Silencing of Gnas R201C in patients with fibrous dysplasia using dCas9

VESCICLES WITH CONSEQUENT GSAR201 DECREASE AND REVERSION OF CELLULAR AND BONE TISSUE PHENOTYPE.

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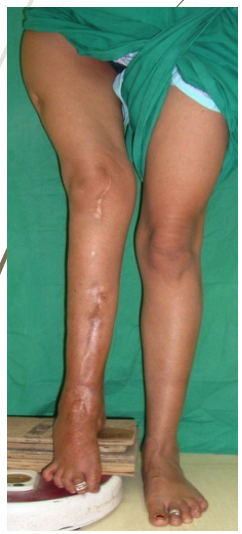
What is Fibrous Dysplasia?

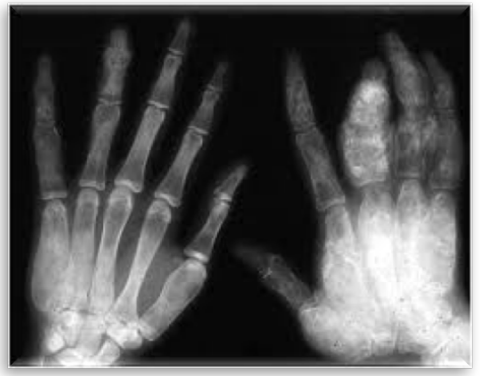
Skeletal progenitor cells in Fibrous Dysplasia (FD) disease aren't able to build wt bone since FD affect osteoclastic and osteoblastic genesis.

FD may affect one or more bones depending on Monostotic or Polyostotic phenotype.

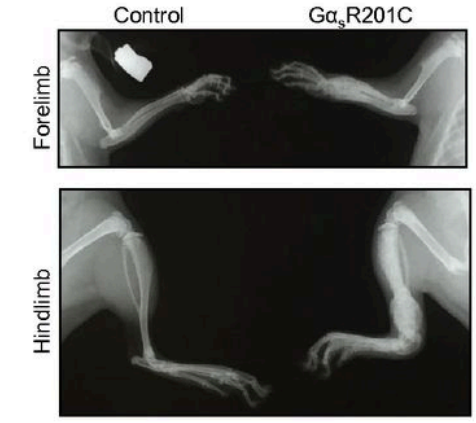


It's difficult to have a early diagnosis because FD has no direct symptoms.

Diagnostic method:
Imaging and histology analysis.
Radiography

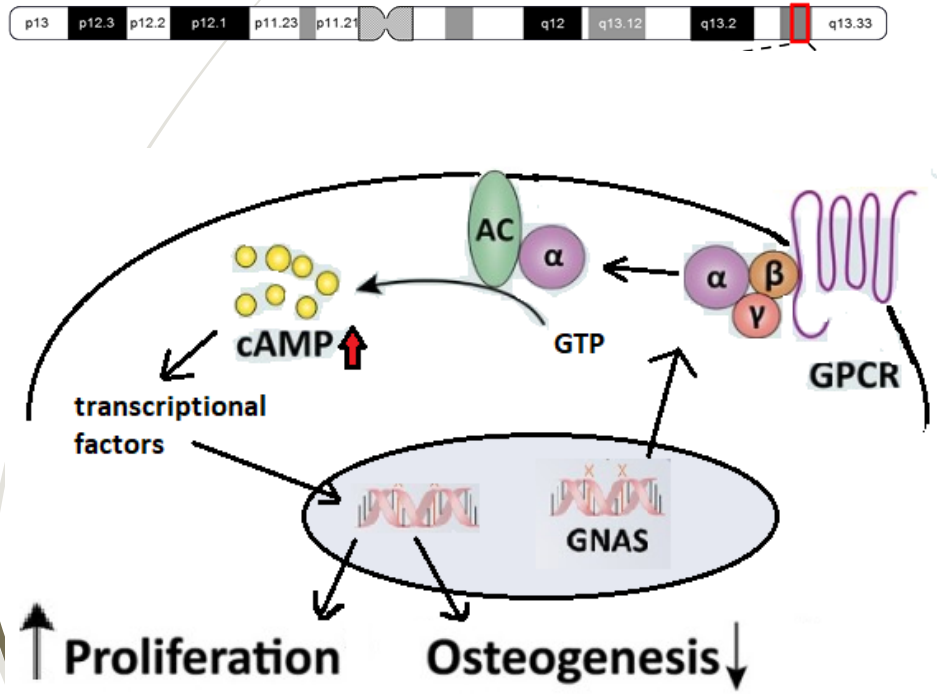


Human radiography



Mice radiography

What are Fibrous Dysplasia genetical mutation?



Fibrous dysplasia is caused by R201C mutation in the gene GNAS1 (gene locus:20q13.32).
R201C is a gain of function mutation and consist in:

-Point mutation in exon 8.

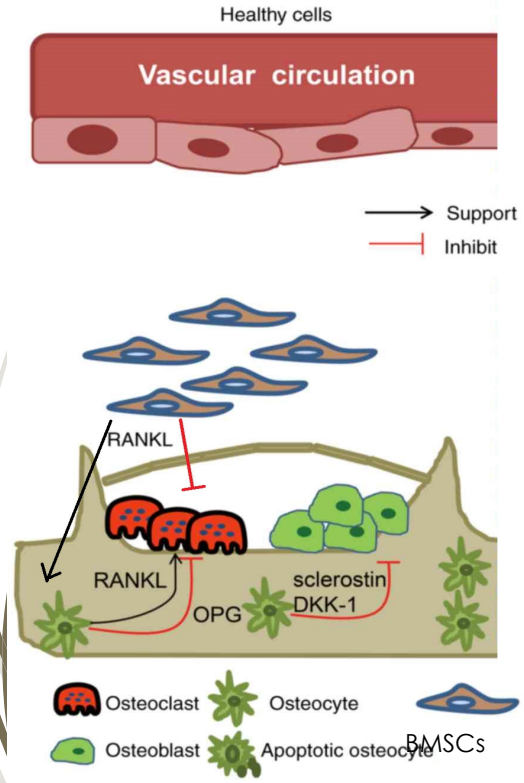
-Arg201 is substituted by an His or an Cys on the peptide structure affecting Gsalpha protein task.

-Specifically alpha subunit in G protein mutated has a decrease in its GTPasic activity

What does the mutated alpha subunit involve?

R201C leads to a constitutively active Gsa protein causing an over stimulation of adenylate cyclase and an high concentration of cAMP

Bone Marrow Stromal Cells (BMSCs)
Pluripotent mesenchymal stem cells



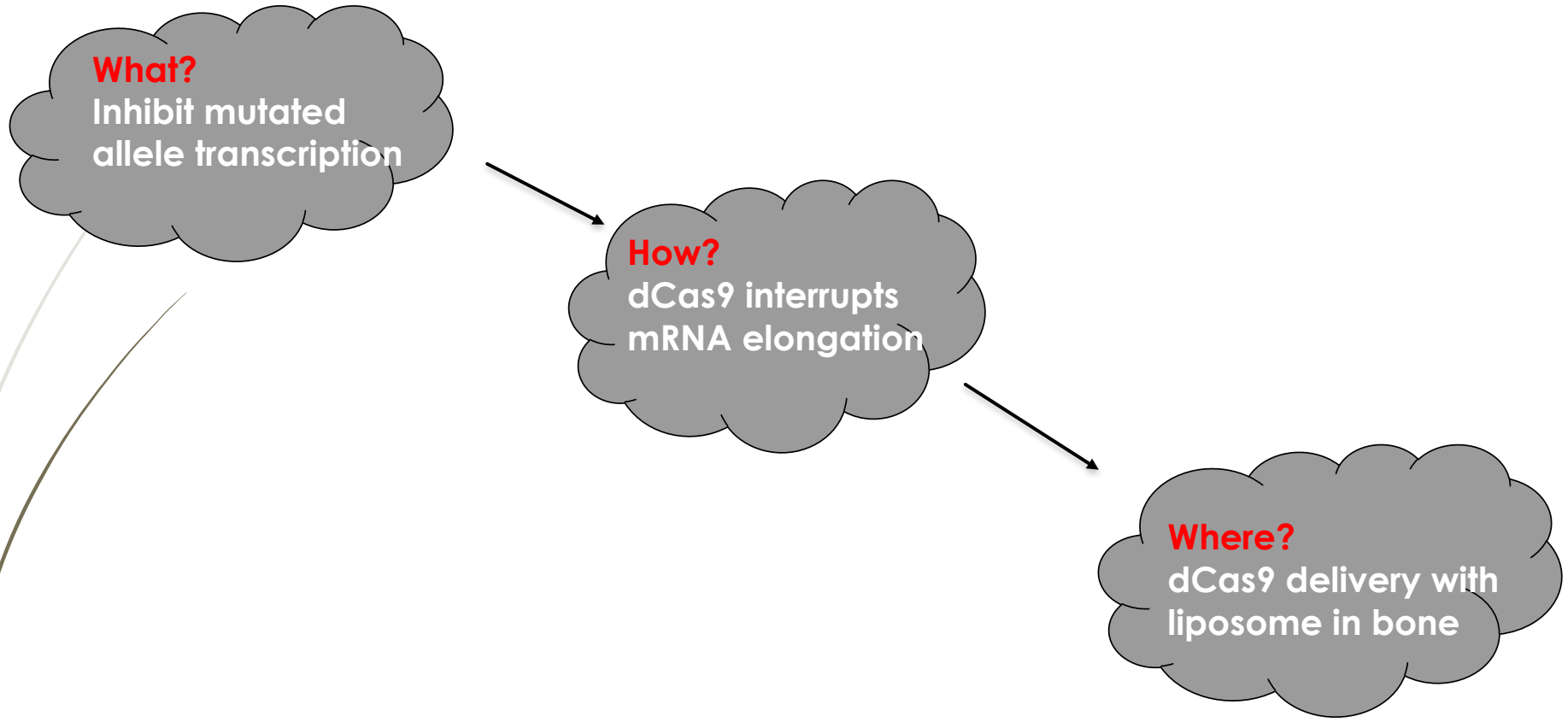
can differentiate in:
Osteoblasts
Chondrocytes
Fibroblasts
Adipocytes

R201C cause differentiation:
-decrease
Osteoblastogenesis
-increase
Osteoclastogenesis
Adipogenesis

BONE DEGRADATION

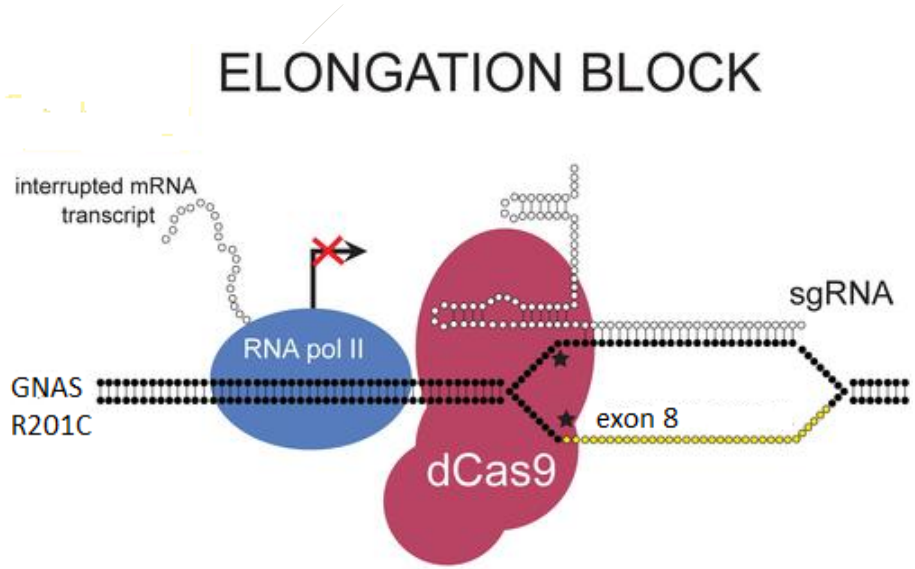
ABNORMAL BONE DEVELOPMENT

Experimental plan



Tool and strategy In vitro

Part 1



dCas9 target GNAS R201C causing steric block that halts transcript elongation by RNA polymerase.

Dna target:
CATGTTTGACGTGGGTGGCCAGCGCGAGGA
ACGCCGCAAGTGTATCCAGTGCTTCAACG

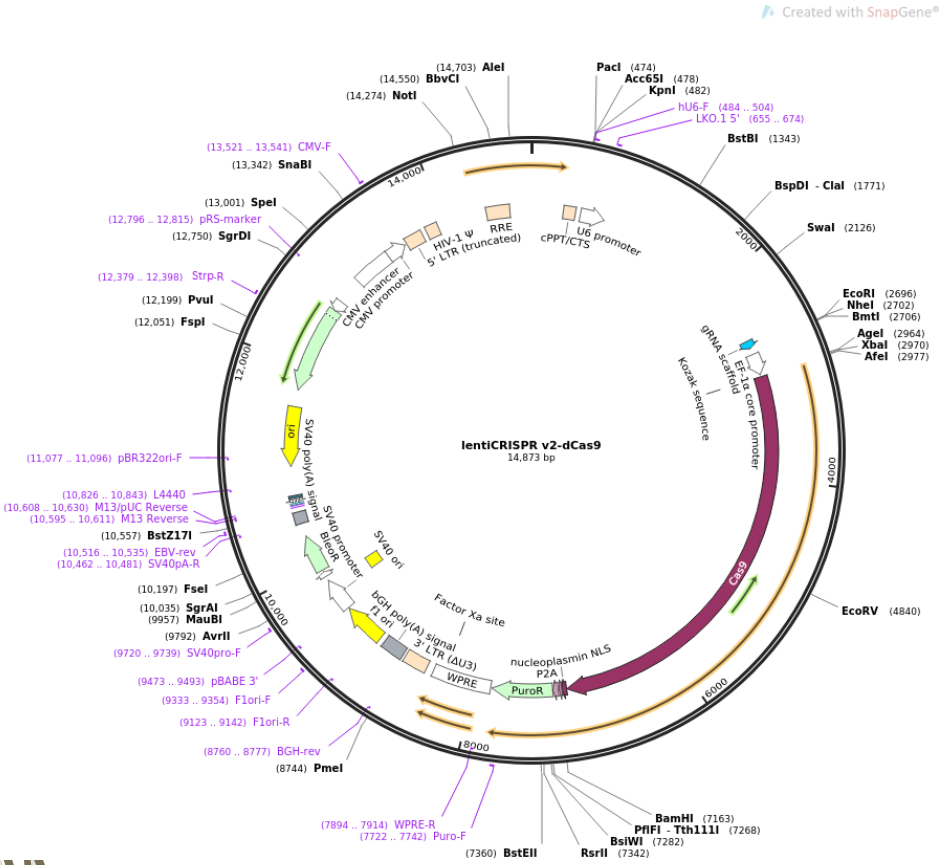
In red:
-Pam (n-GG) sequence
-Point mutation (GTG)

sgRNA:
AACGCCGCAAGUGUAUCCAG

On-target score: 66 Off-target score: 67

Tool and strategy In vitro

Part 2



lentiCRISPR v2-dCas9 that brings dCas9 into murine cells.

BACKBONE:

-Size insert (bp)10000
-Total vector size (bp)14873
-Vector type: Mammalian Expression, Lentiviral, CRISPR
-Selectable markers: Puromycin

Can we revert FD BMSCs in Vitro experiment?

Mouse FD+

We use an engineered lentivirus that carries dCas9 (specific for Gsα^R201C)
and resistance to puromycin (as marker)

LV-dCas9-Gsα^R201C-purR

FD BMSCs

FD BMSCs

Transduced BMSCs are grown in presence of puromycin

purR^+

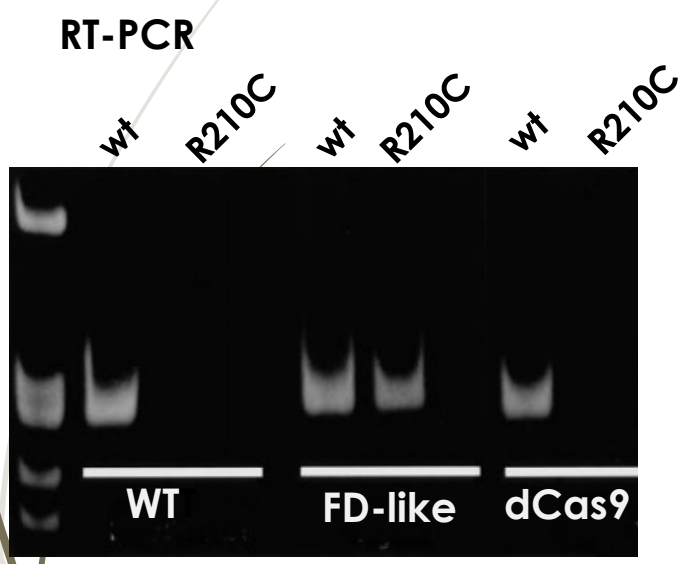
PurR cells should be normal BMSCs.
How can we check it?

purr^+

Mouse wt

We check FD-reversion
Comparing with a mouse wt

IN VITRO ANALYSIS

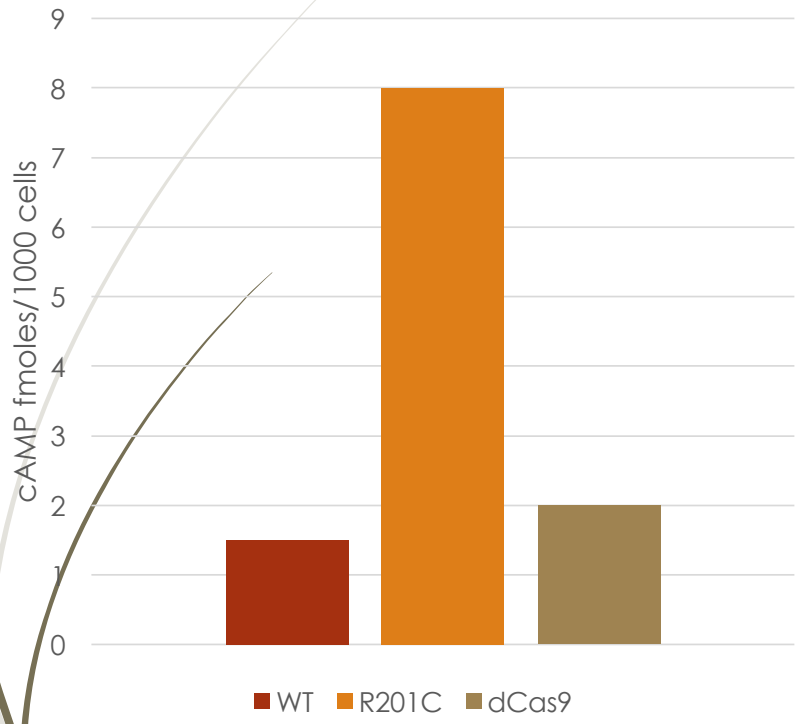


Q-PCR analysis shows restoration of adipogenic and osteogenic markers physiological levels

Adipogenic differentiation

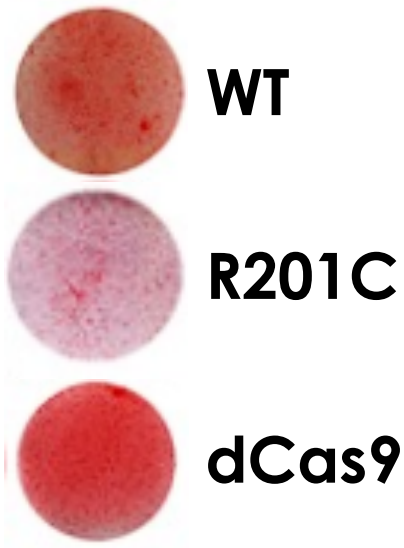
Osteogenic differentiation

cAMP assay



cAMP Direct BioTrak™ EIA (GE healtcare)

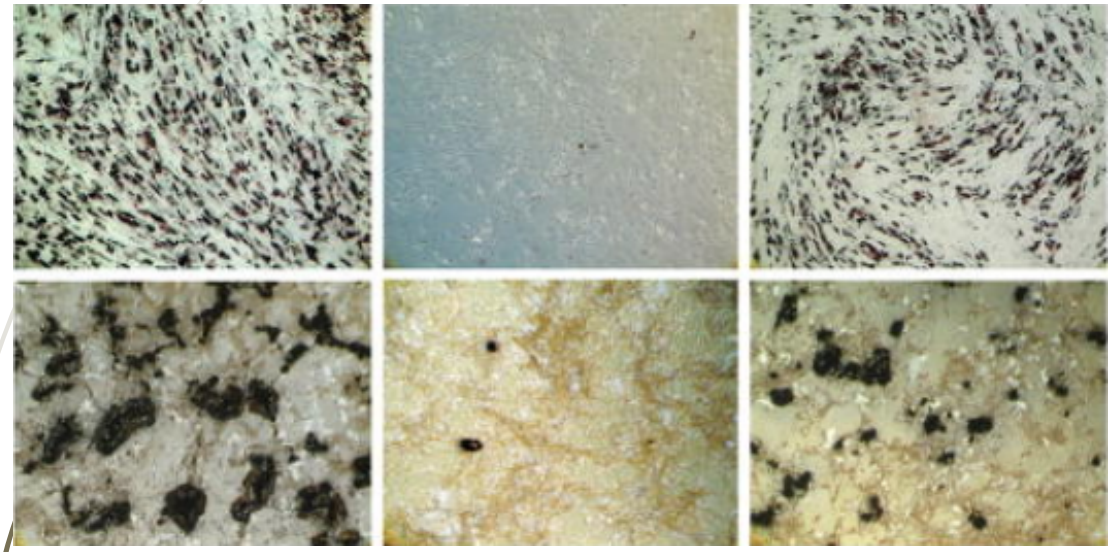
Calcium deposition



Adapted from T. Xiao *et al.* (2018)

Alizarin Red staining

In vitro adipogenic and osteogenic differentiation



Adapted from S. Piersanti

- Adipogenesis
  (Oil red O)
- Osteogenesis
  (von Kossa staining)

EXPERIMENTAL PLAN

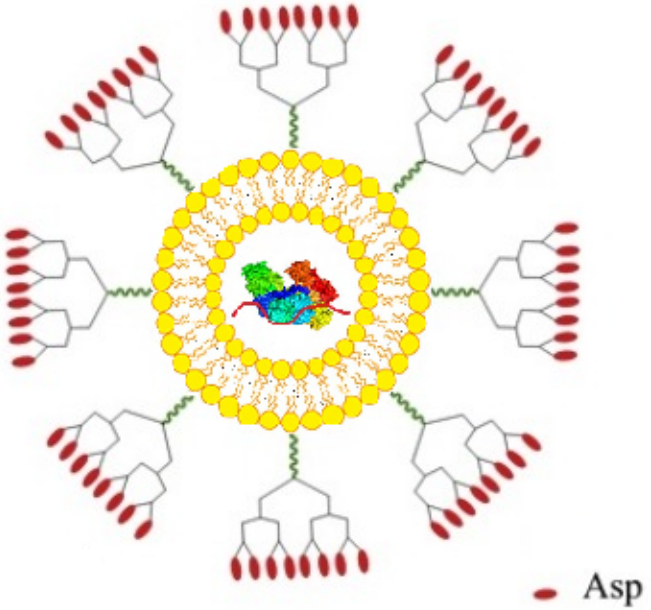
- Preparation of liposome encapsulate by Asp^{+}.
- Local administration.
- After treatment biopsy and radiography.

IN VIVO EXPERIMENT

Delivery of liposomes to bone tissue.

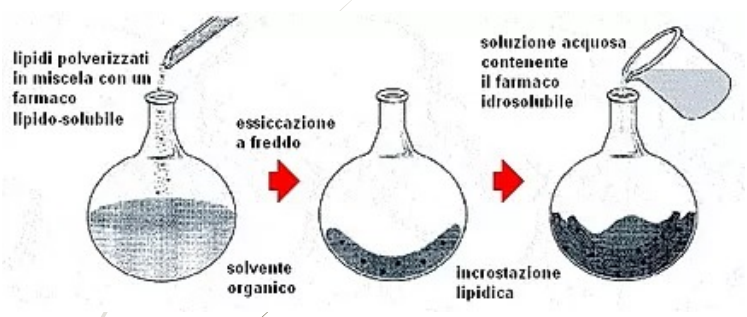
BBL: BIOMINERAL BINDING LIPOSOMES

- high biodegradability and biocompatibility
- coating with aspartic acid residues
- excellent targeting ability



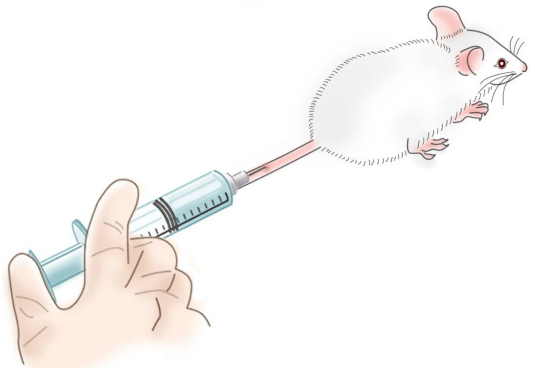
dCas9/sgRNA is encapsulate and deliver by Asp^{+} coated liposomes to bone tissue having high affinity with Bone's hydroxyapatite.

LIPOSOME'S PREPARATION AND ADMINISTRATION



Preparation:

- The lipid material is dissolved in a solvent
- drying and obtaining a thin lipid film from it
- addition of an aqueous solution containing dCas9
- shaking of the solution
- sonication and encapsulation



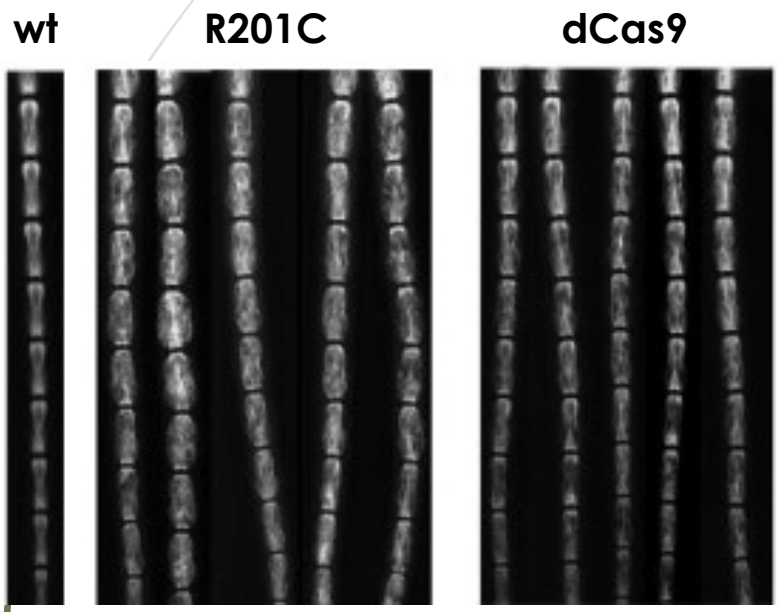
Administration ways:

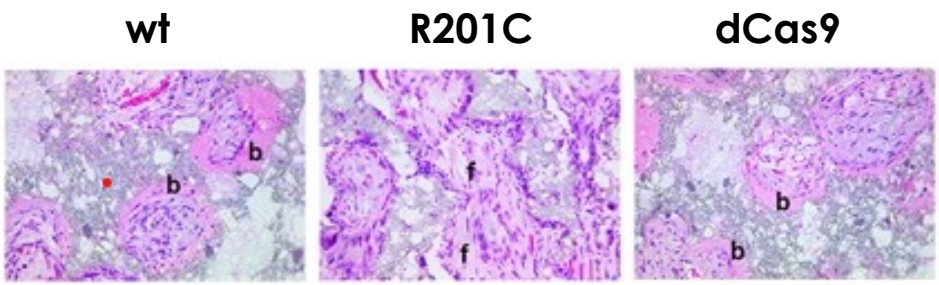
-local

-Mice R201C were treated every 6 days for 60 days

After treatment: radiography and biopsy

In vivo phenotype reversion





Budget

Materials and assays	Cost
Mice C57BL/6	€ 600,00
Mice FD like	---
cAMP assay	€ 299,00
Crispr/cas9 kit	€ 300,00
PCR Kit	€ 300,00
Lentivirus	€ 500,00
dCas9	€ 150,00
sgRNA	€ 200,00
Puromycin	€ 140,00
Stabulation costs	€ 3.000,00 years
Salaries for researches:	€ 80.000,000 years

TOTAL COST: € 85.500

References

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Thanks for your attention