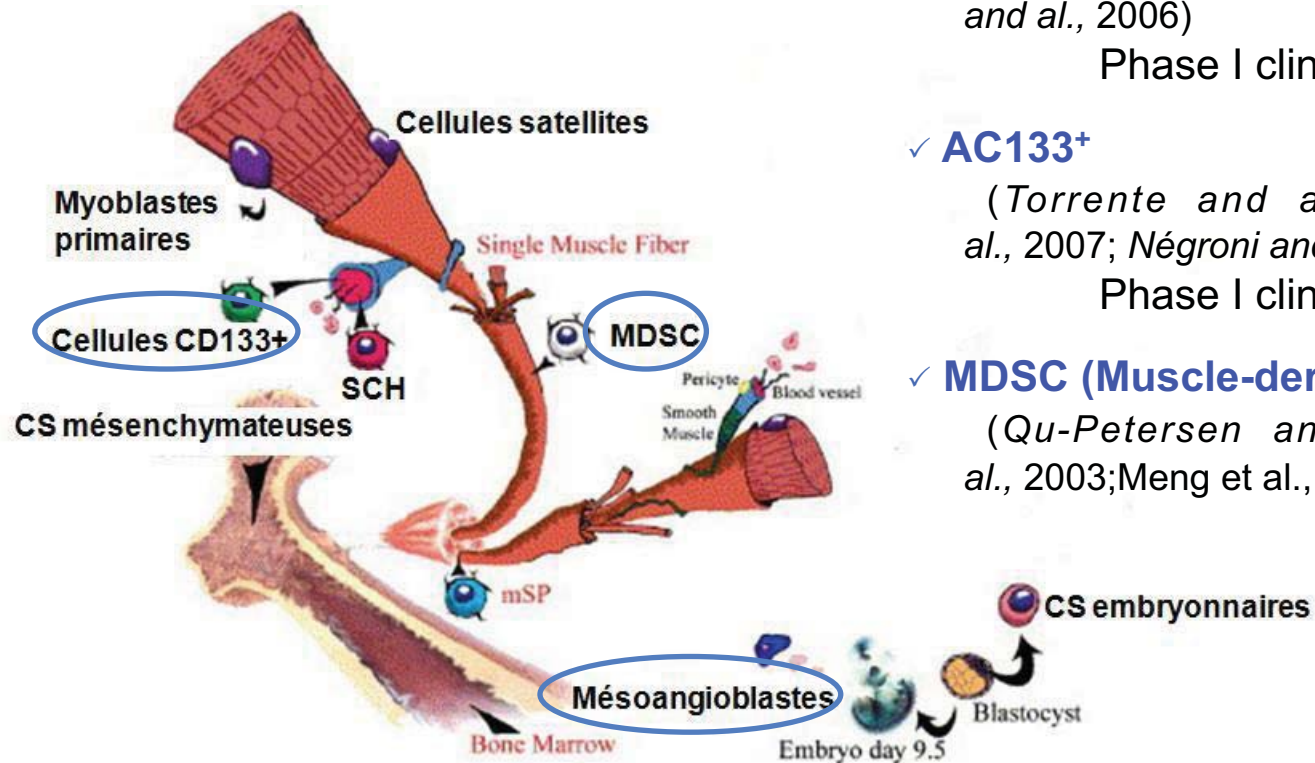
HES



- **No curative treatment**

# DMD Therapeutical approches

- Pharmacological treatments (*Garcia and al., 1990; Odom and al., 2008*)
- Gene Therapy (*Wang and al., 2007; Yuasa and al., 2007*)
- **Cellular therapy**
  - identification of adult stem cells: new therapeutical propositions



## ✓ Mésoangioblasts

(B.G. Galvez and al., 2006; *Sampaolesi and al., 2006*)

Phase I clinical trial (Tedesco et al., 2010)

## ✓ AC133+

(*Torrente and al., 2004; Benchaouir and al., 2007; Négroni and al., 2009*)

Phase I clinical trial (Torrente et al., 2007)

## ✓ MDSC (Muscle-derived stem cells)

(*Qu-Petersen and al., 2002; Torrente and al., 2003; Meng et al., 2011*)

# DMD Proteomic approaches

---

Absence of dystrophin  
in muscle

## Network of implicated proteins



Expression level (total proteins)



Post-translational modifications (phosphorylated proteins)

Aim 1

Identification of global protein perturbations in dystrophic muscle by proteomic approaches

Aim 2

Strategy for the biochemical evaluation of experimental cell therapy

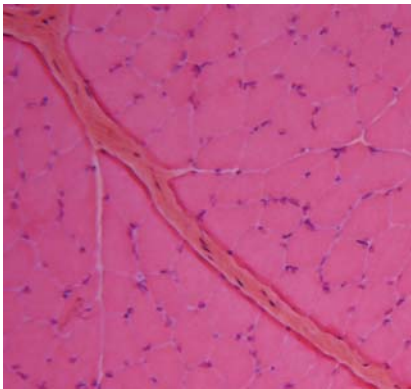
# Animal model



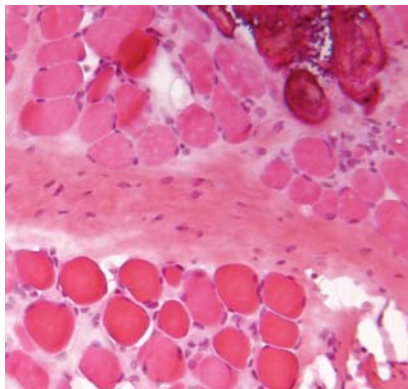
The GRMD dog: a large model,  
The pathophysiology is reminiscent of that of human DMD

Genotype : point mutation in intron 6, stop codon in exon 8  
(dystrophin gene)

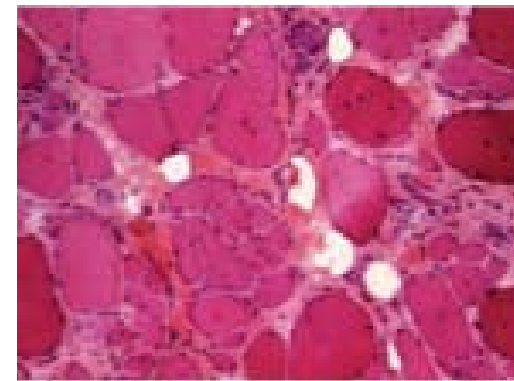
Healthy



GRMD

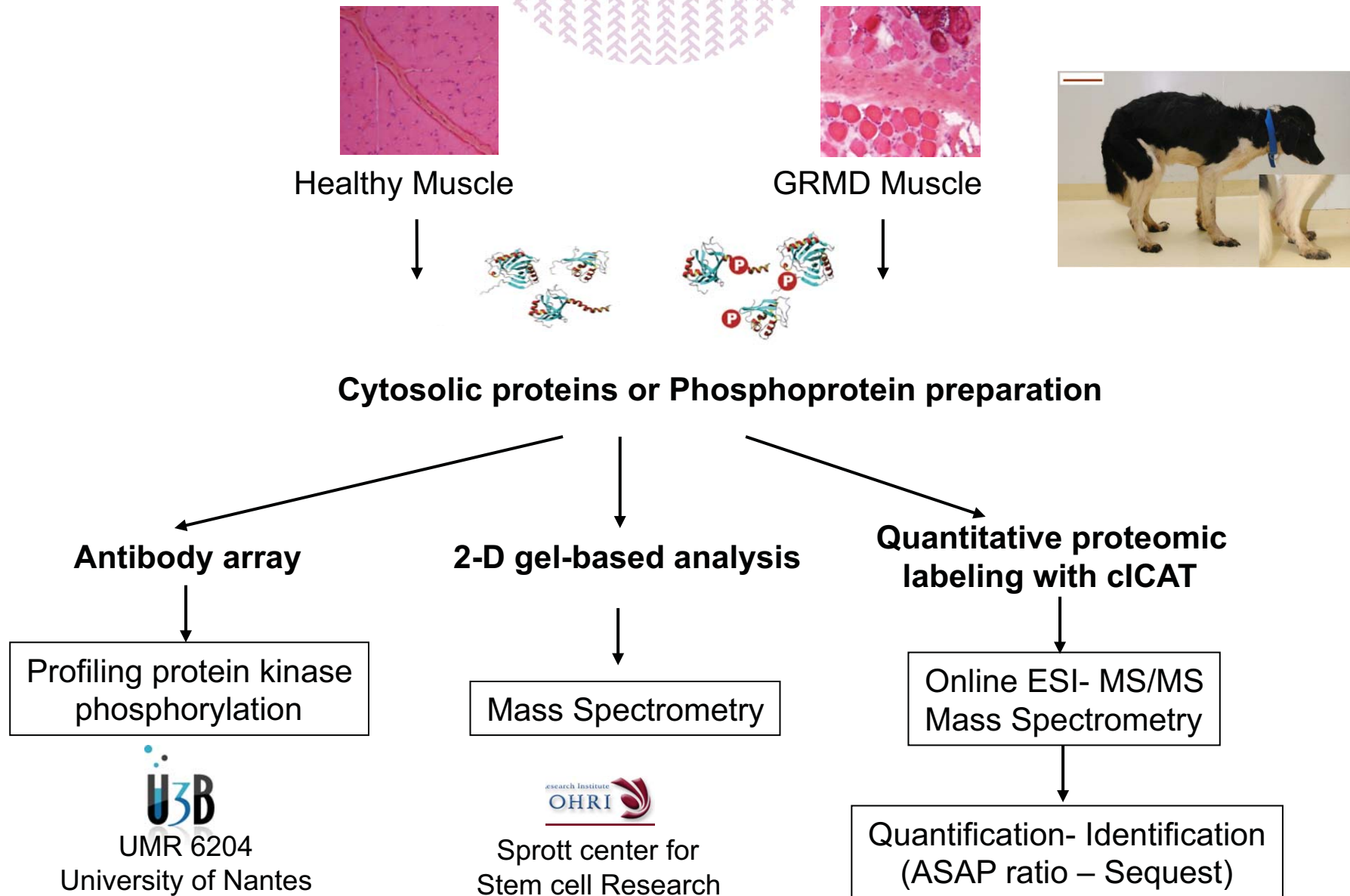


human biopsy DMD

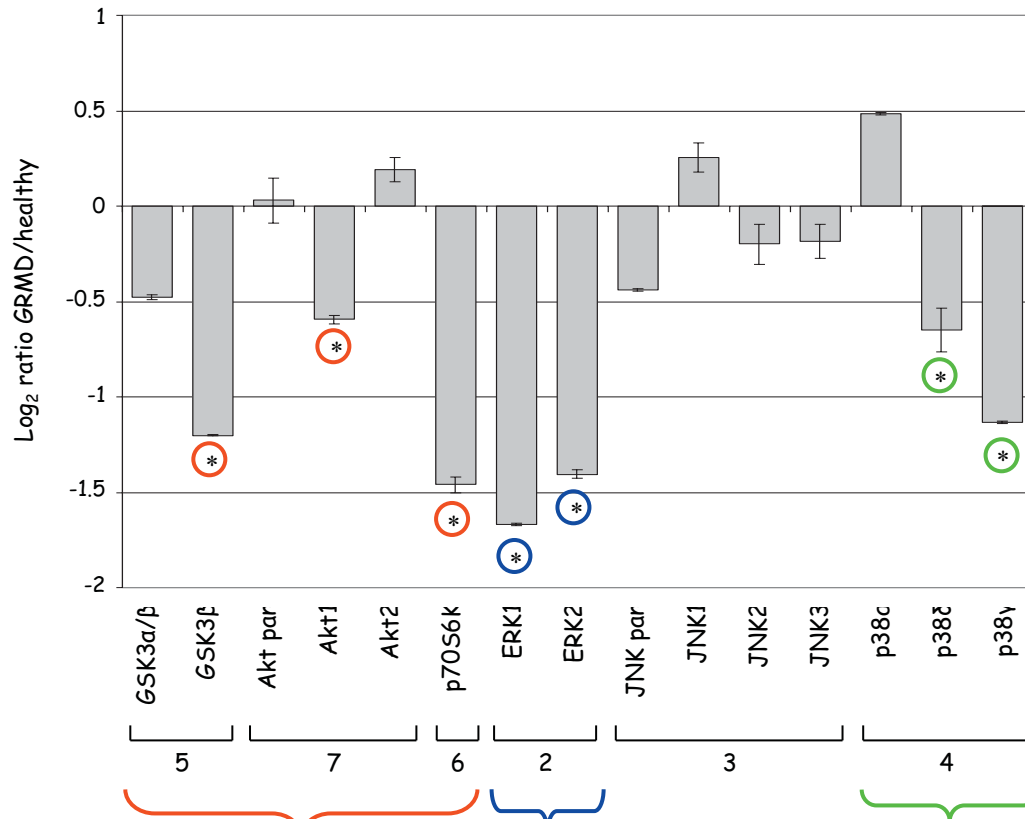


H&E

# Proteomic approaches



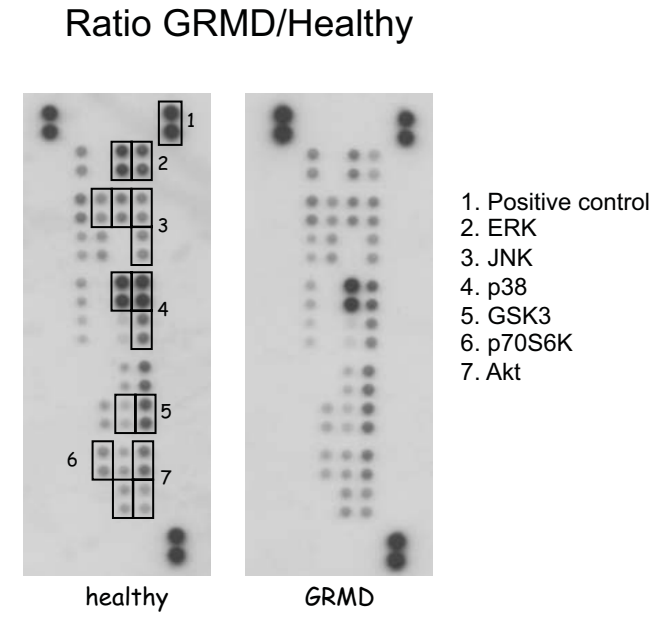
# Profiling protein kinase phosphorylation in skeletal muscle



**PI3K/Akt pathway**  
 Reduced phosphorylation of  
**GSK3β, Akt1 et p70S6K**

**ERK/MAPK pathway**  
 Reduced phosphorylation of  
**ERK1 et ERK2**

**p38/MAPK pathway**  
 Reduced phosphorylation of  
**p38δ et p38γ**

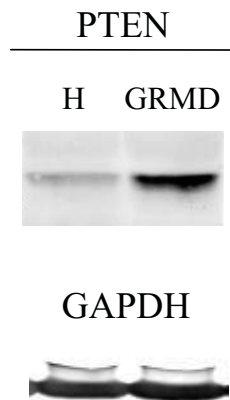


➡ **Global decreased phosphorylation in dystrophic skeletal muscle**

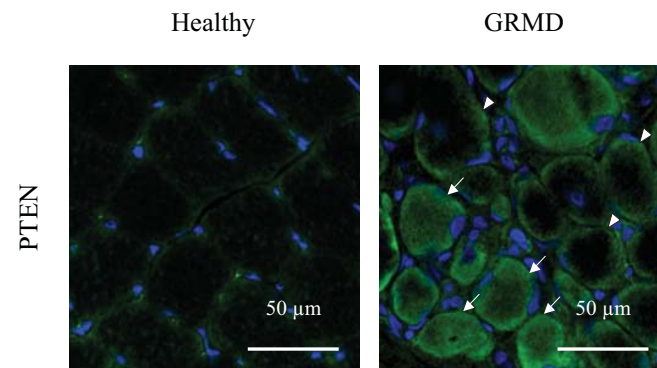
# Increased PTEN expression and activity in GRMD

- ➔ Analysis in key enzymes involved in Akt regulation
- PDK1 (phosphorylates Akt)
  - PP2A and PTEN (dephosphorylates Akt)

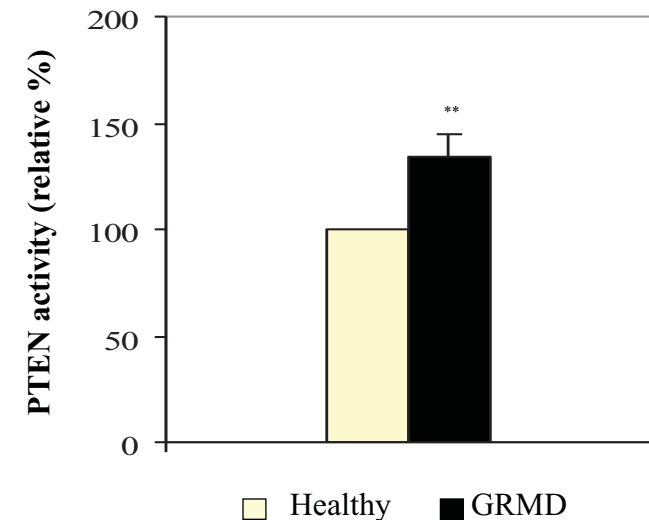
WB (total extract)



Immunohistochemical analysis



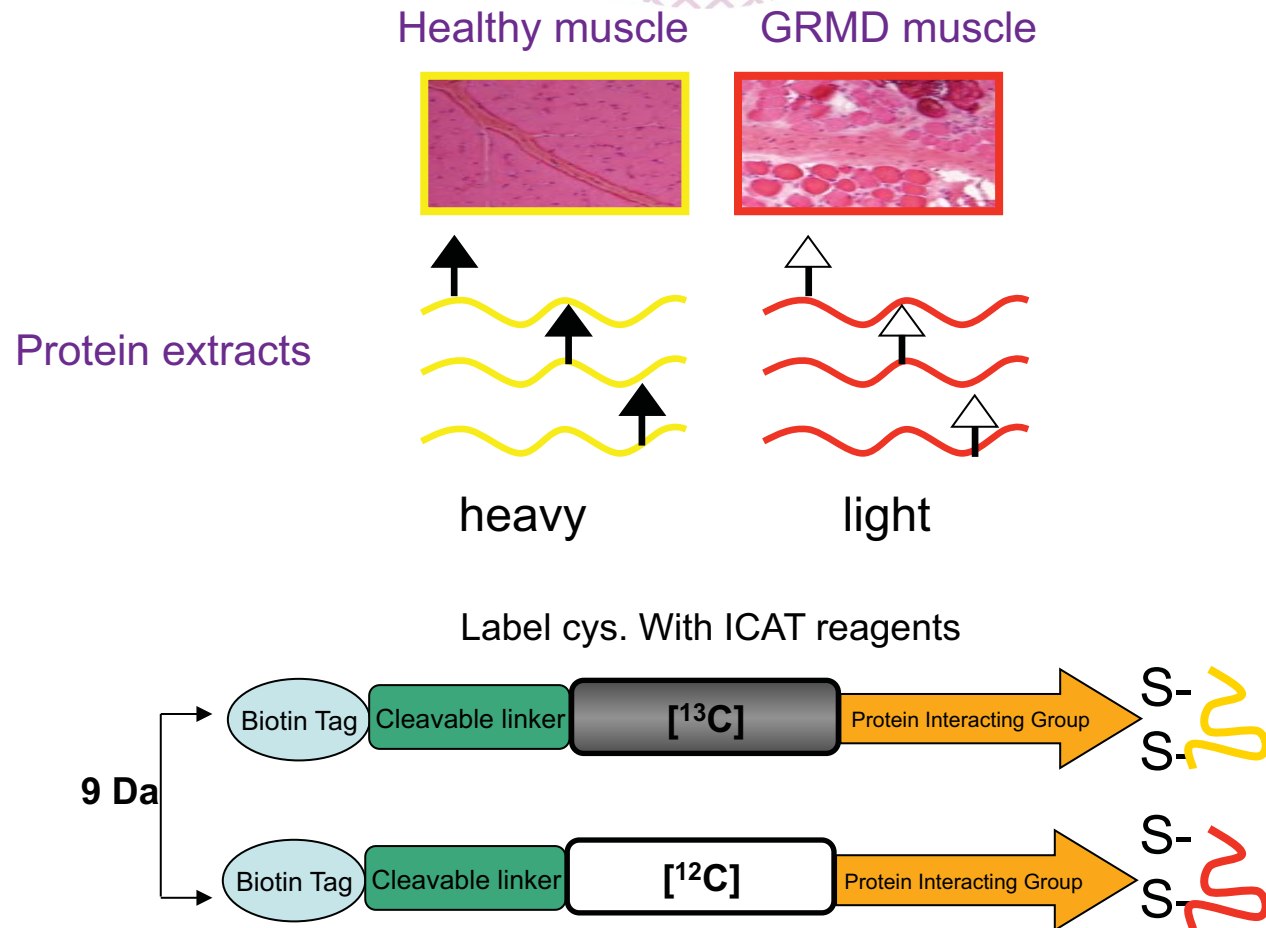
Phosphatase assay



➔ Increased PTEN expression and activity in GRMD muscle could be responsible of the PI3K/Akt signaling deregulation



# Protein profiling by the isotope-Coded Affinity Tags strategy

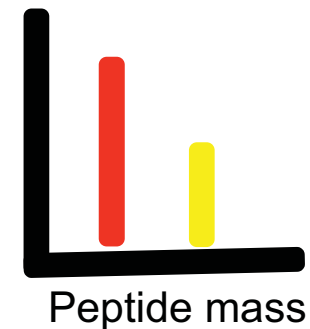
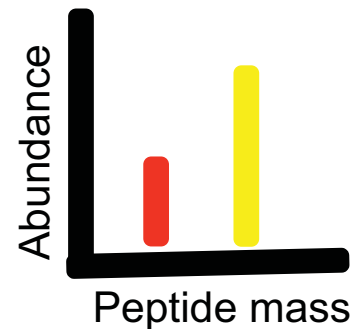
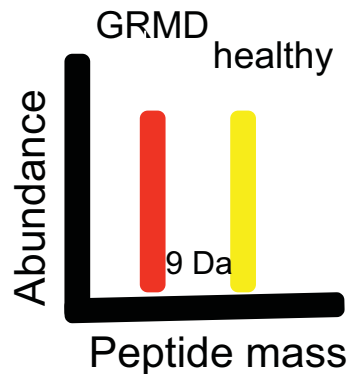


Combine and proteolyse (<sup>12</sup>C ICAT and <sup>13</sup>C ICAT labeled peptides)

Reduced sample complexity

# Protein profiling by the isotope-Coded Affinity Tags strategy

- Labeled peptide analysed by LC MS/MS



- Relative protein abundance from each peptide is quantify  
Identification of the correspondant protein on a computer cluster

Adapted from the Trans-Proteomic Pipeline at the Institute for Systems Biology in Seattle, WA- R Aebersold

**Validation**

PeptideProphet

**Protein  
Annotation &  
Validation**

ProteinProphet

**Quantification  
&  
Validation**

ASAPRatio

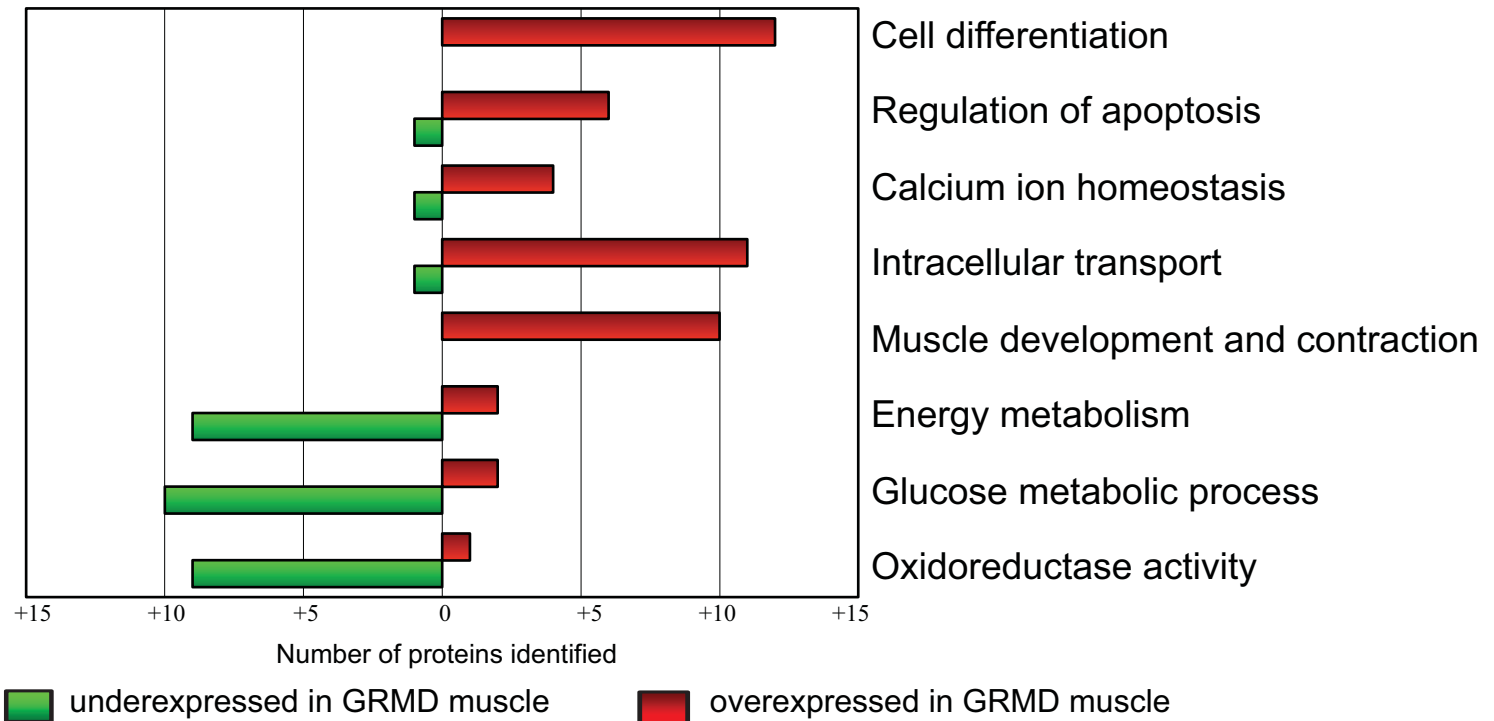
# Differentially expressed proteins revealed by ICAT

★ Cytosolic protein /ICAT

➔ 360 uniq peptides  
83 Identified protein

★ Phosphoprotein /ICAT

➔ 143 uniq peptides  
67 Identified proteins



➔ Global significant change in the analysis

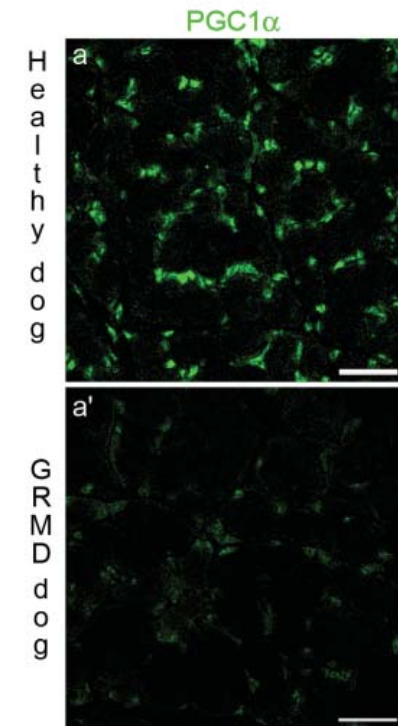
➔ Decreased protein set appeared primarily composed of metabolic proteins

## Decreased protein level of the PGC-1 $\alpha$ targets

Gene Symbol	Description
PGK1	phosphoglycerate kinase 1
PFKM	phosphofructokinase, muscle
PKM2	pyruvate kinase, muscle
MDH2	malate dehydrogenase 2
PGM1	phosphoglucomutase 1
PRDX3	peroxiredoxin 3
ACO2	aconitase 2, mitochondrial
ATP5A1	ATP synthase, H <sup>+</sup> transporting
CYC1	cytochrome c-1
FABP3	fatty acid binding protein 3
UQCRC1	ubiquinol-cytochrome c reductase
ACADS	acyl-Coenzyme A dehydrogenase
AK1	adenylate kinase 1

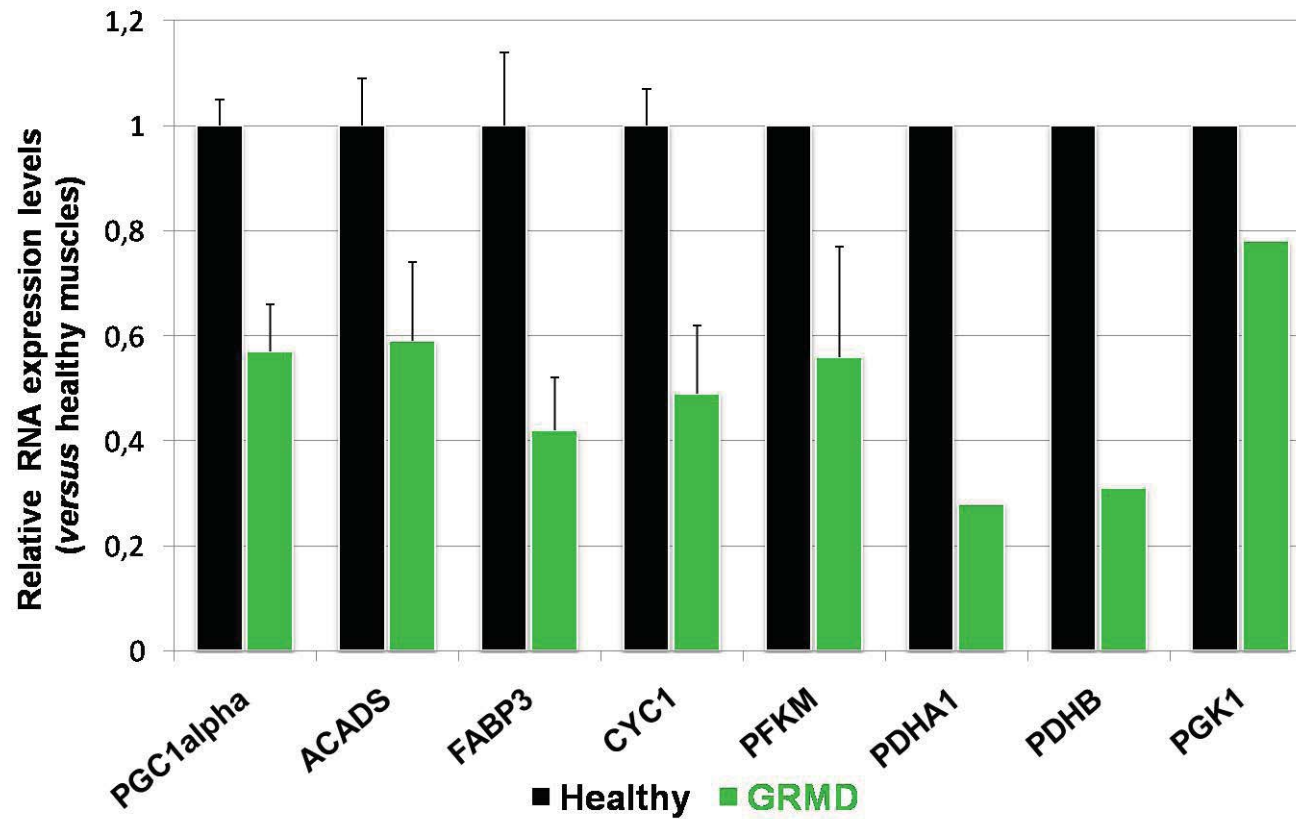
Putative PGC 1alpha related genes

*C. Perez-Iratxeta*



- ➡ 40% of the decreased proteins have been shown to be regulated by PGC-1 $\alpha$
- ➡ PGC-1 $\alpha$  expression is dramatically reduced in GRMD muscle

## Altered transcriptional regulation of PGC-1 $\alpha$



➔ The down-expression of PGC-1 $\alpha$  and most of its downstream putative target genes is significantly validated by real-time RT-PCR

## Conclusion

---

- ➔ Quantitative proteomic provides a sensitive approach to study skeletal muscle and the DMD pathology
- ➔ Defective energy metabolism is a hallmark of the disease progression
- ➔ PGC1 $\alpha$  may be at the origin of the general metabolic crisis that characterize this disease
- ➔ a well-defined set of signature molecule could serve the evaluation of experimental strategy

### 2. Evaluation of the experimental cell therapy

# Can systemic delivery of MuStem cells be efficient?

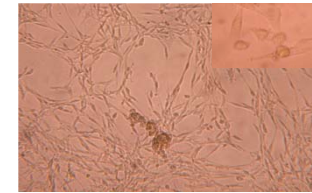
## Cellular Therapy

### Experimental protocol

- 3 immunosuppressed GRMD dogs
- Injection of  $1.10^7$  wild-type MuStem cells/kg
- 5 injections through left femoral artery



Healthy dog



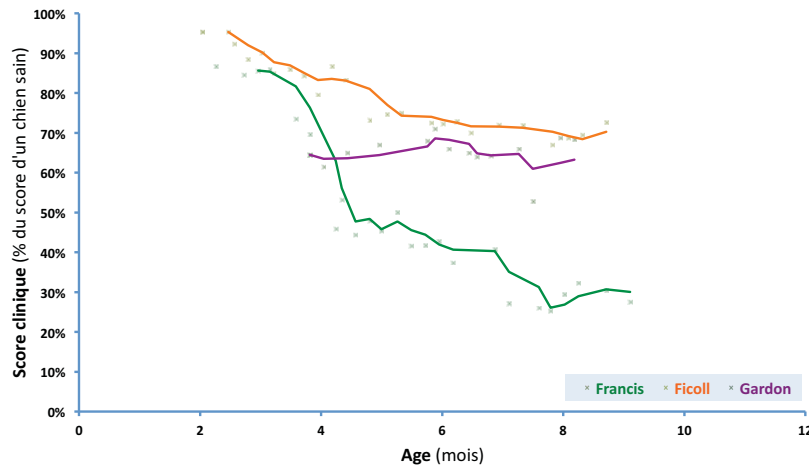
Primary culture with two distinct morphological cell types



GRMD

## Clinical score

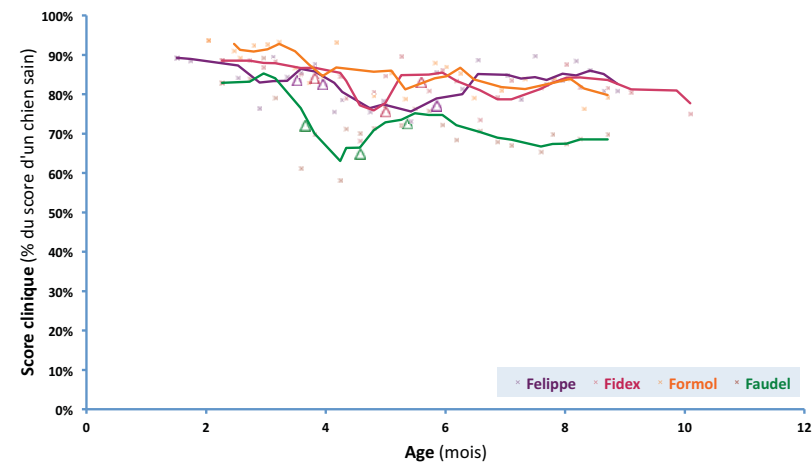
GRMD



Euthanasia: 9 months

## Persistent clinical status stabilization

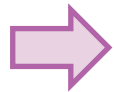
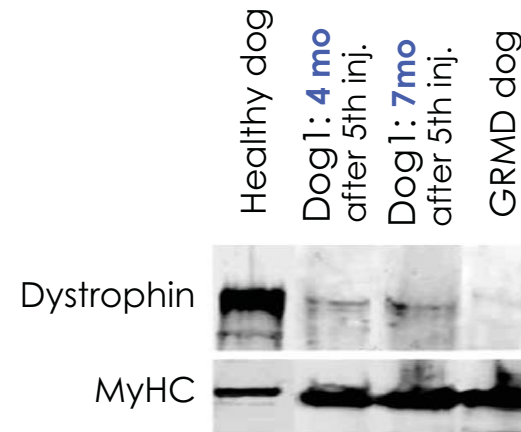
Treated GRMD



## Aim 2: Strategy for the biochemical evaluation of cell therapy

### Cellular Therapy :

Muscle fibers regeneration  
Long-term clinical improvement  
.... Low expression of dystrophin



**MuStem cells: potential candidates for cell therapy of DMD ?**

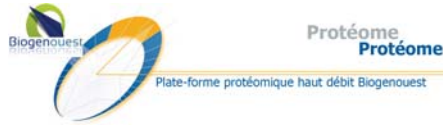
- To determine the molecular mechanism
- To establish a precise characterization of the biomarkers
- To evaluate the muscular regeneration

Proteomic analysis - Transcriptomic analysis - miRNA expression





# Strategy for the biochemical evaluation of cell therapy

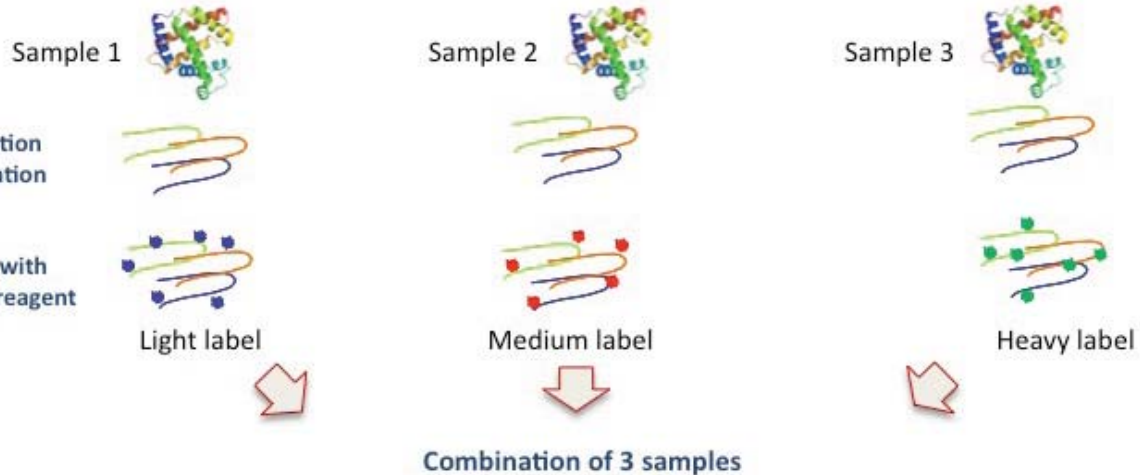


## Quantitative proteomic analysis : ICPL

Healthy n=3

GRMD n=3

Treated GRMD n=3



Reduction of complexity by gel fractionation

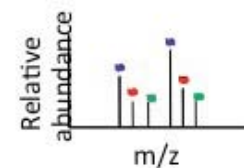
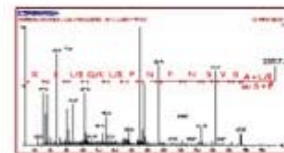


1D electrophoresis

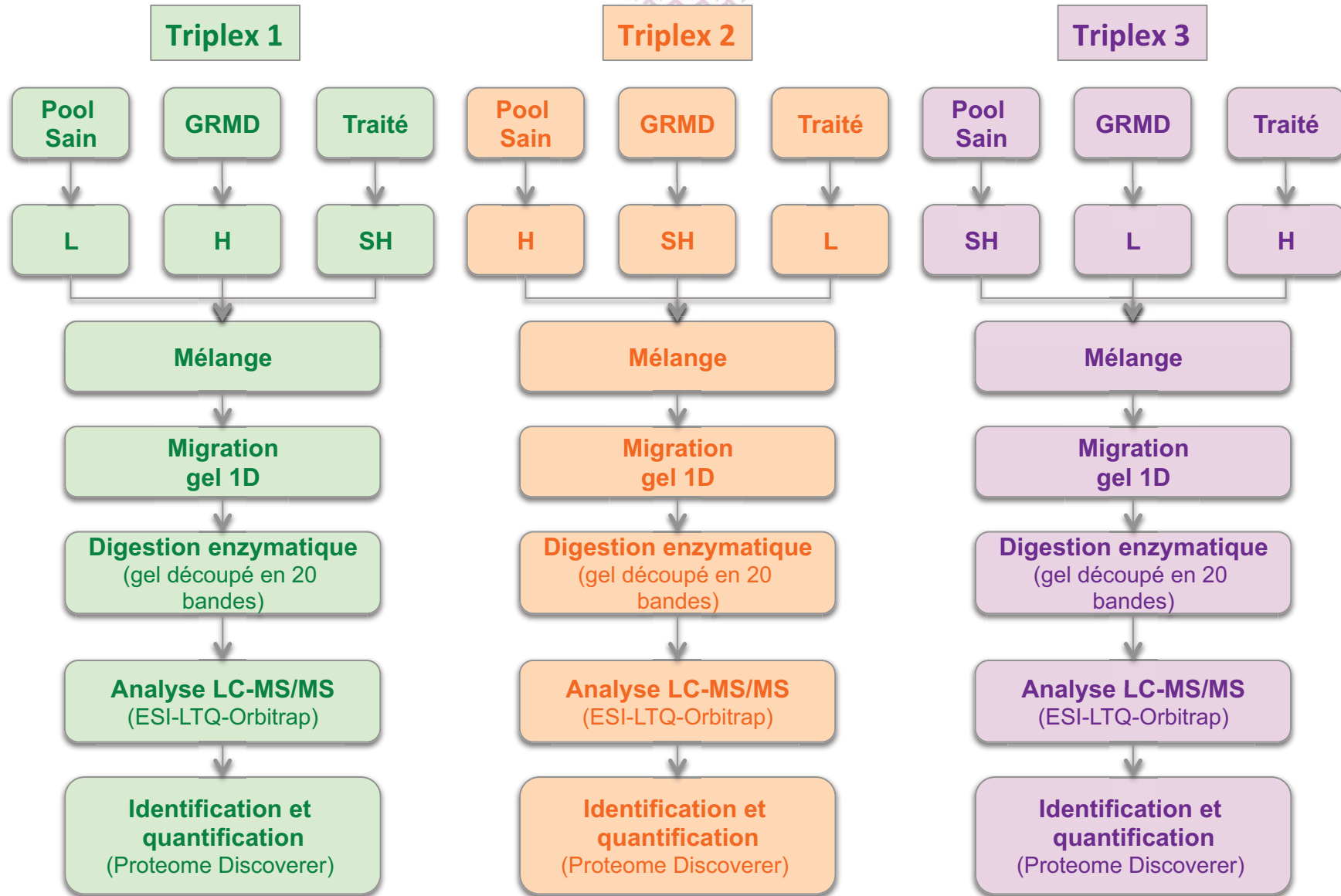
Enzymatic digestion of proteins into peptides

Mass spectrometry (identification & quantification)

LTQ-Orbitrap

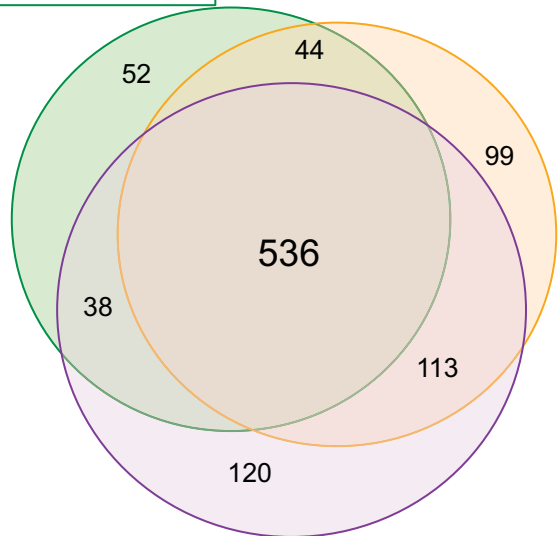


# Experimental design



## Work in progress...

Triplex 1 (670 proteins)



Triplex 2 (792 proteins)

Triplex 3 (807 proteins)

## 536 quantified Proteins (3 Triplex)

GRMD/Sain	Triplex 1	Triplex 2	Triplex 3
Up	114	123	137
Down	112	177	172

Global analysis (3 Triplex):  
79 underexpressed in GRMD  
75 overexpressed in GRMD

Only one variable...

Pool GRMD ( average ratio GRMD/Healthy)

And...

- analyzed ratio Treated GRMD/Healthy
- and Treated GRMD/ average GRMD

Global analysis : Effect of treatment  
8 underexpressed after treatment  
10 overexpressed after treatment

....

# Conclusion

Plateformes  
Biogenouest :  
Protéomique  
Transcriptome  
Génomique

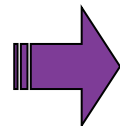
Grants:  
*Européen (FEDER)*  
*AFM*

Centre de Références  
des Maladies  
Neuromusculaires  
Rares  
(*CHU de Nantes;*  
*Pr Y. Péréon*)

This project will allow to:

- ✓ Establish a precise characterization of the physiopathology of DMD
- ✓ Validate the cellular therapy in GRMD dogs
- ✓ Evaluate the proteome after treatments

**hMuStem Cells**



Clinical Trial for DMD



MERCI à TOUTES les personnes impliquées  
dans ce projet



Karl **ROUGER**; Florence **ROBRIQUET**; Thibaut **LARCHER**; Yan **CHEREL**;  
Laurence **DUBREIL**; Judith **LORANT**; Céline **ZUBER**; Isabelle **LEROUX**;  
Mireille **LEDEVIN**; Marie-Anne **COLLE**



Protéome  
Protéome

Charles **PINEAU**; Mélanie **LAGARRIGUE**; Blandine **GUEVEL**



Marie **FERON**; Adrien **HERLEDAN**; Cathy **CHARLIER**

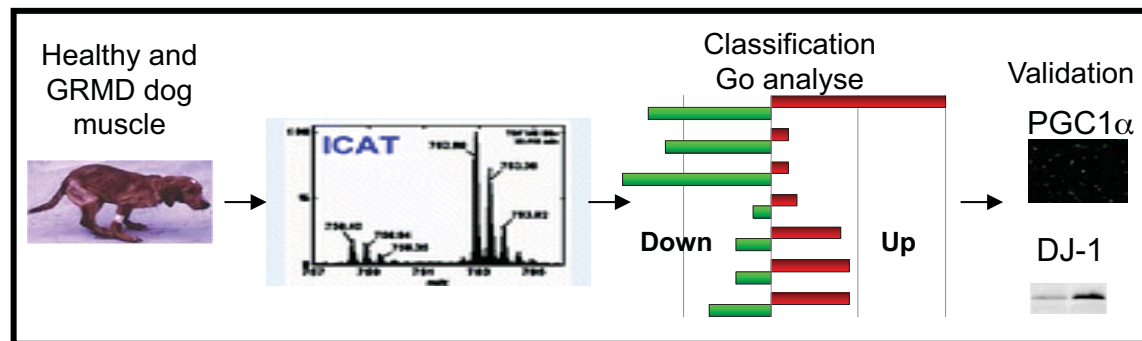


Lynn **MEGENEY**; Marjorie **BRAND**; Carolina **PEREZ-IRATXETA**; Jessie **LAVOIE**





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