Gene therapy for Oculopharyngeal Muscular Dystrophy

Alberto Malerba

World muscle society annual conference 06-10-2017

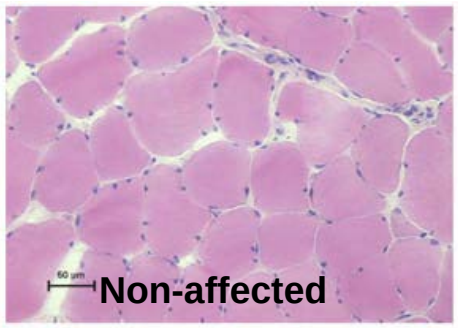
Oculopharyngeal Muscular Dystrophy (OPMD)

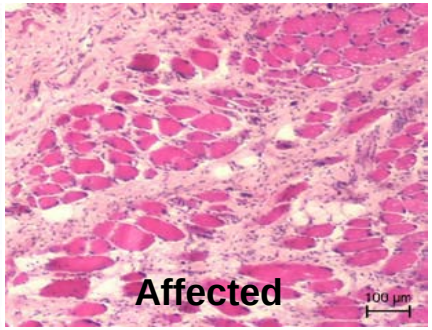
- Autosomal dominant disease: 1:100000 in Europe, 1:1000 in French/canadian population

- Typically onset occurs in the fifth to early sixth decade of life

- Phenotype characterized by:
  a) Progressive eyelid drooping
  b) Swallowing difficulties
  c) Proximal limb weakness

- Histology characterized by:
  - Decrease in fibre number
  - Variation in fibre size
  - Fibrosis
  - Intranuclear inclusions (INIs)



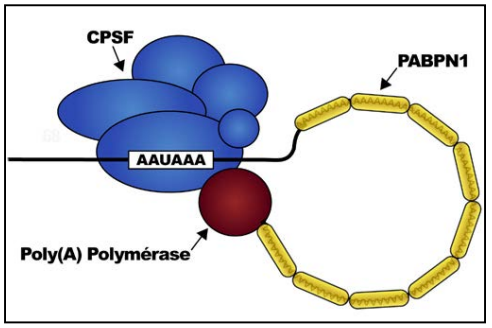


Mutation of PABPN1 leads to INIs

OPMD is due to expansion of the short (GCG) trinucleotide repeat in the coding sequence of the polyA binding protein nuclear 1 (PABPN1)

PABPN1: a ubiquitous protein that controls:

1) The length of mRNA poly(A) tails,
2) The mRNA export from the nucleus,
3) The alternative poly(A) site usage.



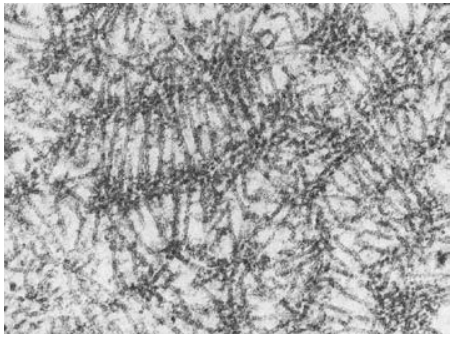
WT ATG $(GCG)_6$ ----------$(GCA)_3$ GCG GGG GCT GCG..
MUT ATG $(GCG)_6$ $(GCG)_{2-7}$ $(GCA)_3$ GCG GGG GCT GCG...--

12-17 ala instead of 10→expPABPN1

↓

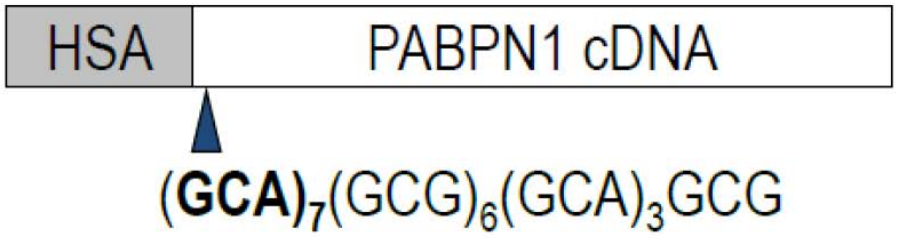
Intranuclear inclusions (INIs)
In skeletal muscle

- Resistant to degradation
- Trapping RNAs, proteins….wtPABPN1!!!



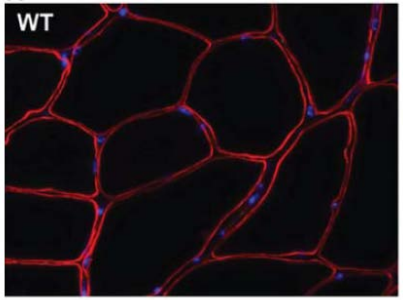
Tomé & Fardeau, 1980

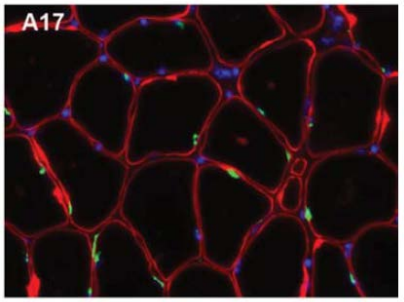
The A17 OPMD mouse model



Created with insertion of an expanded bovine PABPN1 driven by the human skeletal actin promoter

→Massive gene deregulation
→Severe muscle atrophy
→Mimics many pathological observations in human:
- Progressive muscle weakness/ Atrophy/Fibrosis
- Mitochondrial / Ubiquitin-Proteasome defects
- All muscles contain INIs





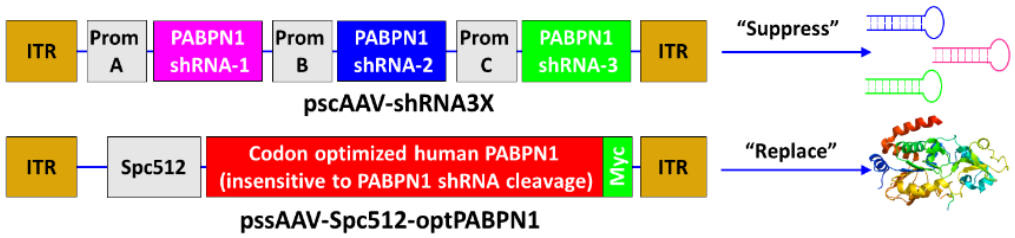
Davies et al, Nature Medicine 2005
Trollet et al, Human Molecular Genetics 2010

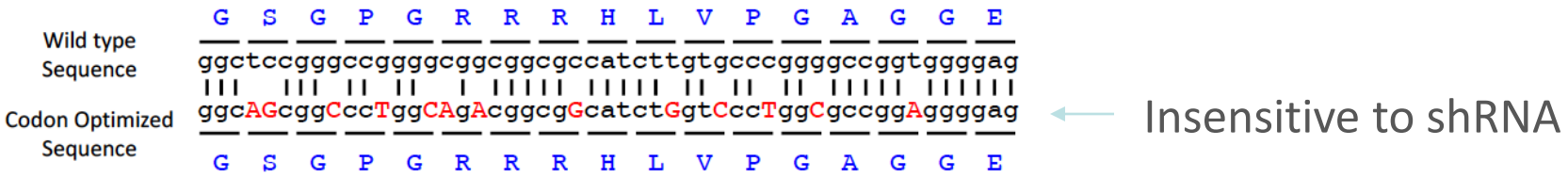
Gene therapy approach

Impossible to specifically target expPABPN1

→Suppression of endogenous PABPN1 (both normal and expanded)

→Replacement with functional optPABPN1

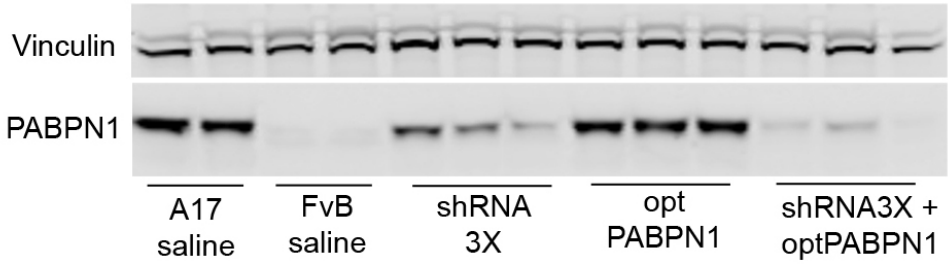


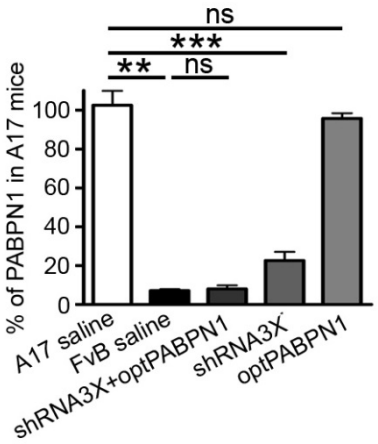


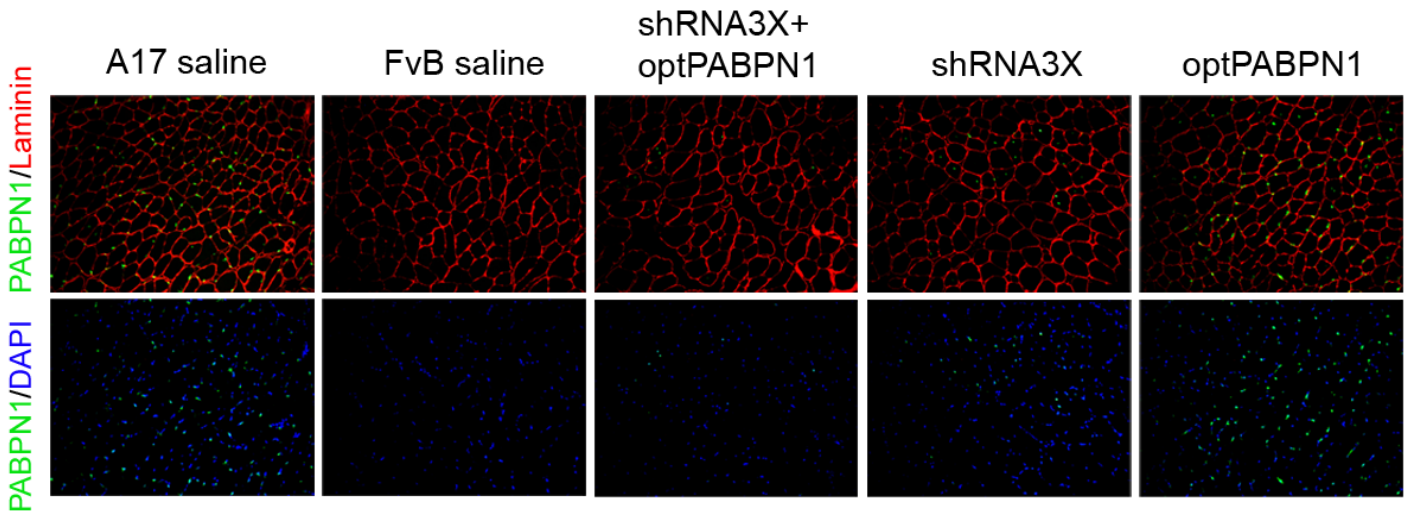
IM Injection in *Tibialis Anterior* (TA) of 10-12 week old A17 mice

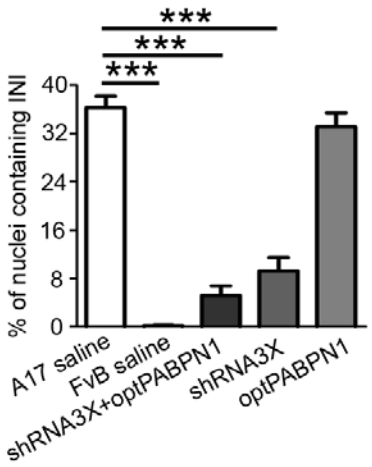
- AAV-shRNA3X ($2.5x10^{10}$ vp/TA)
- AAV-optPABPN1 ($1.3x10^{11}$ vp/TA)
- AAV-shRNA3X ($2.5x10^{10}$ vp/TA) + AAV-optPABPN1 ($1.3x10^{11}$ vp/TA)
- saline injection in TA of A17 and FvB mice as control

Effect on intranuclear inclusions





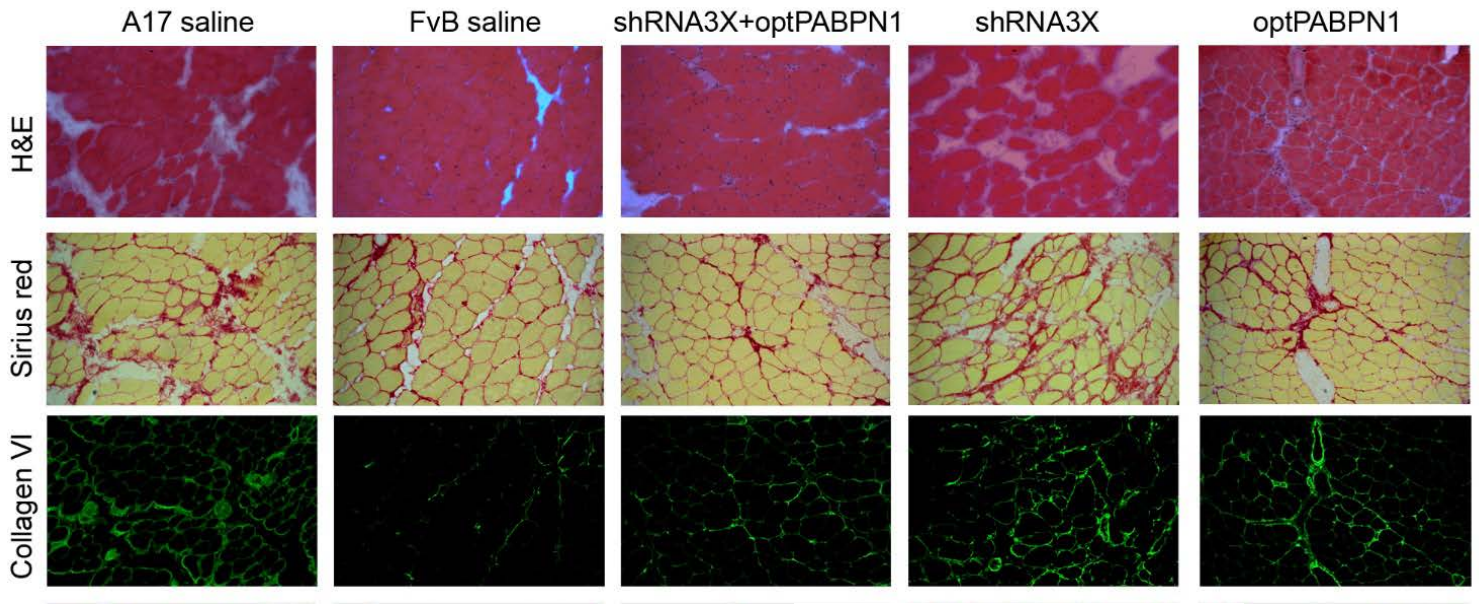


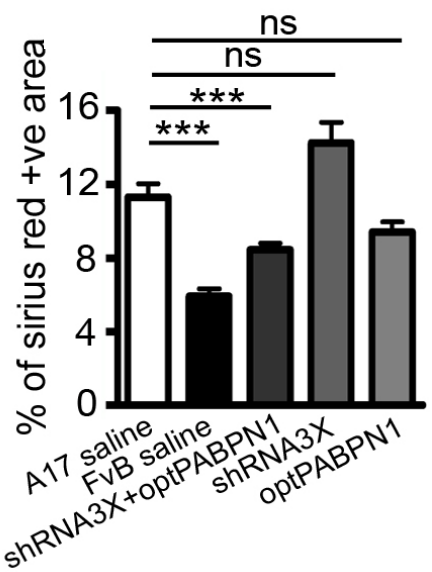


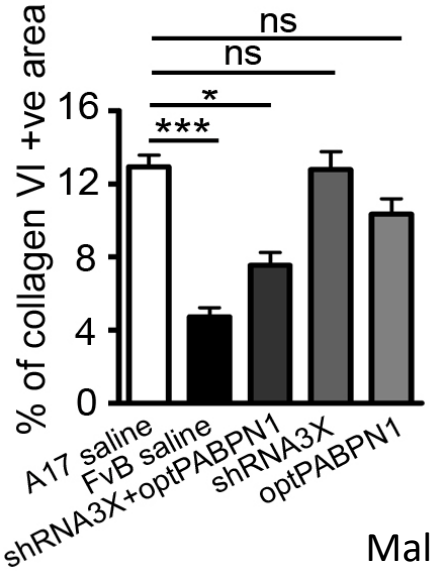
Treatment with KCl 1M to eliminate soluble aggregates

Malerba et al, Nat Comm 2017

GT treatment decreases fibrosis



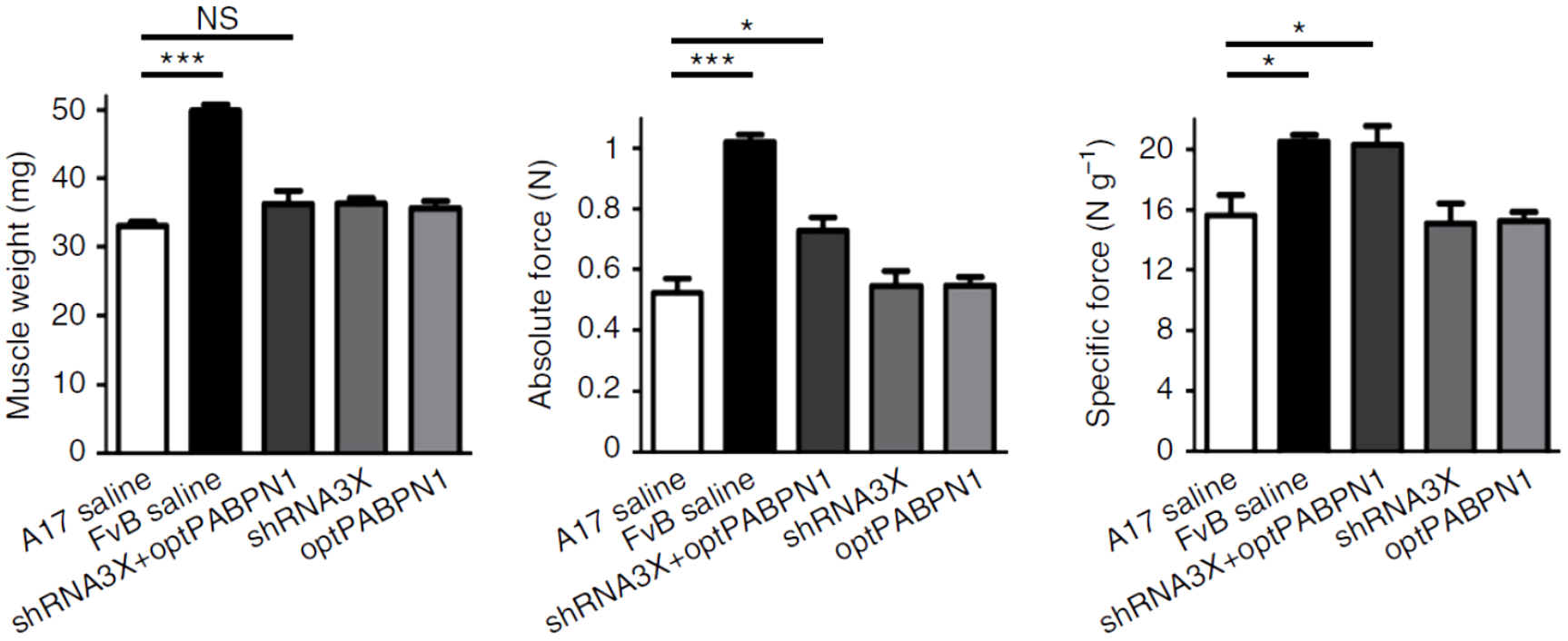




Malerba et al, Nat Comm 2017

GT treatment improves muscle strength

In situ-muscle force measurement



→**Increase in maximal force**

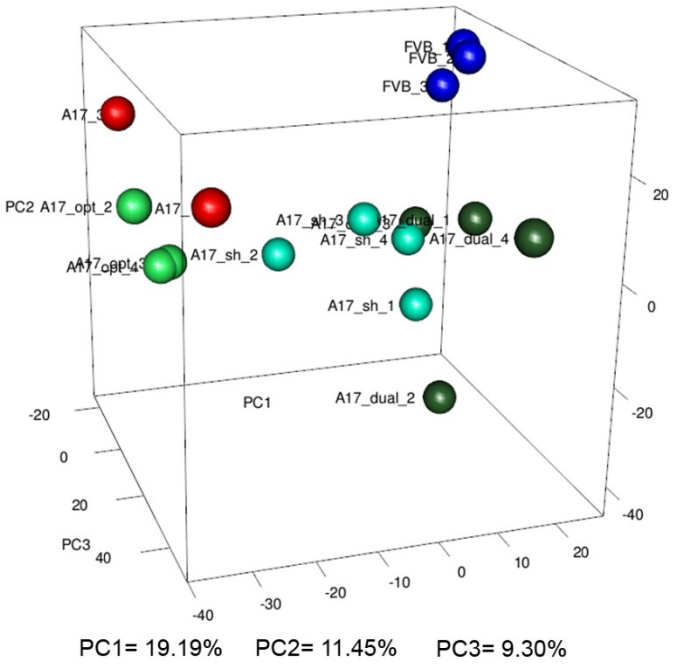
→**Normalization of specific maximal force to wild type level**

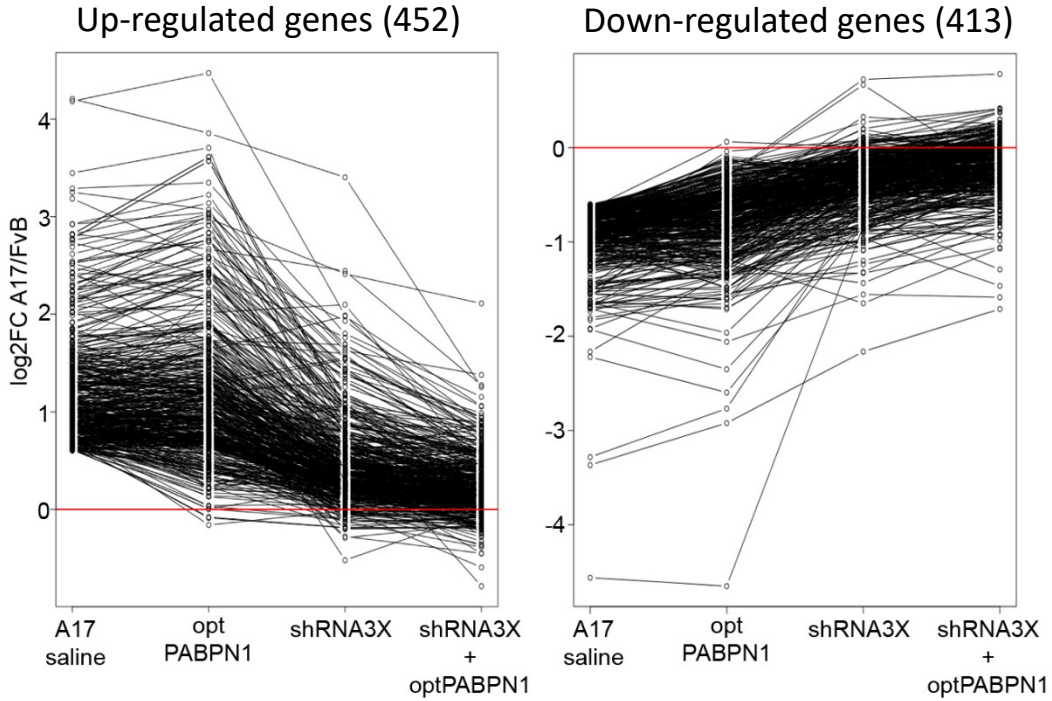
Malerba et al, Nat Comm 2017

GT treatment normalises the transcriptome (Affymetrix analysis)

expPABPN1 expression in A17 mice causes extensive remodelling of muscle transcriptome

(Trollet et al. *HMG* 2010; Anvar et al. *Sk Muscle* 2011; Chartier et al. *Plos Genet* 2015)



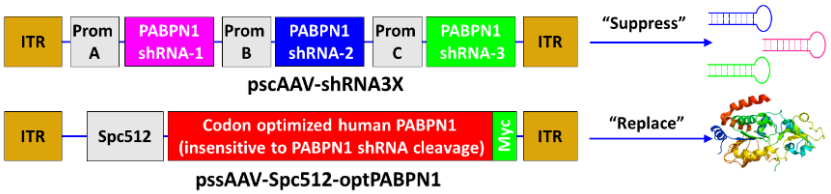


In A17 mice vs FvB, 865 transcripts were deregulated (FC>1.5; p<0.05)

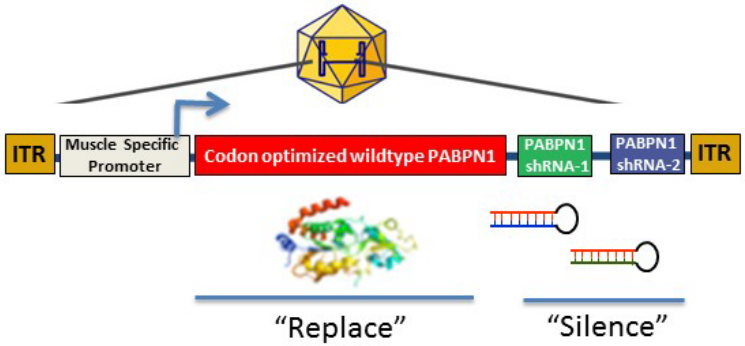
Treatment with shRNA3X+optPABPN1 results in 98% "correction"

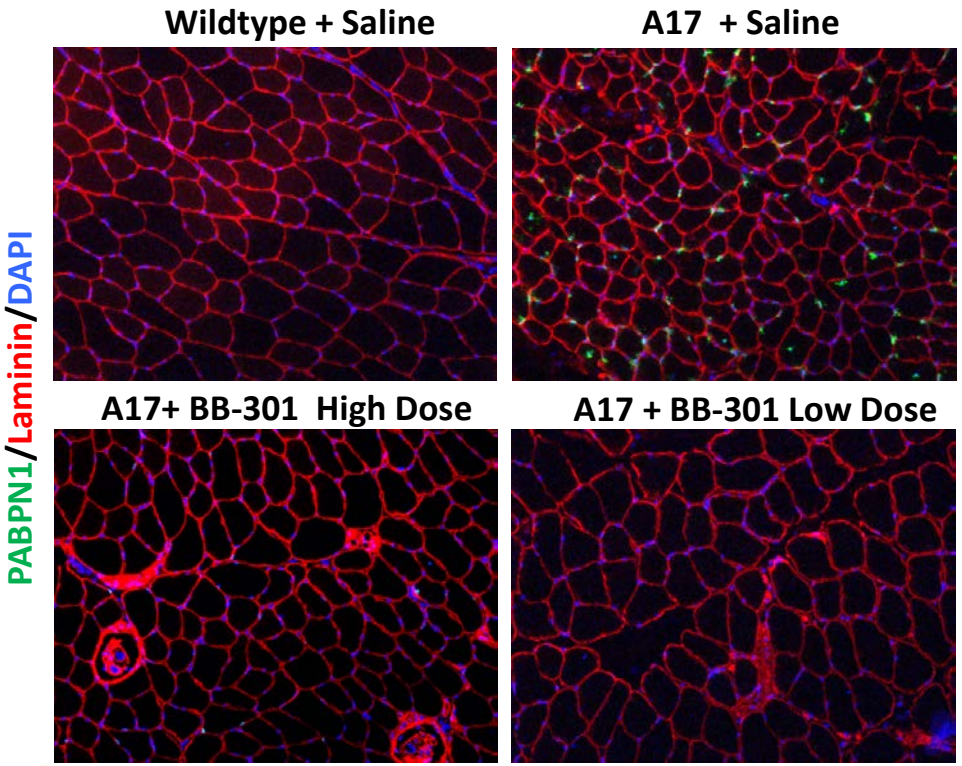
Malerba et al, Nat Comm 2017

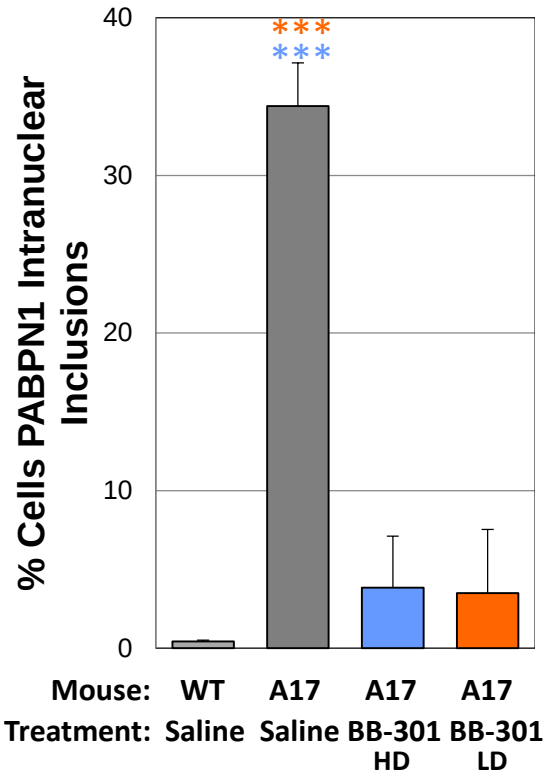
From 2 AAVs to single BB-301 vector



BB-301

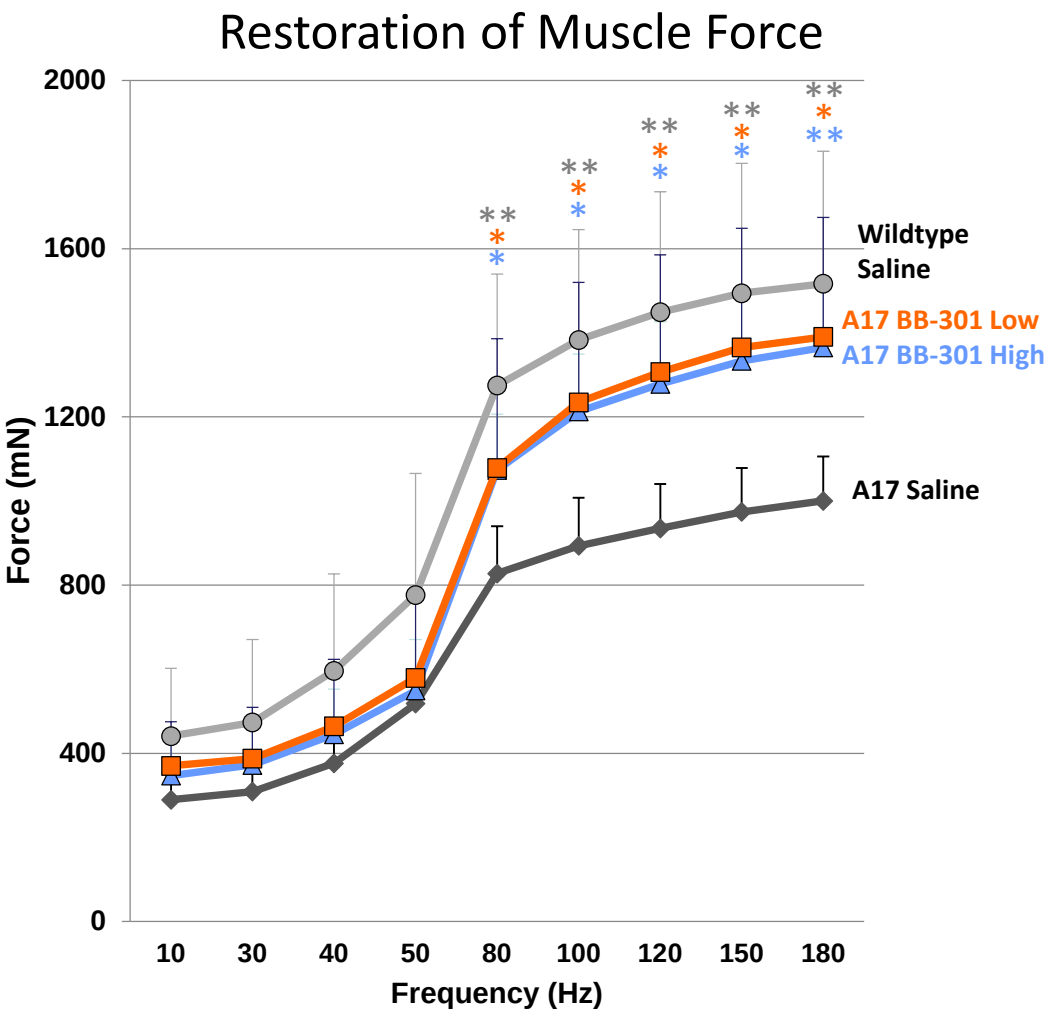


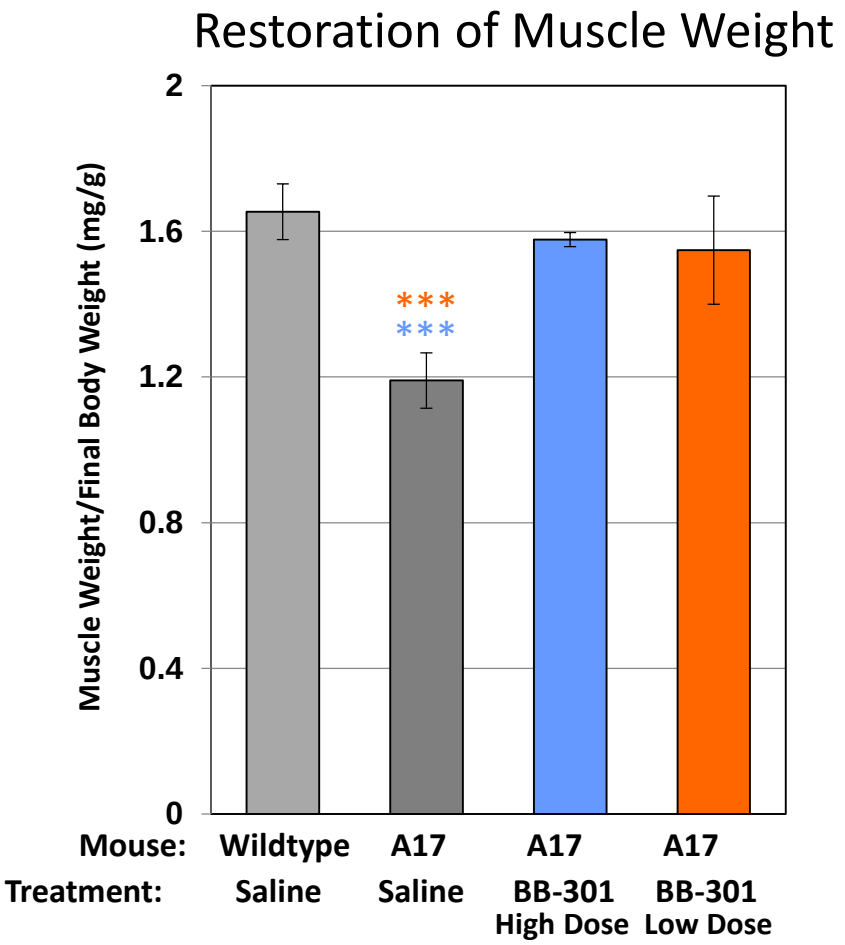




BENITEC BIOPHARMA

BB-301 Treatment Restores Muscle Force and Muscle Weight in A17 Mice





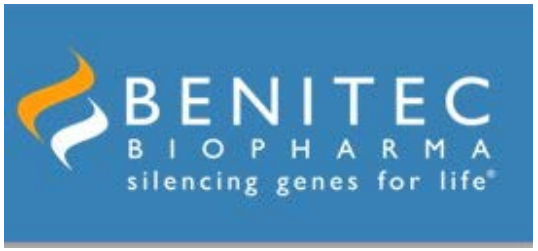
BENITEC BIOPHARMA

Conclusions

The gene therapy treatment:

1) Efficiently down-regulates expPABPN1 without affecting optPABPN1 expression
2) Abrogates insoluble intranuclear aggregates
3) Decreases fibrosis
4) Improves muscle strength
5) Completely recovers muscle mass (BB-301 vector)
6) Nearly normalizes the transcriptome (98% of gene expression is restored)
7) Single BB-301 vector shows great efficacy and allows clinical translation in human

Acknowledgments



Centre for Biomedical Sciences
Prof George Dickson
Dr Ngoc Lu-Nguyen
Dr Houria Bachtarzi
Dr Susan Jarmin
Dr Helena Chaytow
Pradeep Harish
Dr Linda Popplewell

Comparative Biomedical Sciences
Dr Ornella Cappellari

Benitec Biopharma
Dr David Suhy
Dr Vanessa Strings
Dr Michael Graham

Myology Research Center, UMRS974 (Paris)
Dr Capucine Trollet
Prof Arnaud Ferry
Dr Gillian Butler-Browne
Fanny Roth
Dr Pierre Klein