FIBROUS DYSPLASIA: INCREASED MINERALIZATION AGAINST BONE FRAGILITY

Down-regulation of Twist-1 through RNA interference

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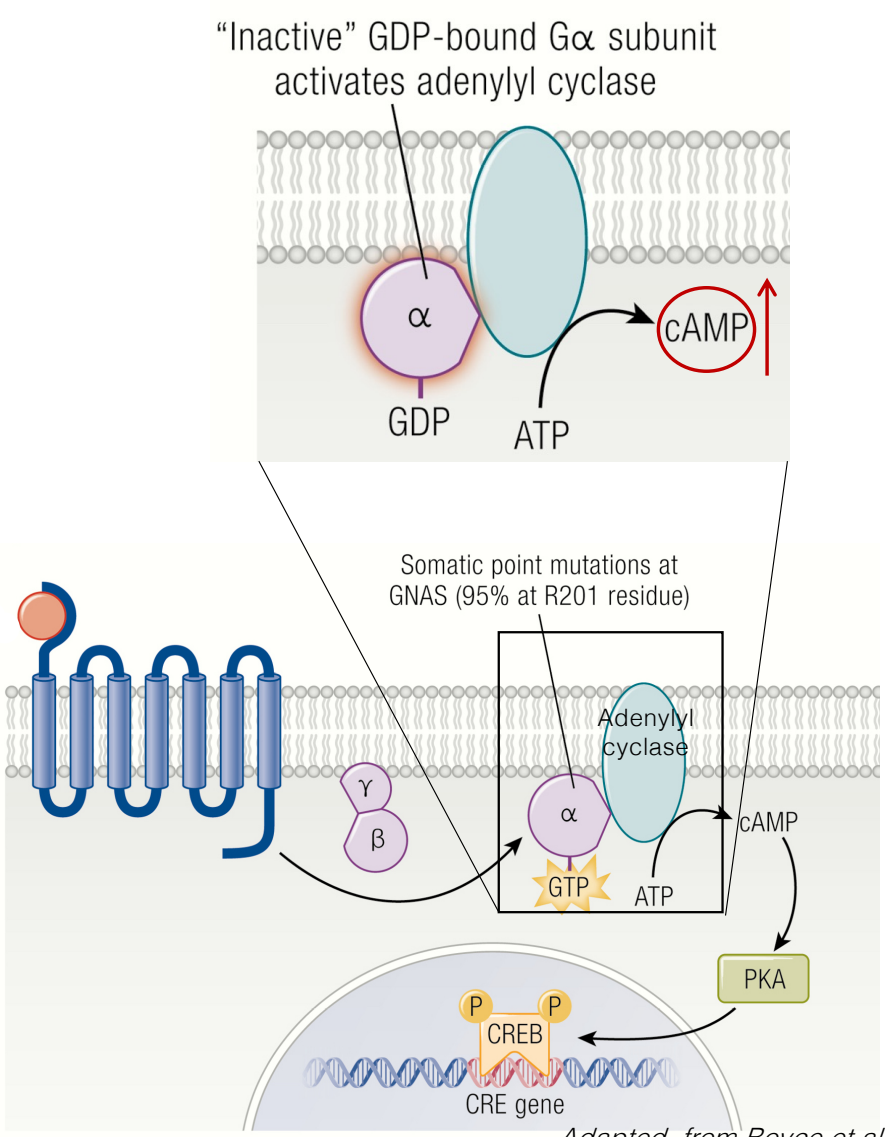
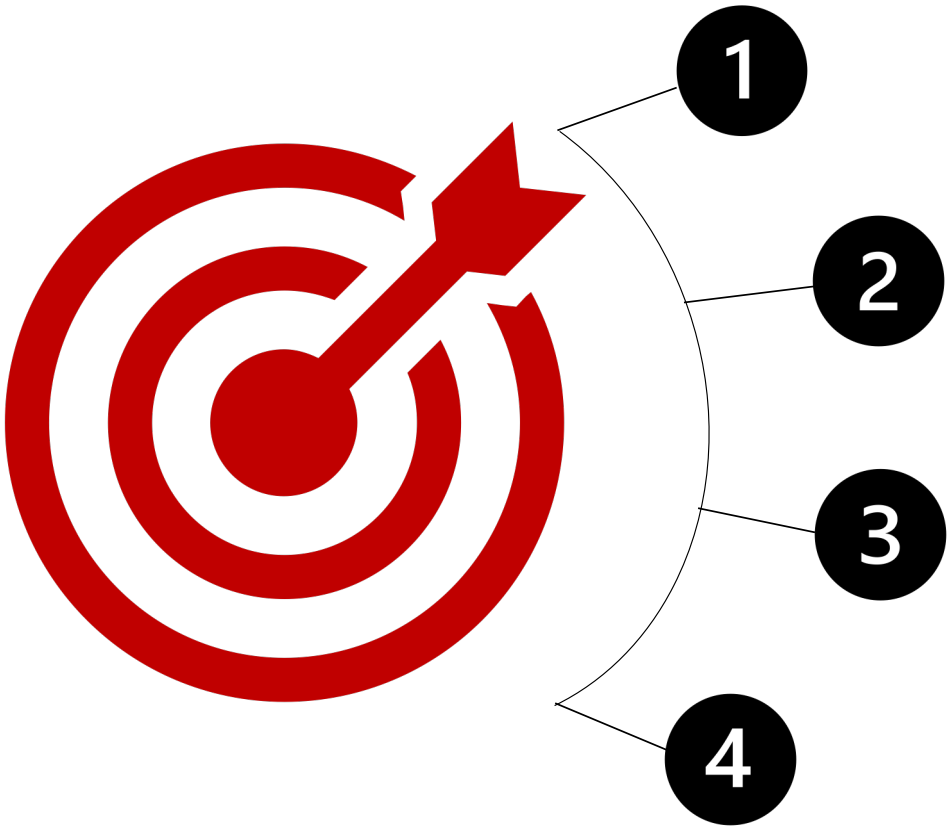
BACKGROUND OF FIBROUS DYSPLASIA

WHAT IS IT?	Genetic disorder characterized by the replacement of normal bone with abnormal fibro-osseous tissue
FROM WHAT IS IT CAUSED?	Activating missense mutations in the GNAS gene, coding for the α-subunit of the stimulatory G-protein
PRODUCTION OF THE MUTATION	A reduced GTP intrinsic activity and excessive cAMP production



Adapted from Boyce et al

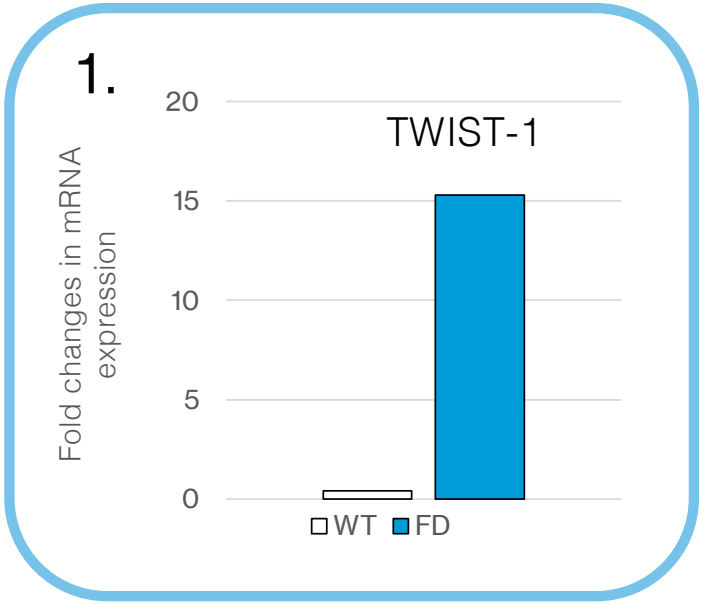
AIMS OF THE PROJECT

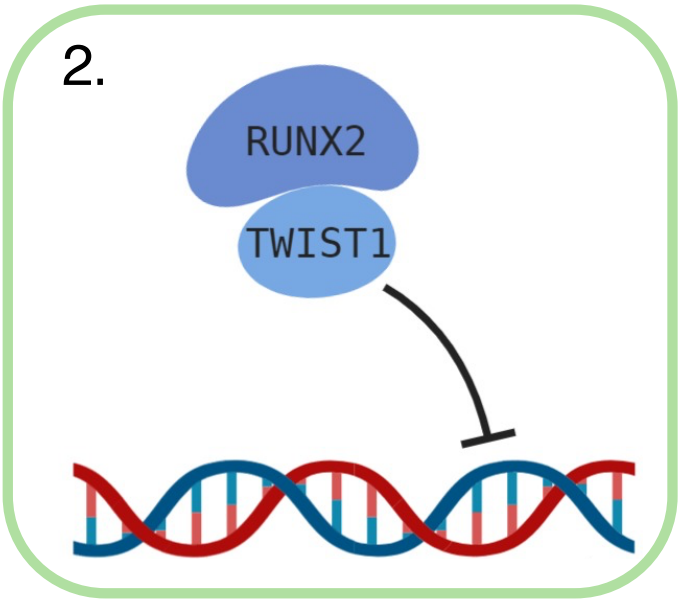


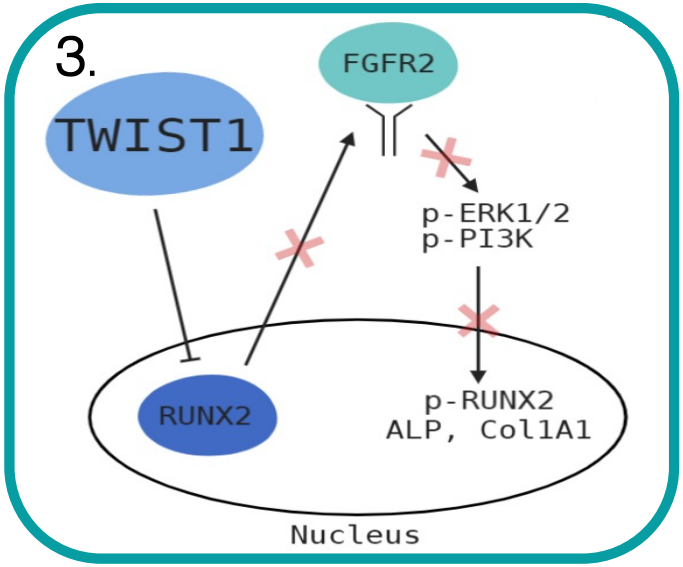
1. siRNA MEDIATED TWIST1 INHIBITION
2. REGULATION OF ATF4 AND RUNX2
3. RESTORING NORMAL EXPRESSION OF OSTEOBLASTIC MARKERS
4. RESTABILIZING BONE REMODELLING AND MINERALIZATION

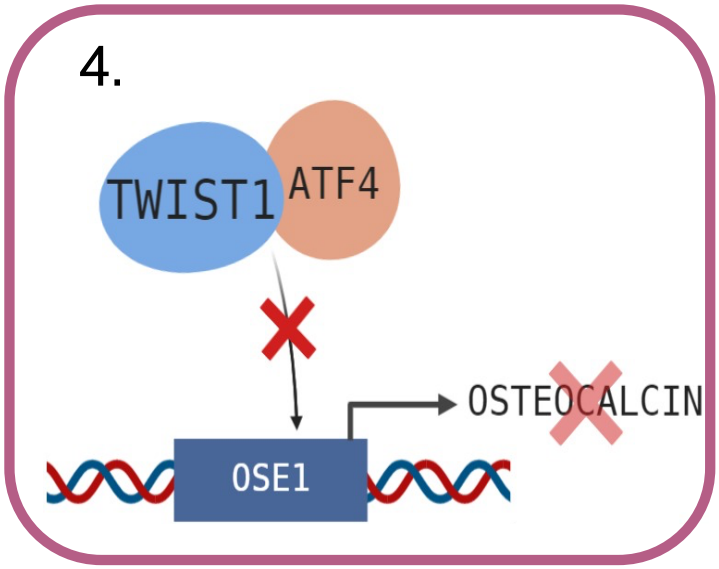
WHY TWIST-1?

1. IT IS OVEREXPRESSED IN FD
2. IT BINDS RUNX2, MAJOR OSTEOBLAST-SPECIFIC GENES
3. IT INHIBITS FGFR2
4. IT ACTS ON ATF4 BY INHIBITING THE EXPRESSION OF OSTEOCALCIN



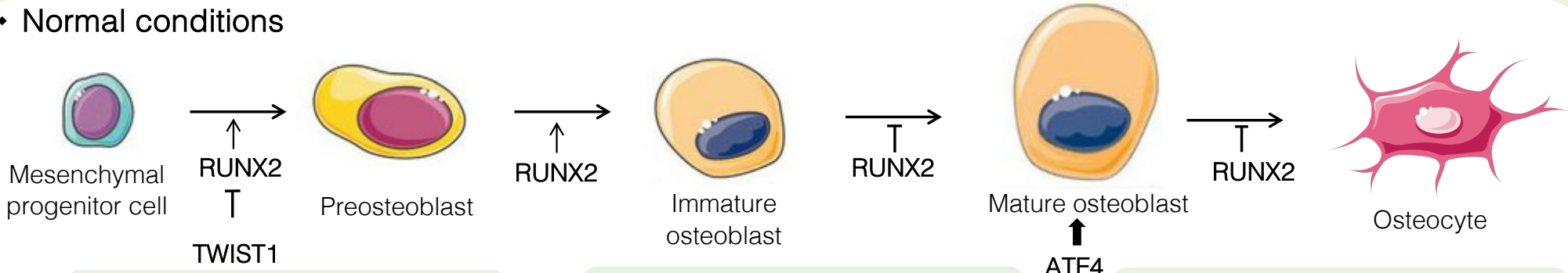






OSTEOBLAST DIFFERENTIATION IN WT e FD

❖ Normal conditions

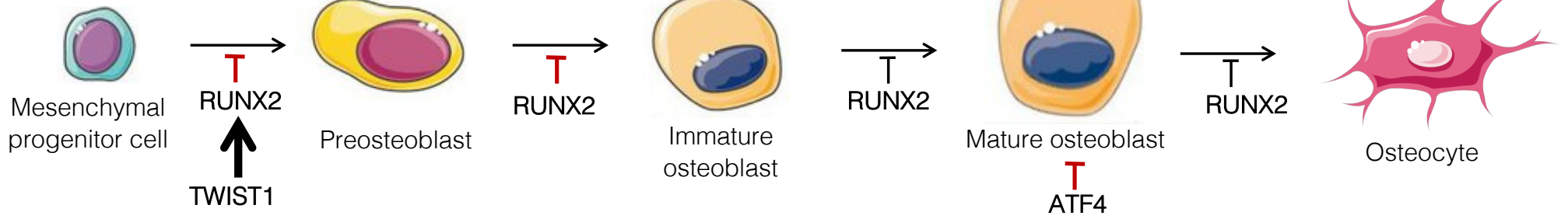


↑ Promotion of osteoblast differentiation

⊤ Inhibition of osteoblast differentiation

⬆ Promotion of the expression of bone matrix protein genes

❖ In Fibrous Dysplasia: upregulation of TWIST1



⬆ Not promote bone matrix formation

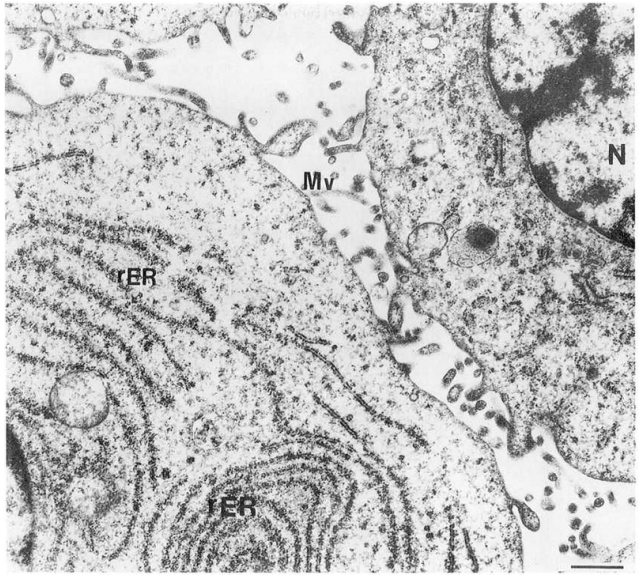
⊤ Inhibition of osteoblast differentiation

⊤ Inhibition of the expression of bone matrix proteins gene

MODELS

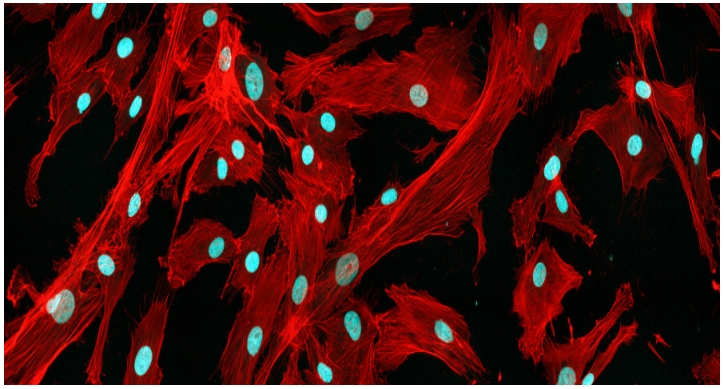
Cell ROS17/2.8

- Osteosarcoma cell line
- Can mineralize a matrix in vivo
- Markers for immature and differentiated osteoblasts



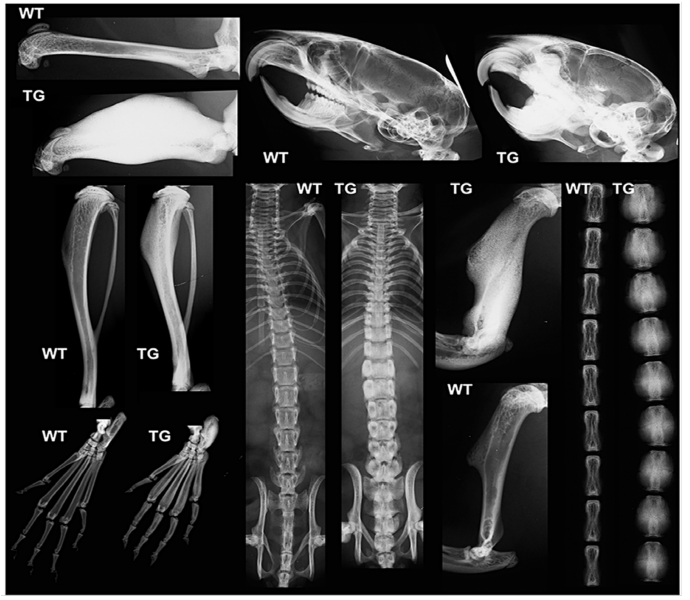
MESENCHYMAL CELLS

- Semi-fibroblastic morphology
- Multipotent Stem Cells
- Important for the production and repair of skeletal tissue
- CD73, CD90, CD105



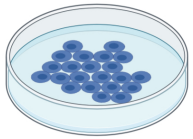
TRANS-GENIC MICE

- Marrow fibrosis
- Osteolysis
- Fracture
- Osteomalacia



EXPERIMENTAL PLAN

ROS17/2.8



DAYS

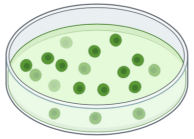
0

1. SiTwist1A
2. **SiTwist1B-sense**
3. SiTwist1C-antisense
4. Empty vector

10

RT-PCR e WesternBlot

MSC



DAYS

0

INJECTION OF BEST SIRNA

3

CELL PROLIFERATION ASSAY
APOPERCENTAGE ASSAY

10

RT-PCR
MACRO-ARRAY

21

HISTOLOGICAL DIFFERENTIATION CHECK (ALIZARIN RED S)

MICE

DAYS

0

IV TAIL INJECTION

30

RT-PCR

120

X-rays and µCT

180

BONE BIOPSY

210

SACRIFICE and HISTOLOGICAL STAINING (VON KOSSA)

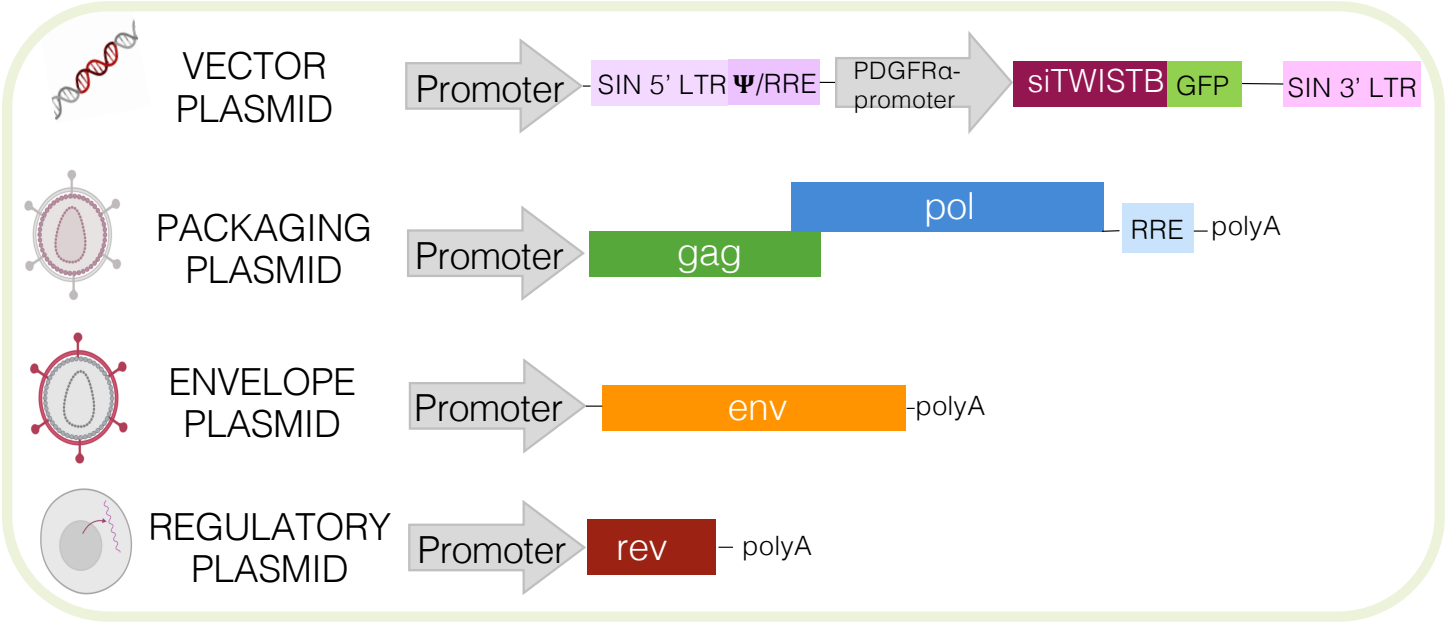
STRATEGY

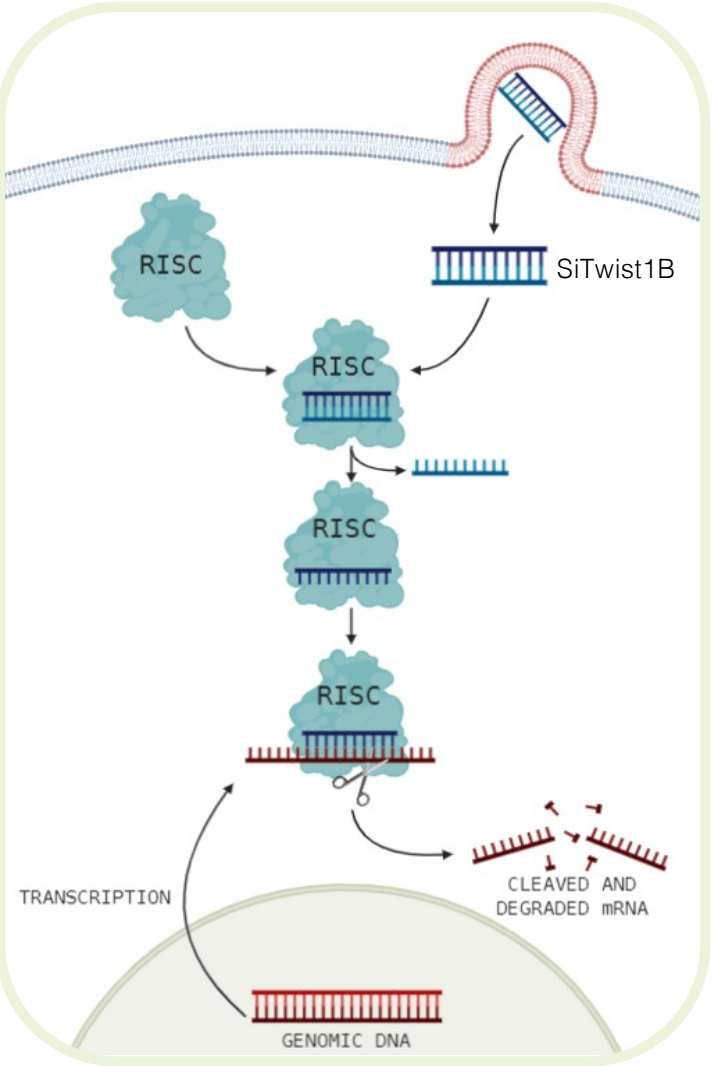
① Cell colture: **SiTwist1A**- sense 5′-GGACAAGCUGAGCAAGAUU-3′

② Cell Colture: **SiTwist1B**-sense 5′-GCGACGAGCUGGACUCCAA-3′

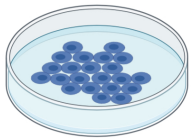
③ Cell colture: **SiTwist1C**-antisense 5′-UUGGAGUCCAGCUCGUCGCUU-3′

④ Cell colture: **Empty vector**





IN VITRO EXPERIMENTAL ANALYSIS



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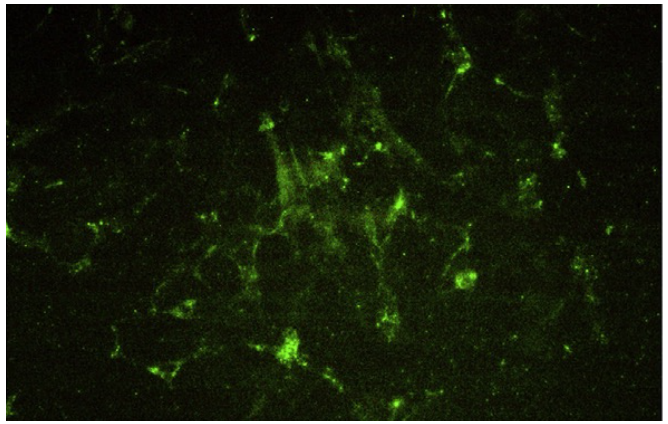

GFP-siRNA was used to test the transfection efficiency

Adapted from Zhang et al

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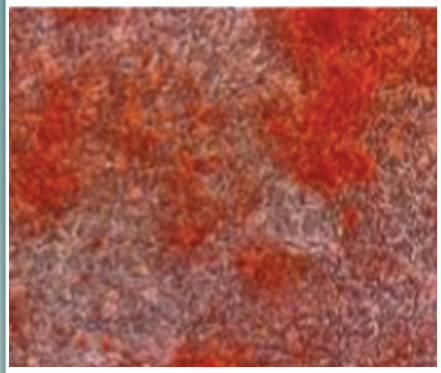
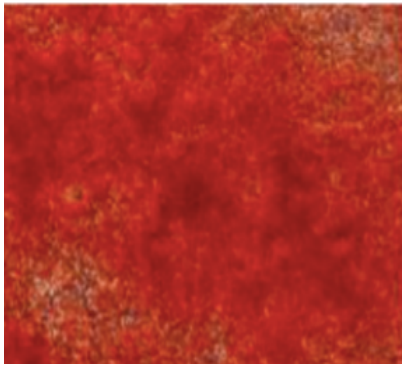


EV



SiTwist1B

Matrix mineralization with Alizarin Red S

Adapted from Qi et al

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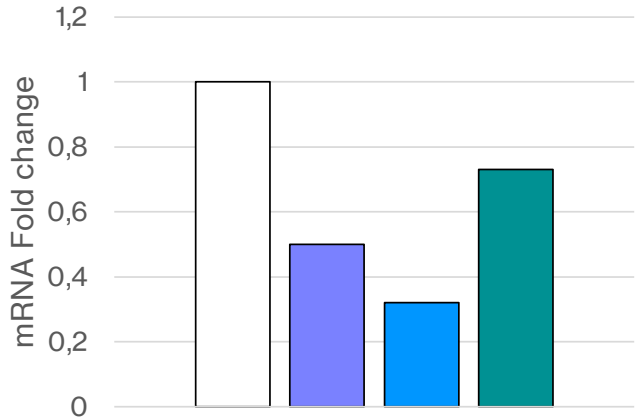

Quantitative analysis to choose the best siRNA that reduced Twist1 mRNA expression by about 68%

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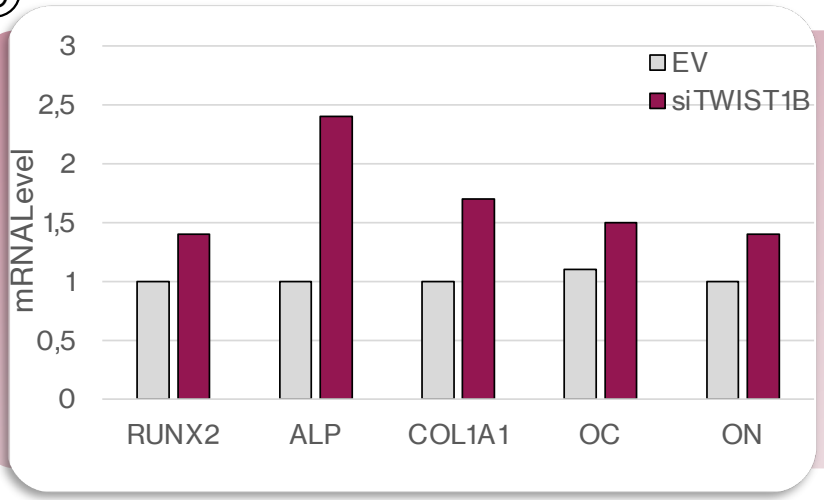

RT-PCR showing that siTwist1B increased the expression of osteoblast markers

IN VIVO EXPERIMENTAL ANALYSIS

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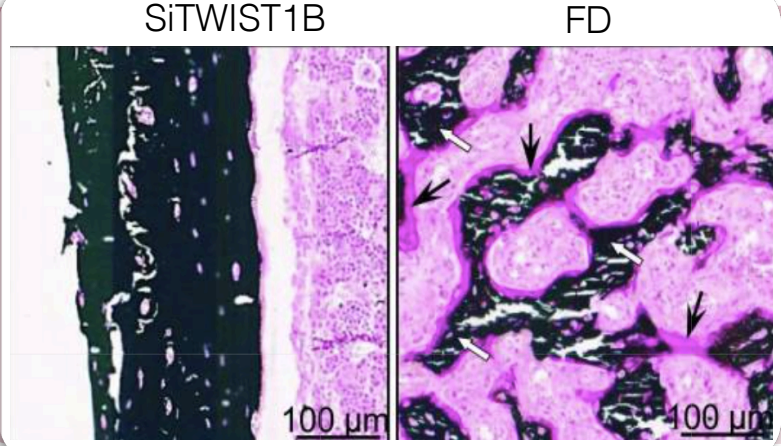

Von Kossa Staining

Adapted from Zhao et al

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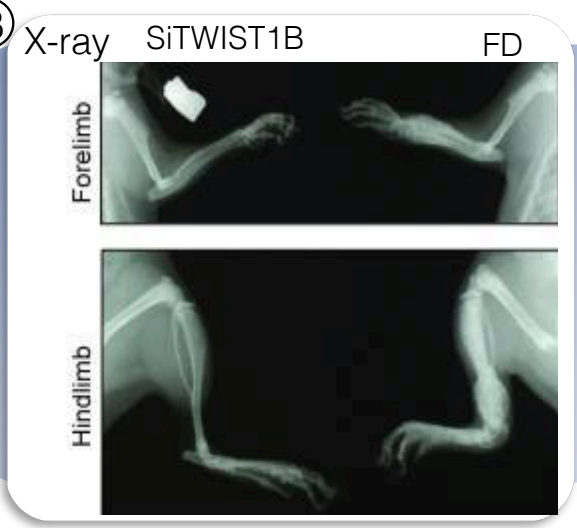

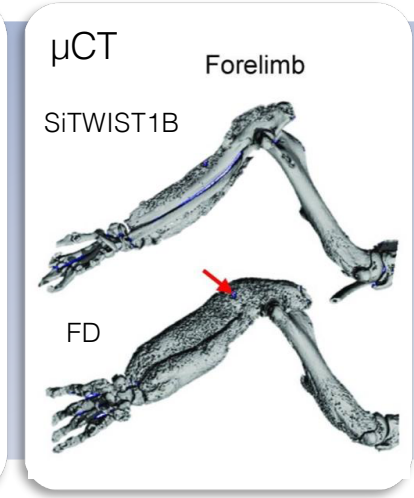

Adapted from Zhao et al

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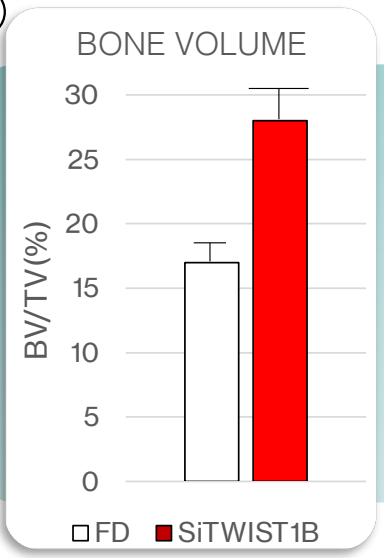

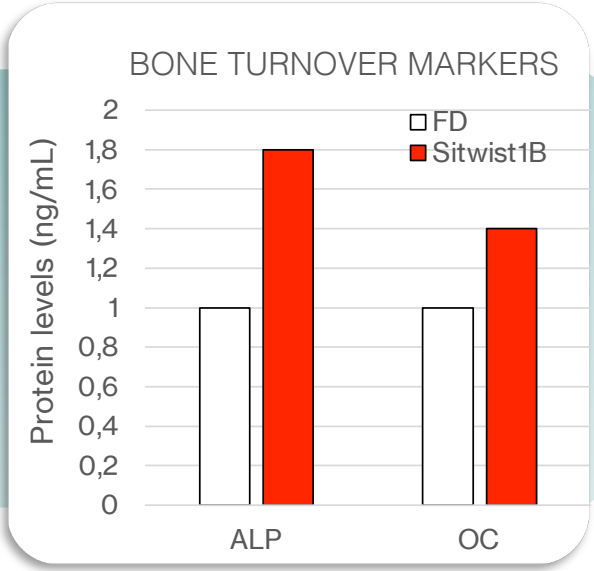

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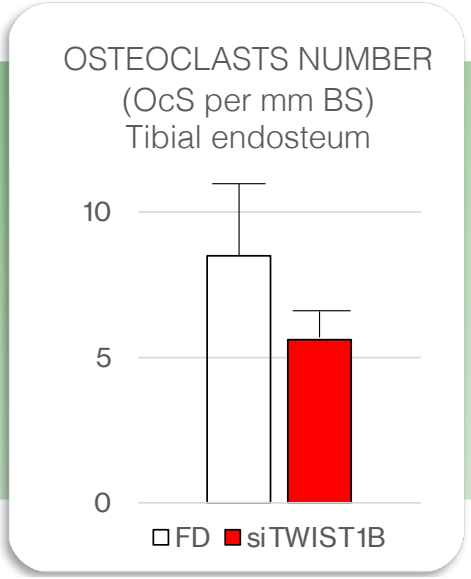
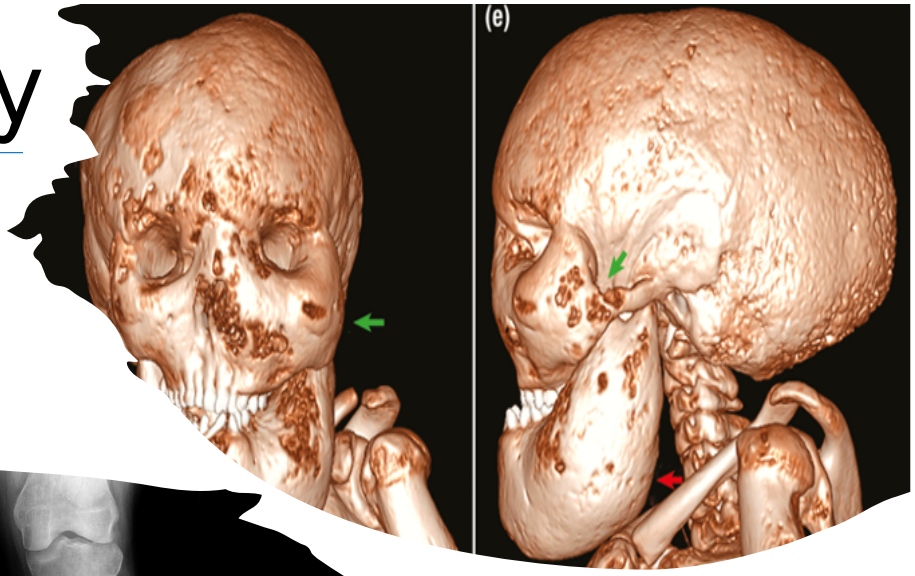


Current therapies use Bisphosphonates, these are a class of drugs that prevent the loss of bone density

NEXT STEP: human therapy

Similar results to the ones observed in vitro have been found in human clonal bone marrow derived mesenchymal cells (Oyajobi et al., 1999)



- ✓ Current therapies are palliative cures
- ✓ Lentiviral transfection offers a high efficiency for good expression levels
- ✓ Secure and effective

- x Low insertional mutagenesis possibility
- x Possible immune response

MATERIALS & COSTS

TYPE OF COST	PLANNED COSTS	WHERE
MICE	1200 €	SaggioLab
MICE STABULATION	1000 € x mice	SaggioLab
CELL ROS 17/2.8	450 € $1x10^6$ cells/vial	As one international, inc.
MSC CELLS	650 € $1x10^6$ cells/vial	As one international, inc.
LENTIVIRUS	490 €	Santa cruz biotechnology
SiTWIST	270 € x 10µ	Santa cruz biotechnology
PLASMID	1499 ($2x10^9$ TU/ml x 1ml)	vectorBuldier
VAN KOSSA STAIN KIT	200 € (x250 tests)	abcam
ALIZARIN RED S	50 € (x25 g)	abcam
cDNA SINTESIS	536 € (x50 kit)	thermo Fisher
RT PRIMERS	120 €	origene
TAQ-MAN KIT	776 € (x40 kit)	thermo fisher
X-ray	40 € (x radiography)	External laboratory
µCIT	50 €	Saggio LAb
BLOOD TESTS	800 €	
OTHERS	5000 €	
RESEARCH TEAM	150.000 x year	

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