The secret of stronger bones: less FGF23

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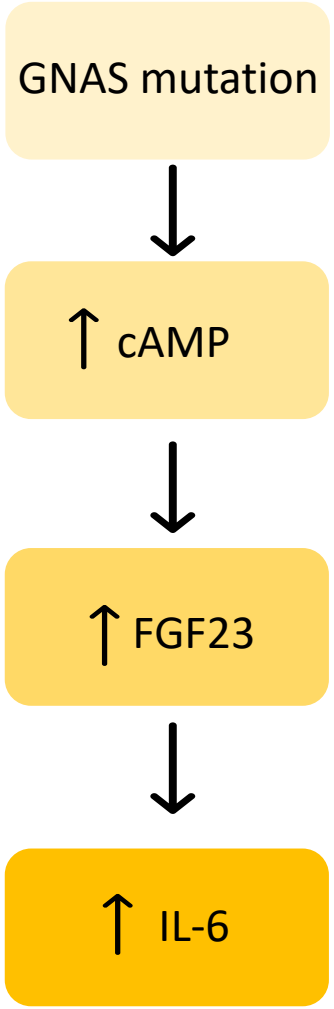
Background

Fibrous Dysplasia is a benign non-heritable disease in which healthy bone marrow is replaced by fibrous tissue resulting in pain, deformity, functional impairment and fractures. The two sites most involved are the skull and the femur. The pathology is characterized by the mutation of the GNAS gene (at level of R201C and R201H), which encodes the α-subunit of the G_s protein leading to a significant reduction of the G_s a GTPase activity and that produces before an excess of cAMP and then of FGF23. The mutation has been seen mainly in the BMSC, causing their failure to differentiate into osteoblasts. In murine model the symptoms can be observed from 3 months of life.

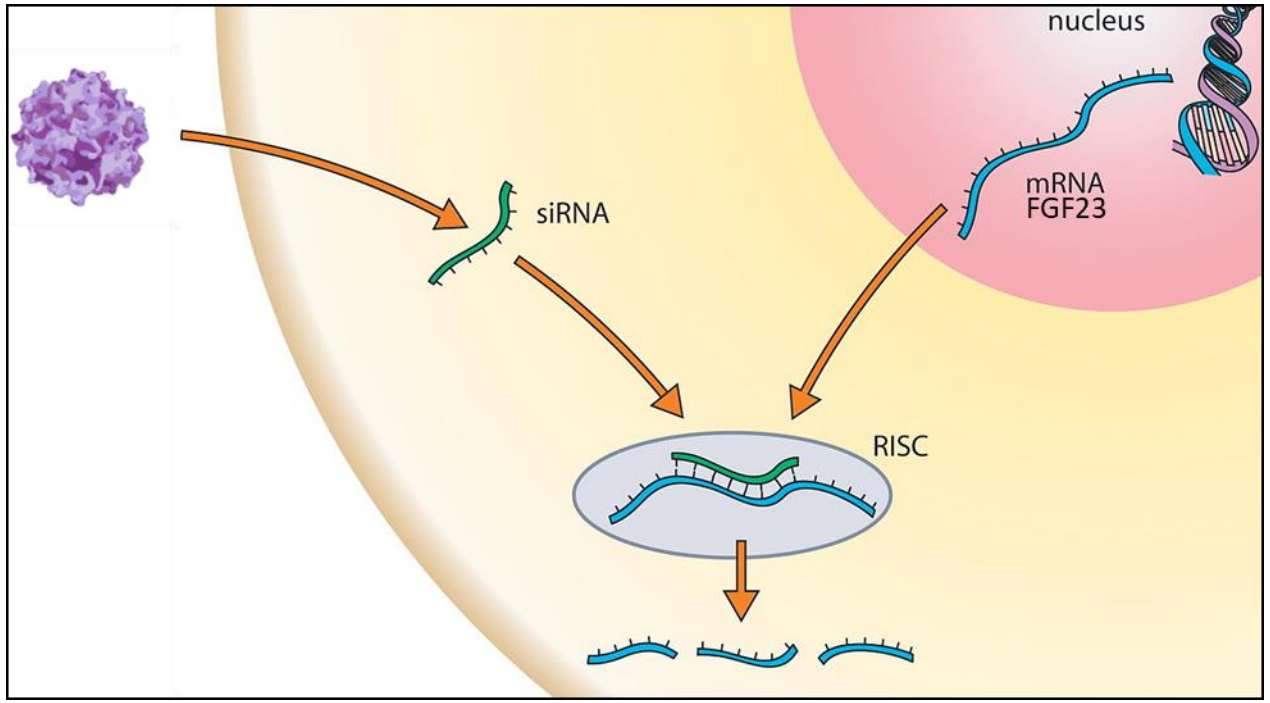
Why FGF23?

- FGF23 plays a role as hormone secreted mainly by osteoblasts;
- It regulates phosphate and vitamin D homeostatis, reducing phosphate reabsorption and absorption in kidneys and in intestine, respectively;
- High levels of FGF23 doesn't allow bone mineralization, BMSC differentiation in osteoblasts and stimulates inflammatory cytokines like IL-6.

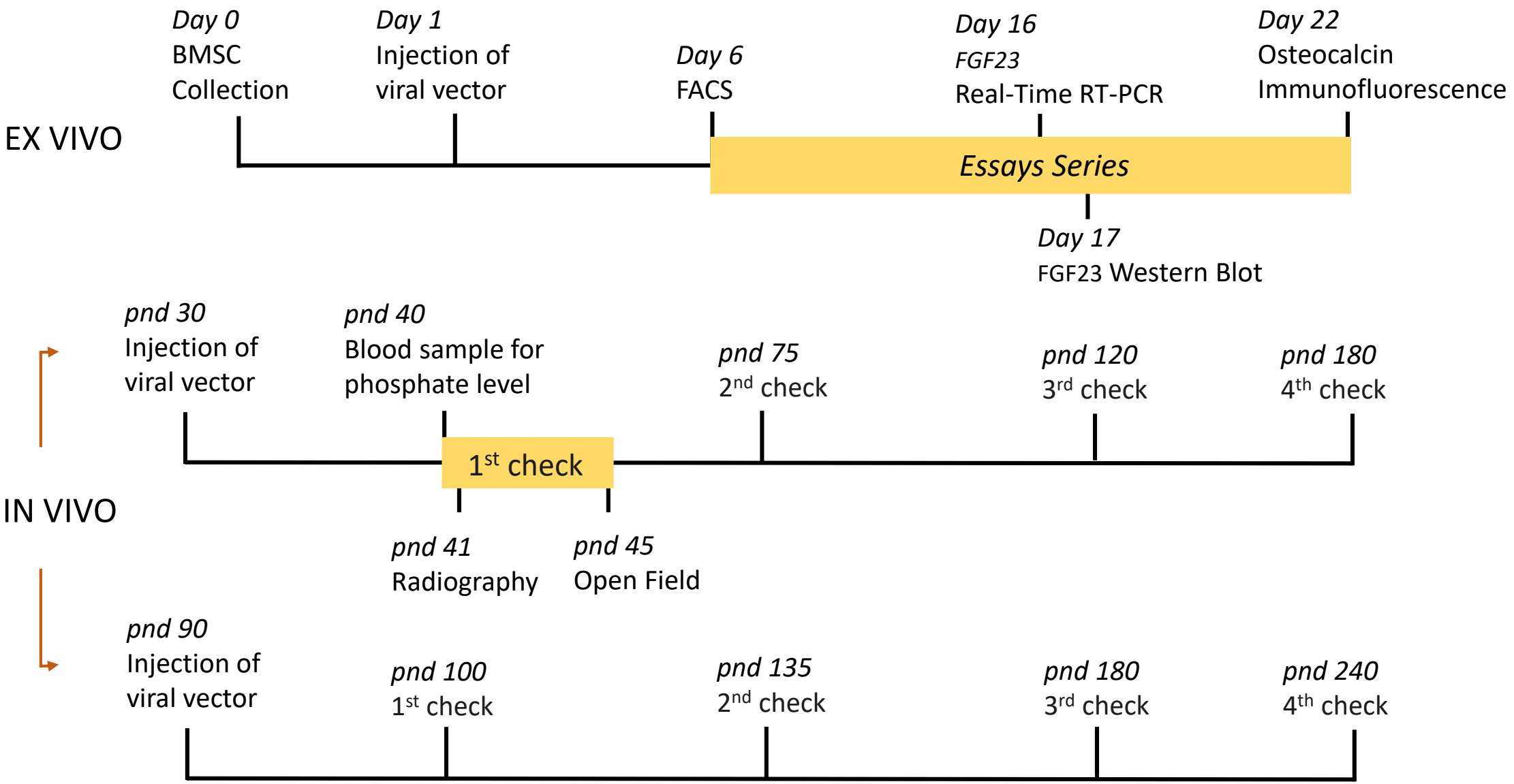


What we want to do?

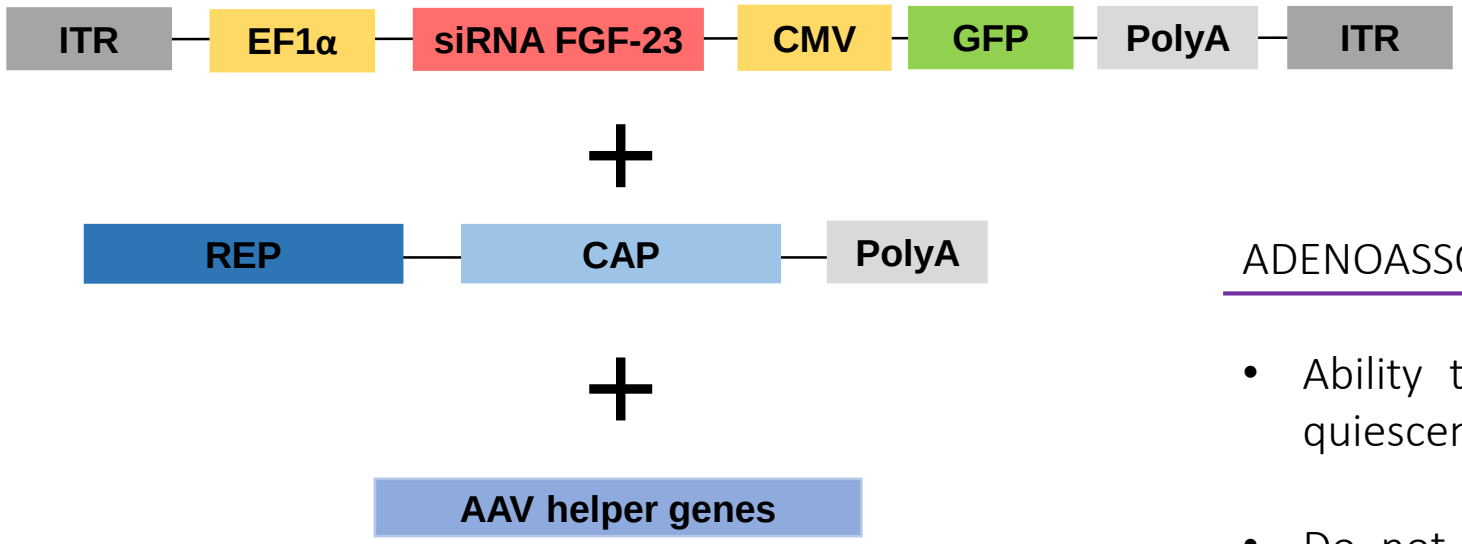
Knock Down of *FGF23* in BMSC and then in the accepted FD mouse model using Rna Interference and Adenoassociated viral vector



The project

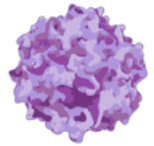

EX VIVO
Day 0
BMSC
Collection
Day 1
Injection of
viral vector
Day 6
FACS
Day 16
FGF23
Real-Time RT-PCR
Day 22
Osteocalcin
Immunofluorescence
Essays Series
Day 17
FGF23 Western Blot
IN VIVO
pnd 30
Injection of
viral vector
pnd 40
Blood sample for
phosphate level
pnd 75
2nd check
pnd 120
3rd check
pnd 180
4th check
1st check
pnd 41
Radiography
pnd 45
Open Field
pnd 90
Injection of
viral vector
pnd 100
1st check
pnd 135
2nd check
pnd 180
3rd check
pnd 240
4th check

Method – viral construct



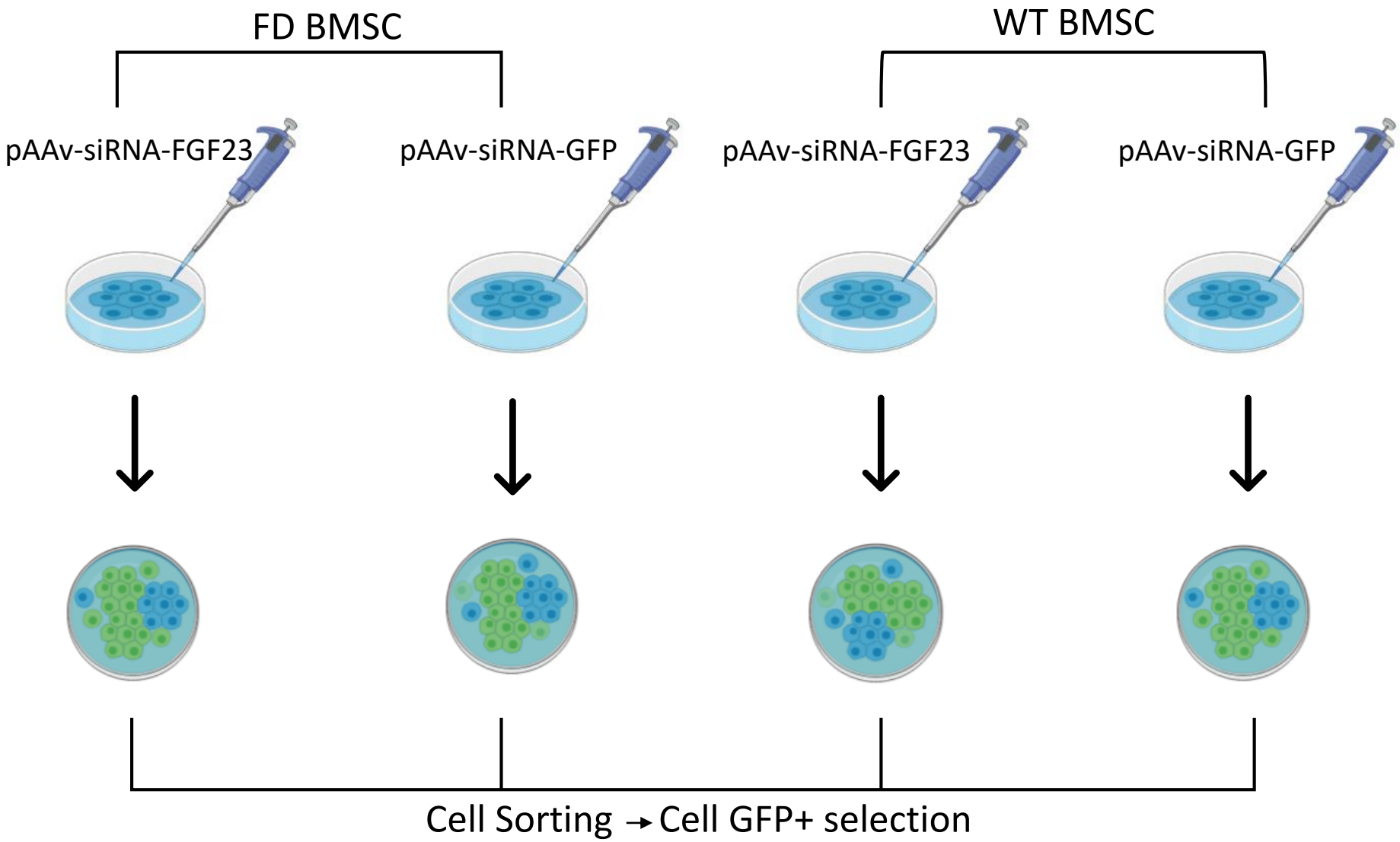
- *Cells incubated for 24h at 37°C and 5% CO_2 in a 24-well plate;*
- *Infected with 0,3 ml/well of 10^9 GC/ml, with MOI of 500;*
- *AAV/DJ8 serotype.*

ADENOASSOCIATED VIRUS PRO

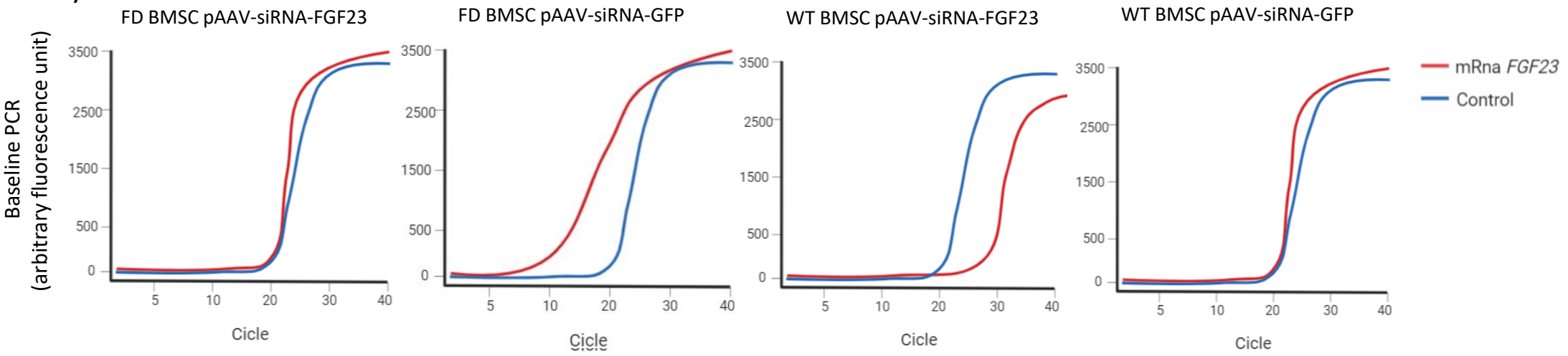


- Ability to transduce both proliferating and quiescent cells;
- Do not elicit significant immune responses and has low pathogenicity;
- No integration into the host genome;
- Transient effect, but long-term expression in non-dividing cell.

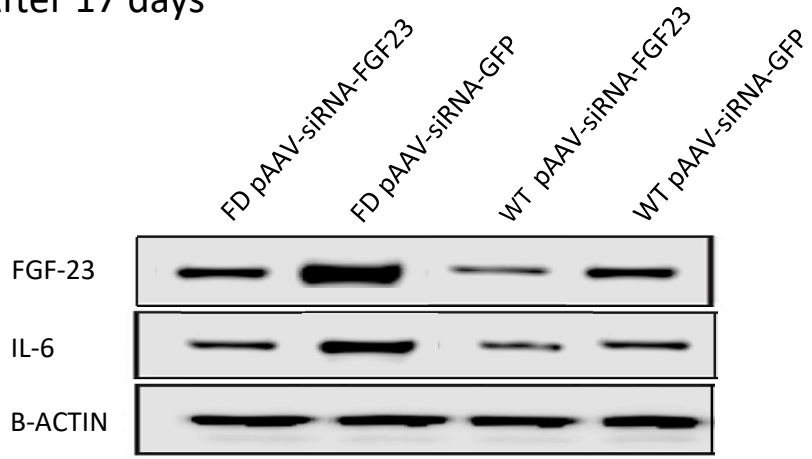
Method – ex vivo



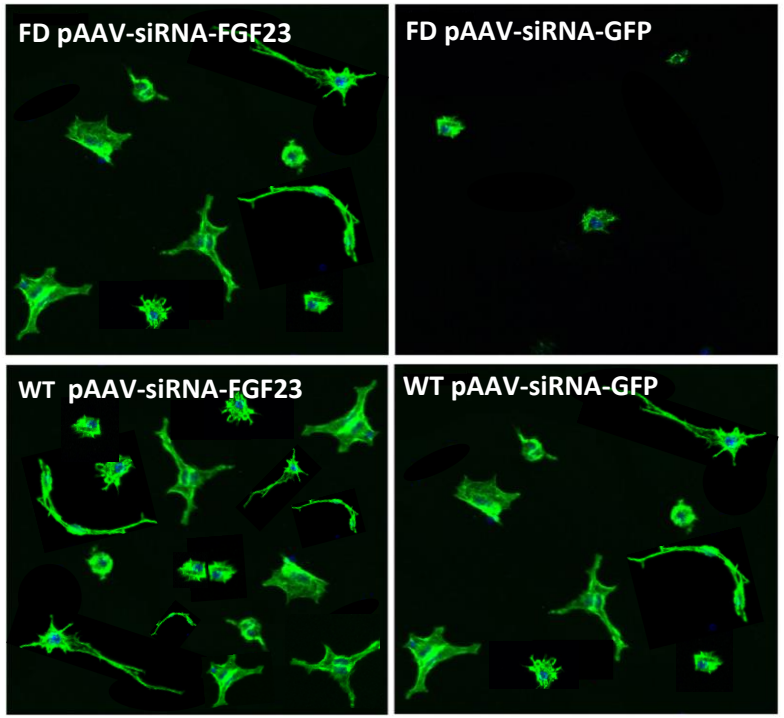
After 16 days



After 17 days



After 22 days



Method – in vivo

1st cohort
Protocol start
at pnd 30

FD mouse model

pAAv-siRNA-FGF23

pAAv-siRNA-GFP

WT

pAAv-siRNA-FGF23

pAAv-siRNA-GFP

2nd cohort
Protocol start
at pnd 90

FD mouse model

pAAv-siRNA-FGF23

pAAv-siRNA-GFP

WT

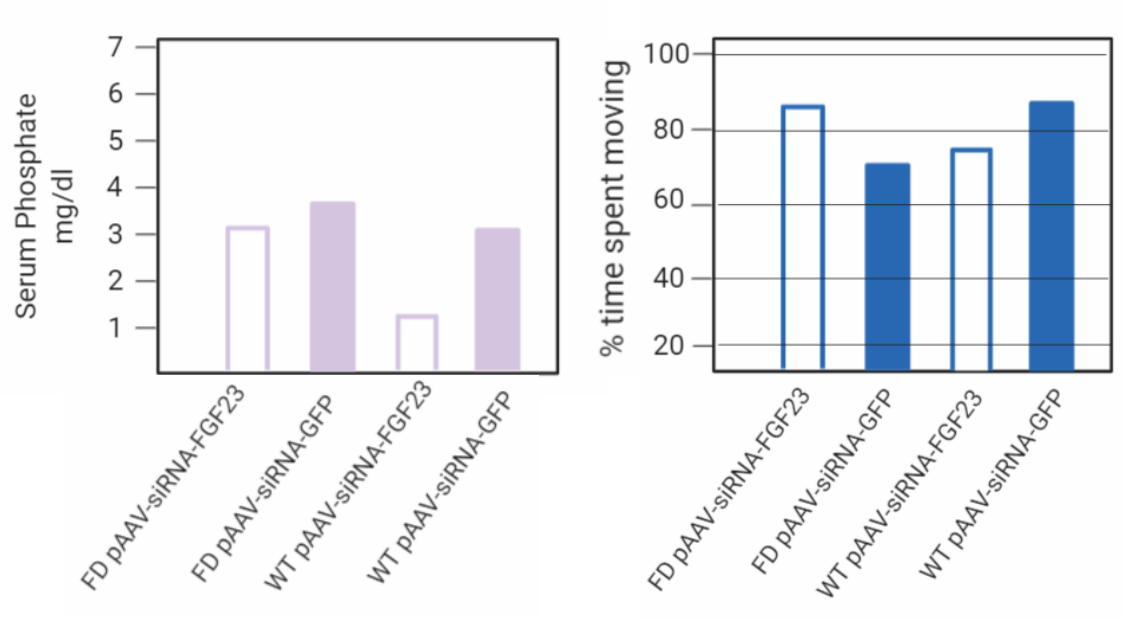
pAAv-siRNA-FGF23

pAAv-siRNA-GFP

Injection into bone marrow

1° Check

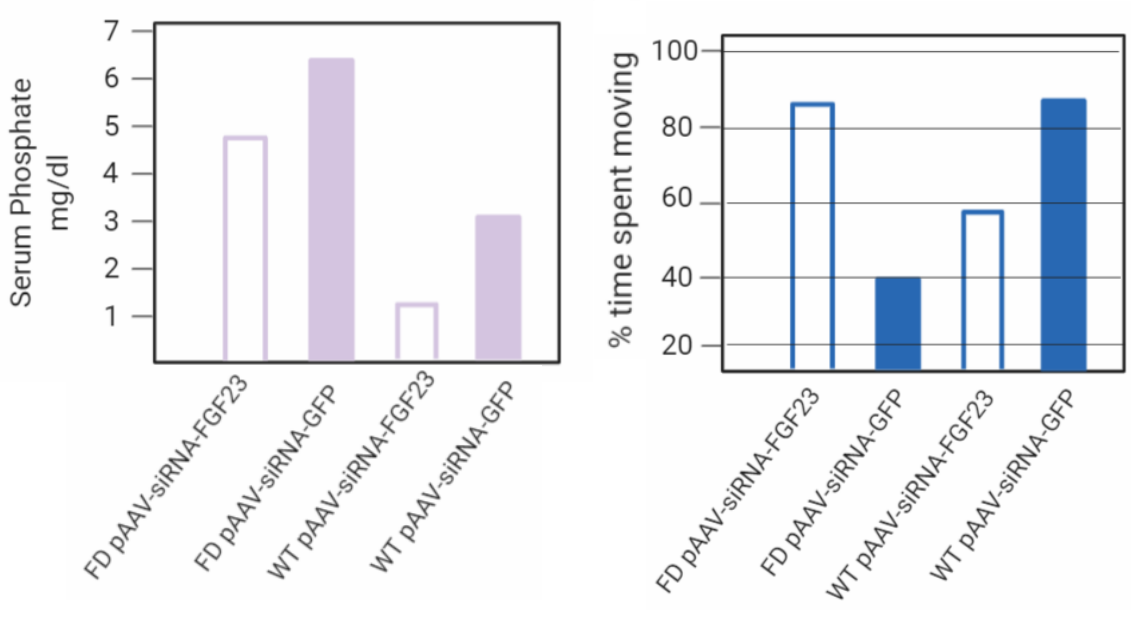
1st cohort



Femur Radiography

Adapted from Saggio et al., 2014

2nd cohort

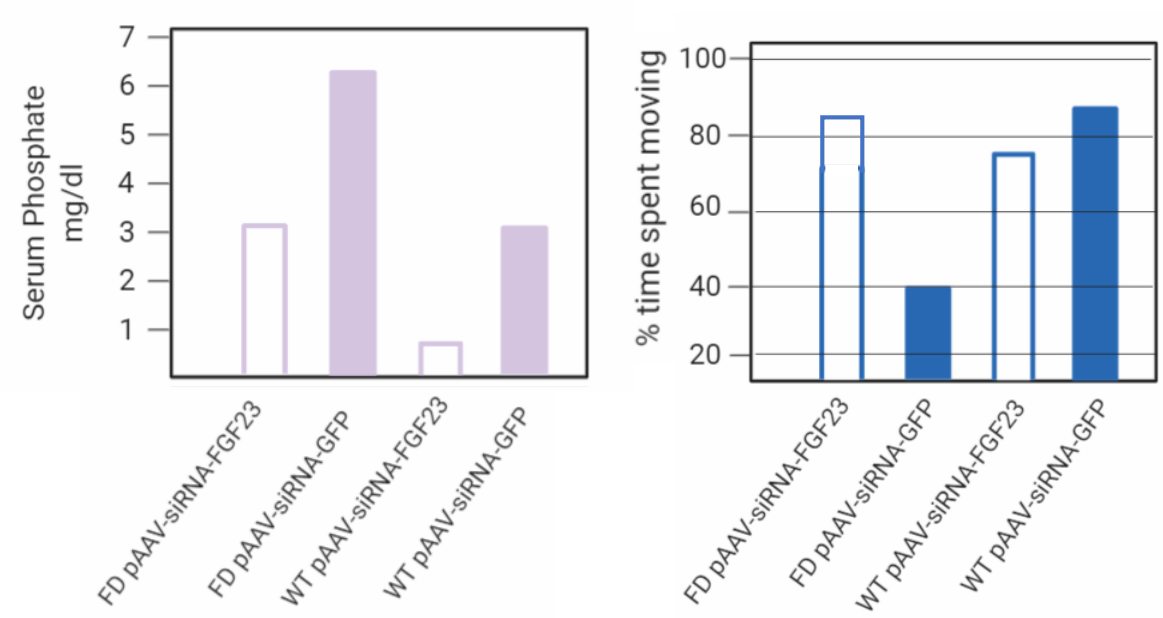


Femur Radiography

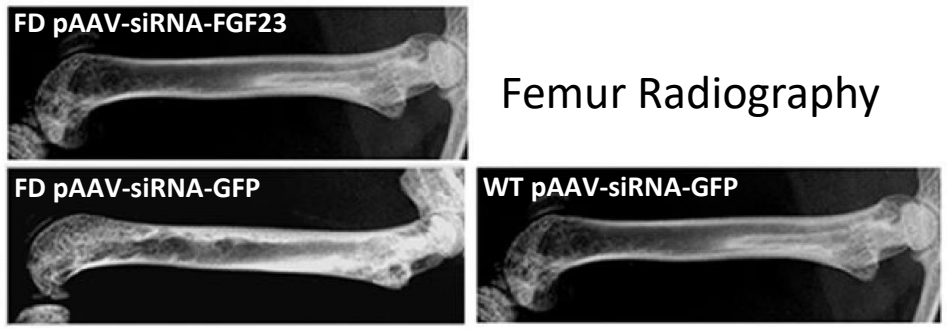
Adapted from Saggio et al., 2014

3° Check

1st cohort



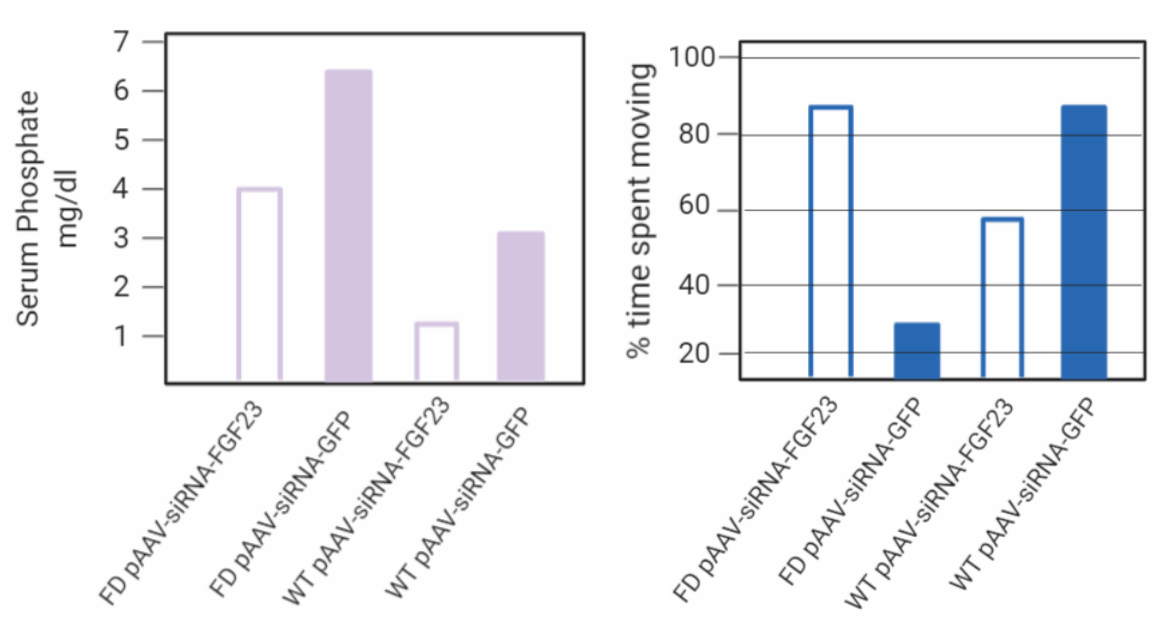
Serum Phosphate normal range: 2,5 – 4,5 mg/dl



Femur Radiography

Adapted from Saggio et al., 2014

2nd cohort



Femur Radiography

Adapted from Saggio et al., 2014

Conclusions

The effect of the treatment tends to decrease after 5 months because it's transient, but we have repeated the successful treatment obtaining the same results.

As future prospects, we want to investigate on a FGF23 important receptor, Klotho, as another possible therapy and we will test this treatment together with an antibody Anti-FGF23 to avoid an excessive dose of viral vector.

The next step is to carry out this treatment in human, in which we expected the same results

Materials costs

Cells culture	1.200,00 €	
Viral vector	395,00 €	Abm®
Scrambled	202,50 €	Abm®
RNeasy Mini kit	287,50 €	Qiagen©
Clean-Blot™ IP Detection Kit (HRP)	371,00 €	Thermo Scientific™
Anti-bodies	540,00 €	MyBioSource
Mice	1.200,00 €	Saggiolab
Mice stabulation	6.000,00 €	
Blood tests	800,00 €	
Ketamine	168,00 €	Drugs.com
Others	1.000,00 €	

References

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