New Horizons on bone targeted therapies

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CBM University

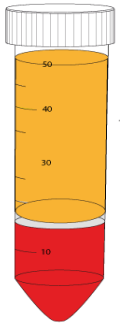
Rome

…we have specific *in vitro models* of bone cells

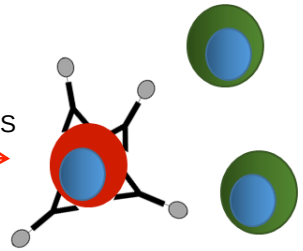
IN VITRO MODELS OF BONE CELLS

PRIMARY HUMAN OSTEOCLASTS

PBMCs ISOLATION

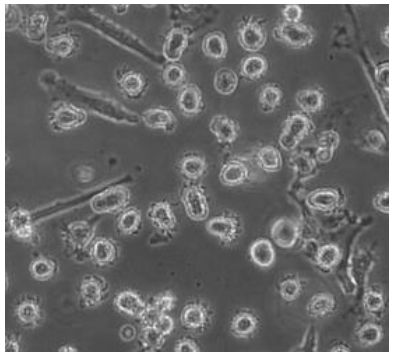


CD14 + CELLS LABELING WITH MICROBEADS



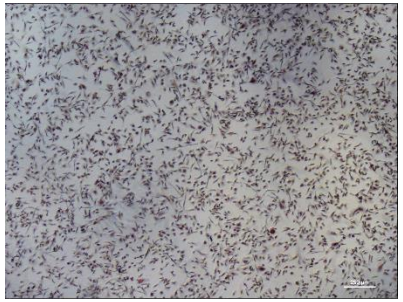
MAGNETIC ISOLATION

SEEDING

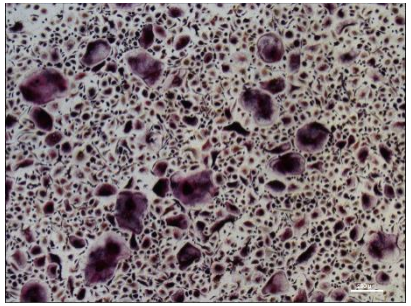


male healthy donors

DIFFERENTIATION (TRAP ASSAY)



UNDIFFERENTIATED

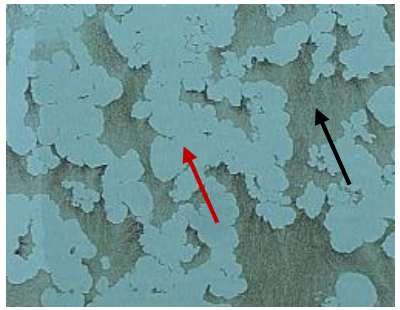


DIFFERENTIATED

Cultured for 12 days with M-CSF and RANKL

ACTIVITY (OSTEOASSAY)

UNDIFFERENTIATED

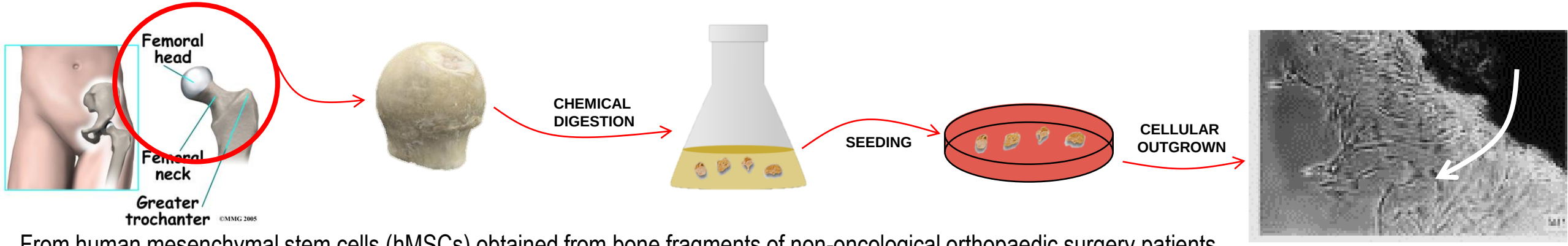


DIFFERENTIATED

BONE LAYER
RESORBED AREA

IN VITRO MODELS OF BONE CELLS

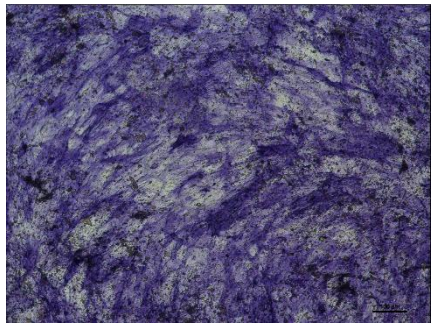
PRIMARY HUMAN OSTEOBLASTS



From human mesenchymal stem cells (hMSCs) obtained from bone fragments of non-oncological orthopaedic surgery patients

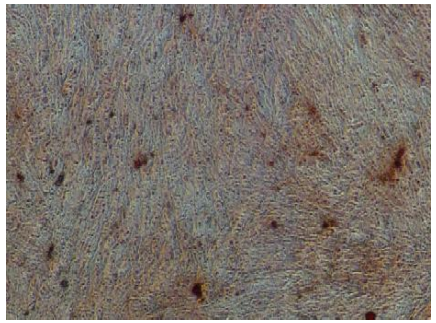
DIFFERENTIATION (ALP ASSAY)

UNDIFFERENTIATED

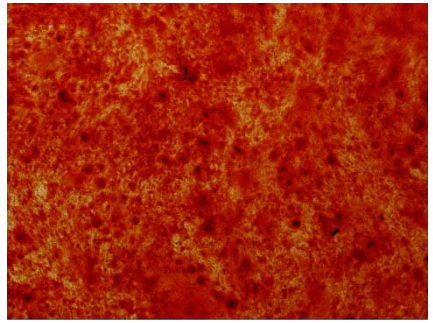


DIFFERENTIATED

ACTIVITY (ALIZARIN RED ASSAY)



UNDIFFERENTIATED



DIFFERENTIATED

OBL differentiation was monitored by alkaline phosphatase (ALP) staining, and bone matrix deposition as a marker of OBL activity by Alizarin Red staining

Abiraterone and bone microenvironment

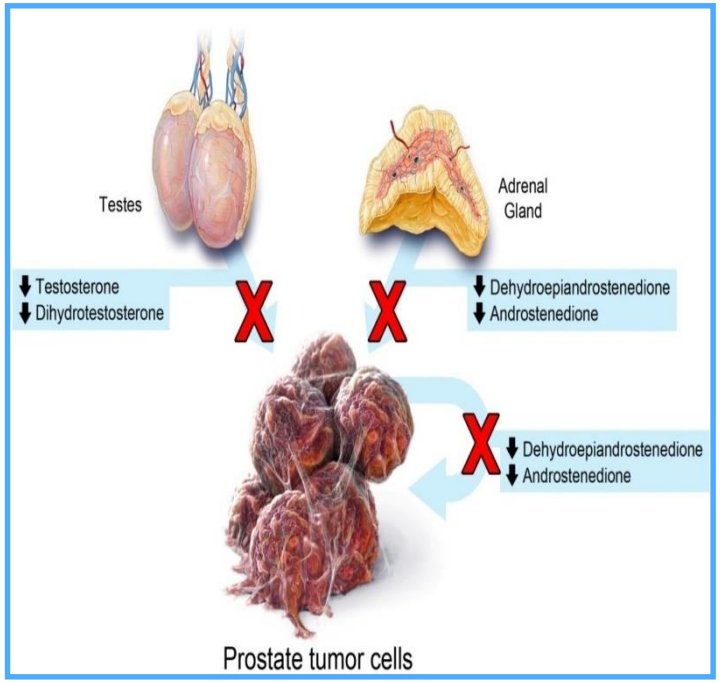
Starting from preclinical and clinical evidence we designed an *in vitro*/translational study in order to investigate a potential direct effect of Abiraterone in our models of primary human OCLs/OBLs

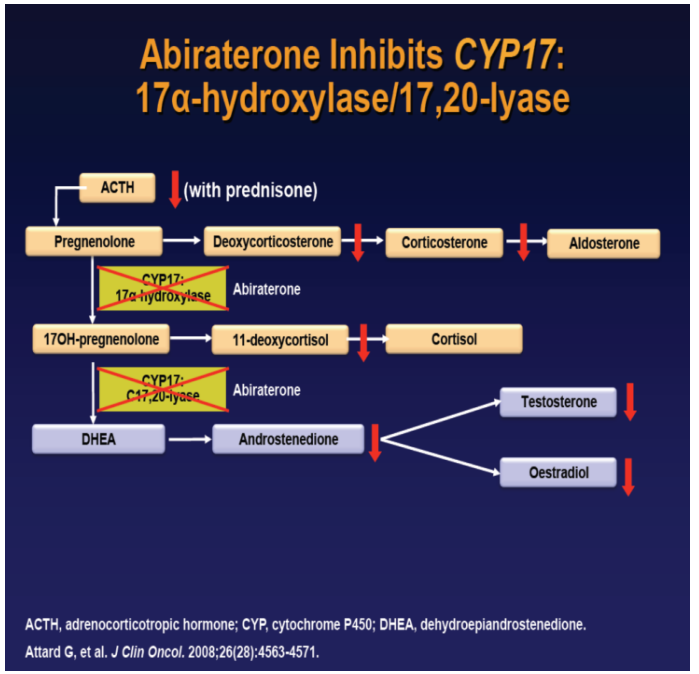
Abiraterone Inhibits Androgen Biosynthesis Through CYP17

" New therapeutic targets in the bone microenvironment for treatment of bone metastasis"

- Androgens produced at 3 critical sites:
  - Testes
  - Adrenal gland
  - Prostate tumor cells

- Abiraterone inhibits biosynthesis of androgens that stimulate tumor cell growth





1. Attard G et al. J Clin Oncol. 2008;26:4563-4571; 2. Attard G et al. J Clin Oncol. 2009;27:3742-3748; 3. Reid AH et al. J Clin Oncol. 2010;28:1489-1495; 4. Ryan CJ et al. J Clin Oncol. 2010;28:1481-1488; 5. Danila DC et al. J Clin Oncol. 2010;28:1496-1501; 6. de Bono JS et al. Ann Oncol. 2010;21(suppl 8): Abstract LBA5.

www.impactjournals.com/oncotarget/ Oncotarget, Advance Publications 2015

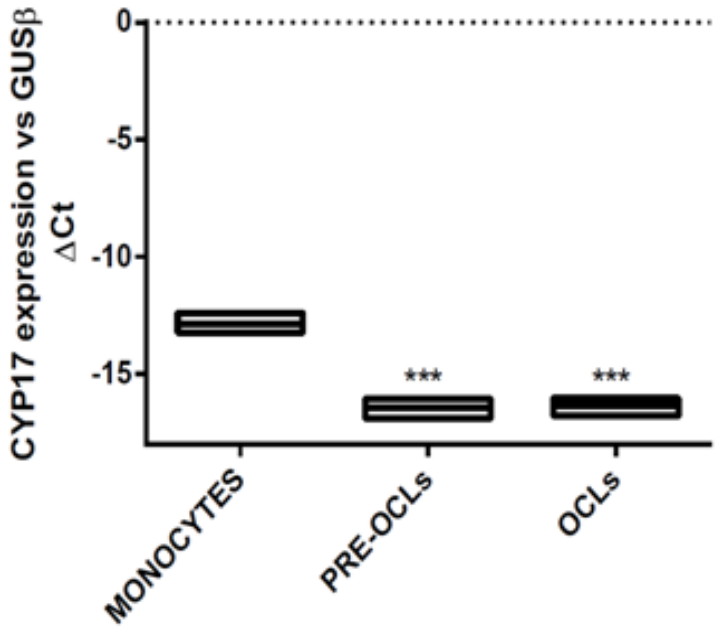
Biological and clinical effects of abiraterone on anti-resorptive and anabolic activity in bone microenvironment

Michele Iuliani[1,*], Francesco Pantano[1,*], Consuelo Buttigliero[2], Marco Fioramonti[1], Valentina Bertaglia[2], Bruno Vincenzi[1], Alice Zoccoli[1], Giulia Ribelli[1], Marcello Tucci[2], Francesca Vignani[2], Alfredo Berruti[3], Giorgio Vittorio Scagliotti[2], Giuseppe Tonini[1] and Daniele Santini[1]

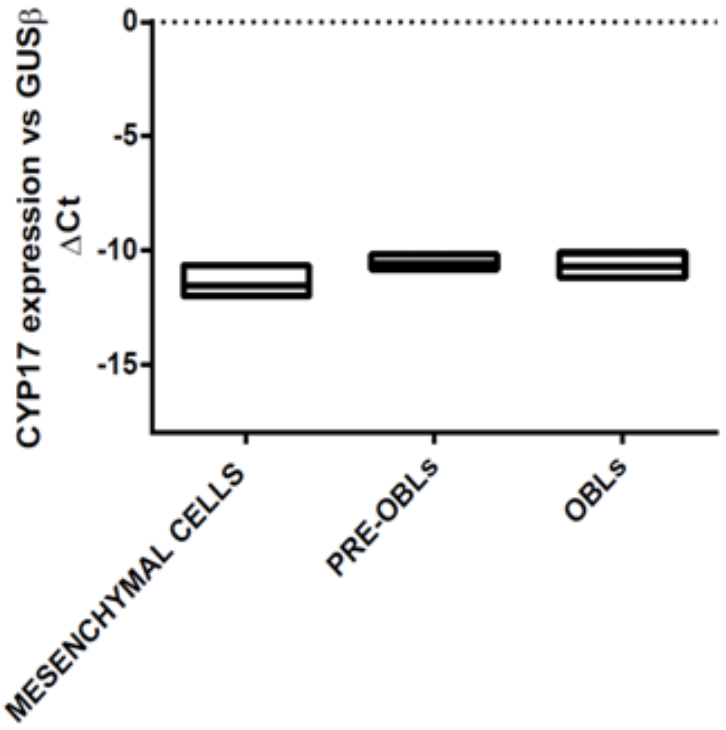
Molecular characterization of abiraterone targets in human primary osteoclast/osteoblast

REAL TIME PCR

Osteoclasts



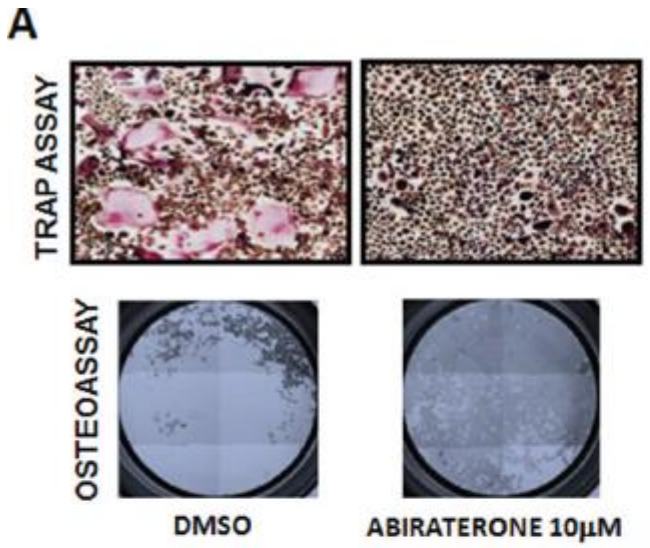
Osteoblasts

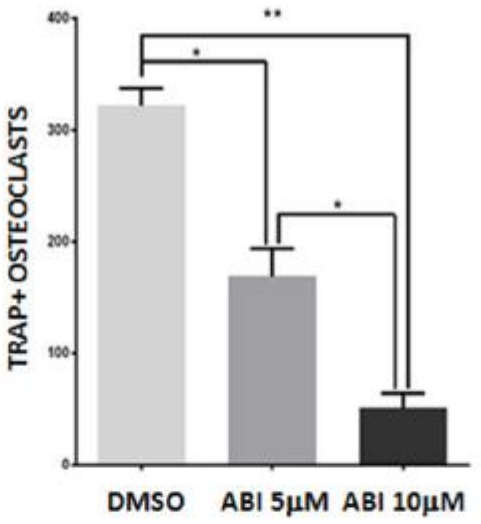


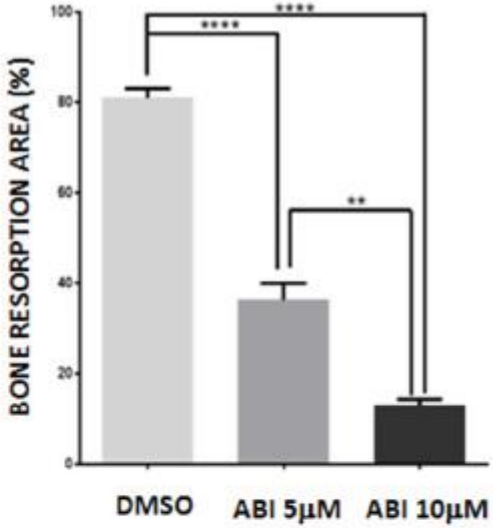
CYP17A1 is expressed in primary human osteoclast/osteoblast

Abiraterone treatment inhibits osteoclast differentiation and activity both in presence and absence of steroids

Steroids







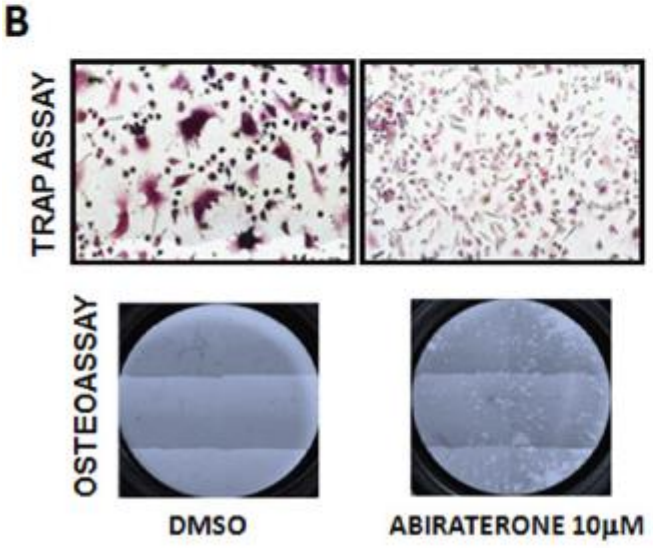
* P < 0.05

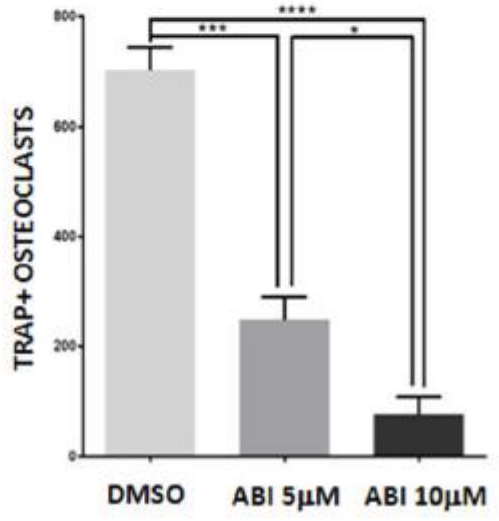
** P < 0.01

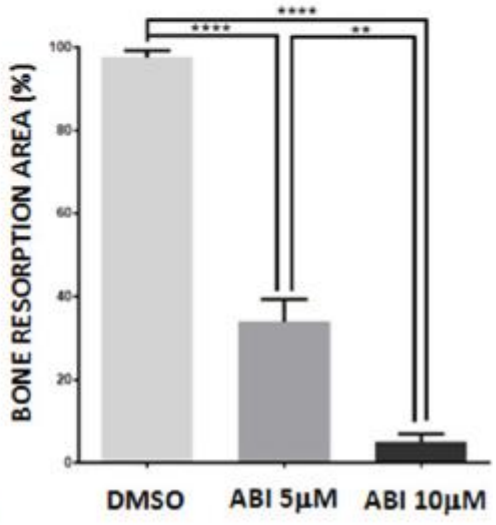
*** P < 0.001

**** P < 0.0001

NO Steroids

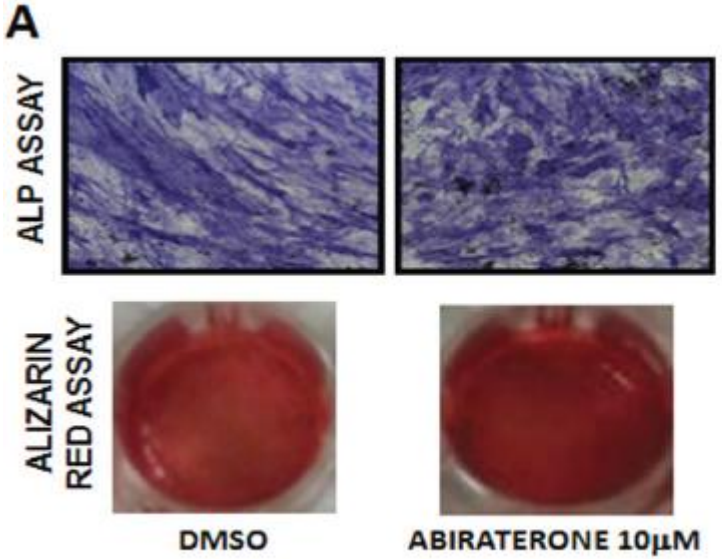


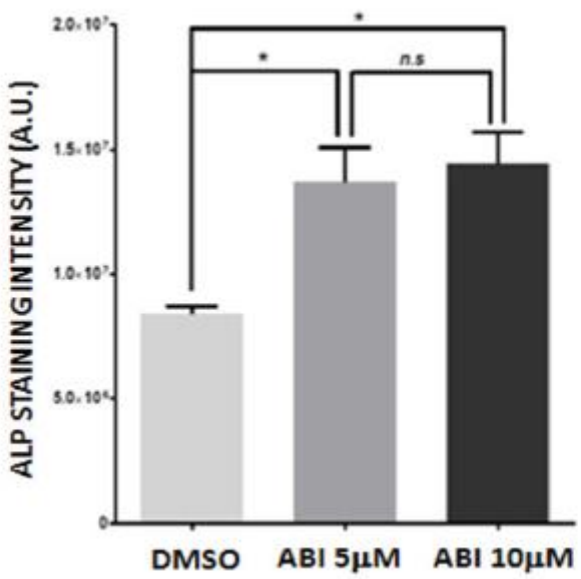


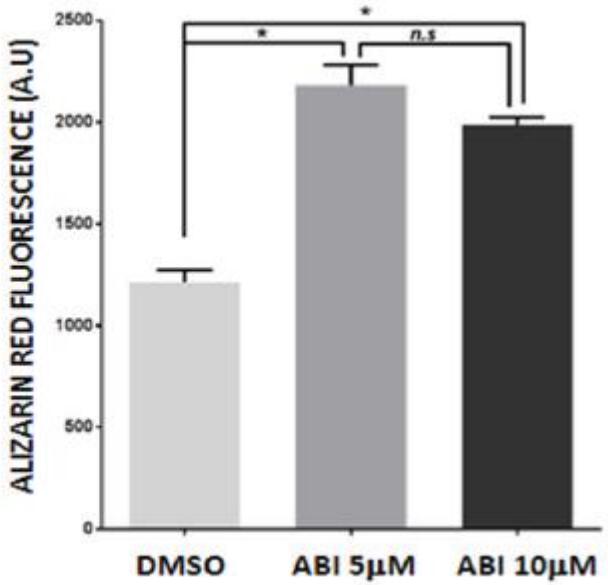


Abiraterone treatment increases osteoblast differentiation and activity both in presence and absence of steroids

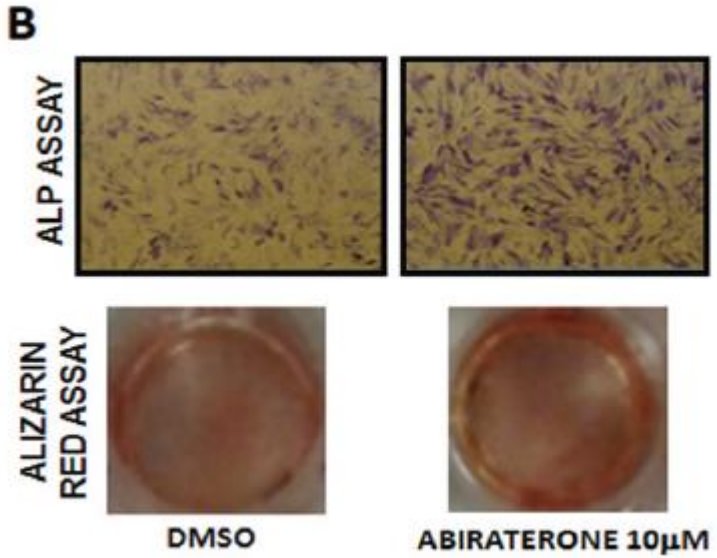
Steroids

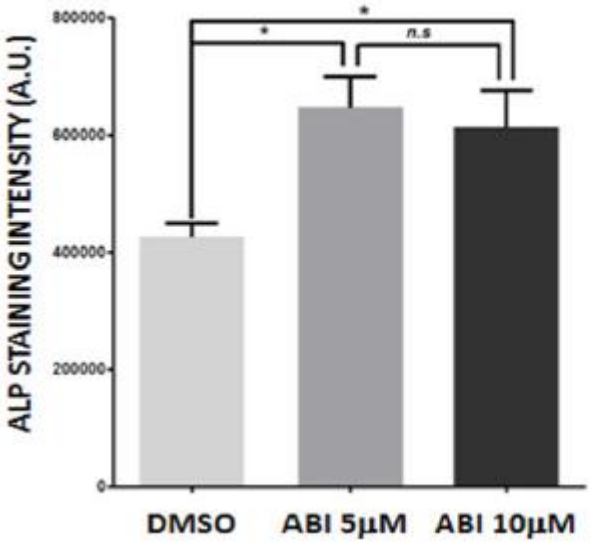






NO Steroids

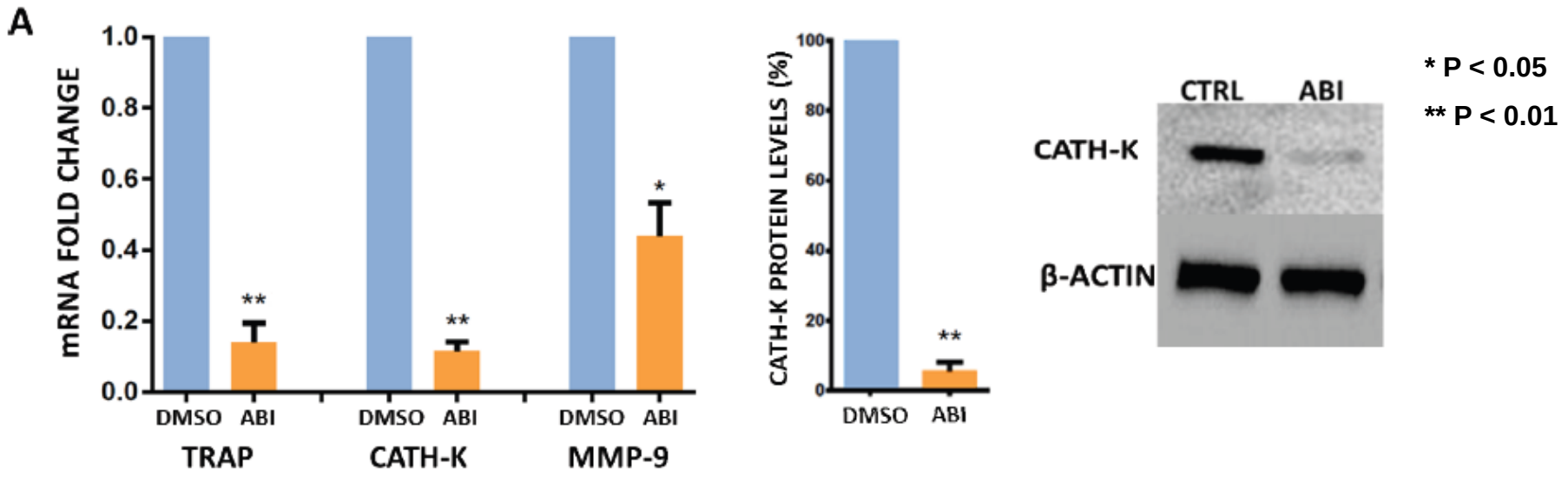




* P < 0.05

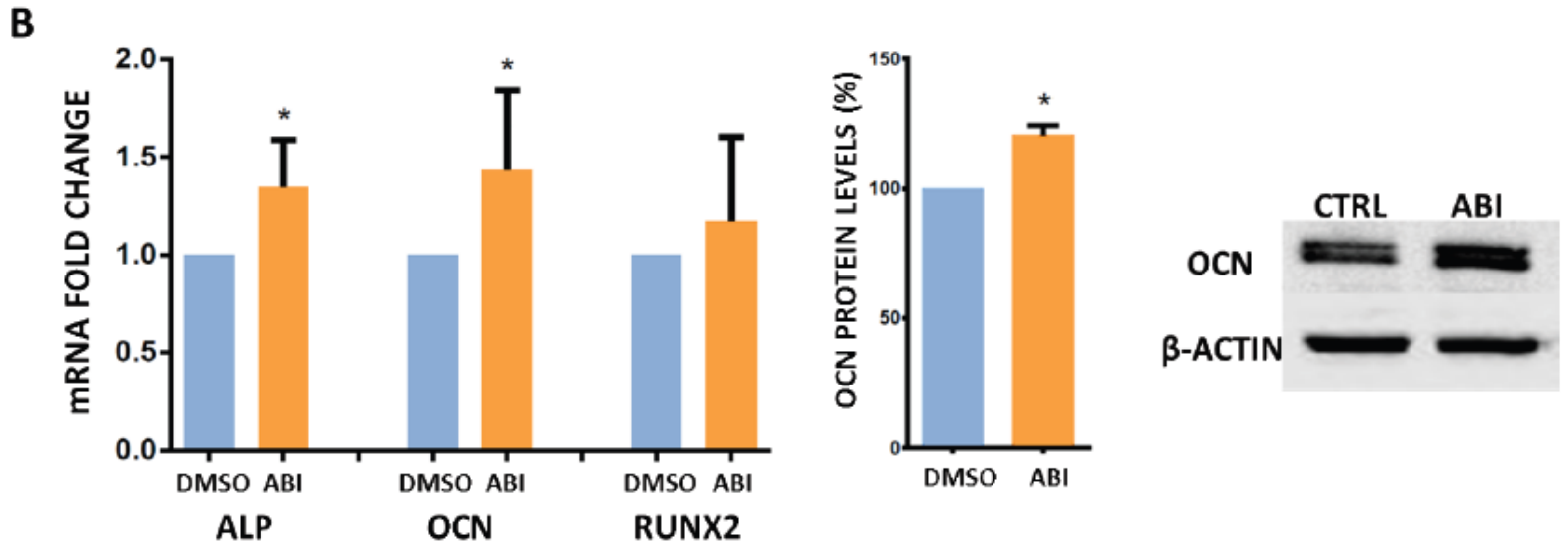
Abiraterone down-modulates osteoclasts marker genes (at gene and protein level)

Osteoclastic gene markers:
TRAP
Cathepsin K (Cath-k)
Metalloproteinase-9 (MMP-9)



Abiraterone up-regulates osteoblasts marker genes (at gene and protein level)

Osteoblastic gene markers:
ALP
Osteocalcin (OCN)
Runx2



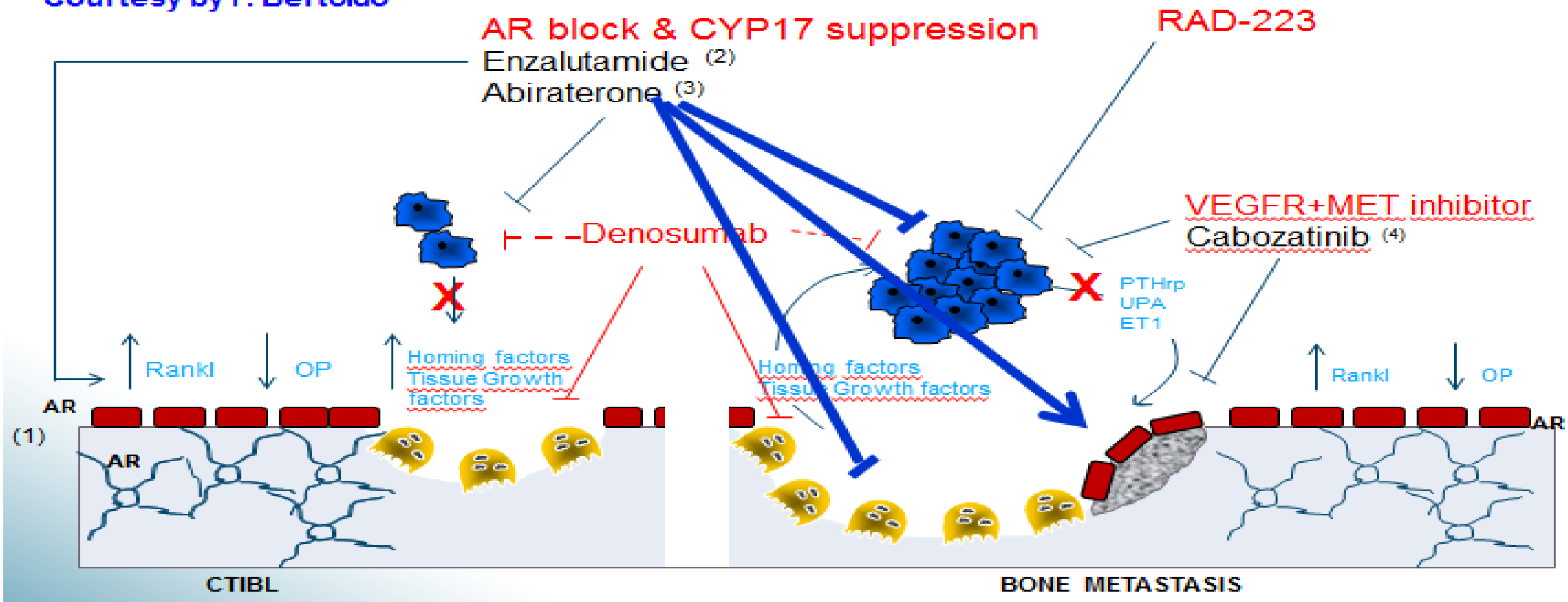
A significant decrease of CTX values and an increase of ALP was found in serum of 49 mCRPC patients treated with Abiraterone post-docetaxel

Table 2: Difference in median level of bone resorption and formation markers

CTX	Baseline ng/mL	Three months ng/mL	Six months ng/mL	Nine months ng/mL
Median, 95% IC	0.86, (0.84-1.25)	0.78, (0.67-1.01)	0.61, (0.73-1.19)	0.66, (0.38-0.71)
p (compare to baseline)		p=0.077	p=0.027	p=0.006
ALP	Baseline U/L	Three months U/L	Six months U/L	Nine months U/L
Median, 95% IC	123, (126-261)	143, (255-382)	126, (200-327)	190, (172-344)
p (compare to baseline)		p=0.01	p=0.62	p=0.28

Abiraterone: TUMOR TARGET AND BONE TARGET THERAPY

Courtesy by F. Bertoldo



(1) Monolagas S et al, Nature Rev Endocrinol 2013 2; (2) Scher HI, et al. N Engl J Med 2012; (3) Fizazi K, et al. Lancet Oncology 2012; (4) Smith DC et al J Clin Oncol 2013; (5) Parker C, et al. N Engl J Med 2013.

Cabozantinib and bone microenvironment

Starting from preclinical and clinical evidence we designed an *in vitro*/translational study in order to investigate a direct effect of CBZ in our models of primary human OCLs/OBLs

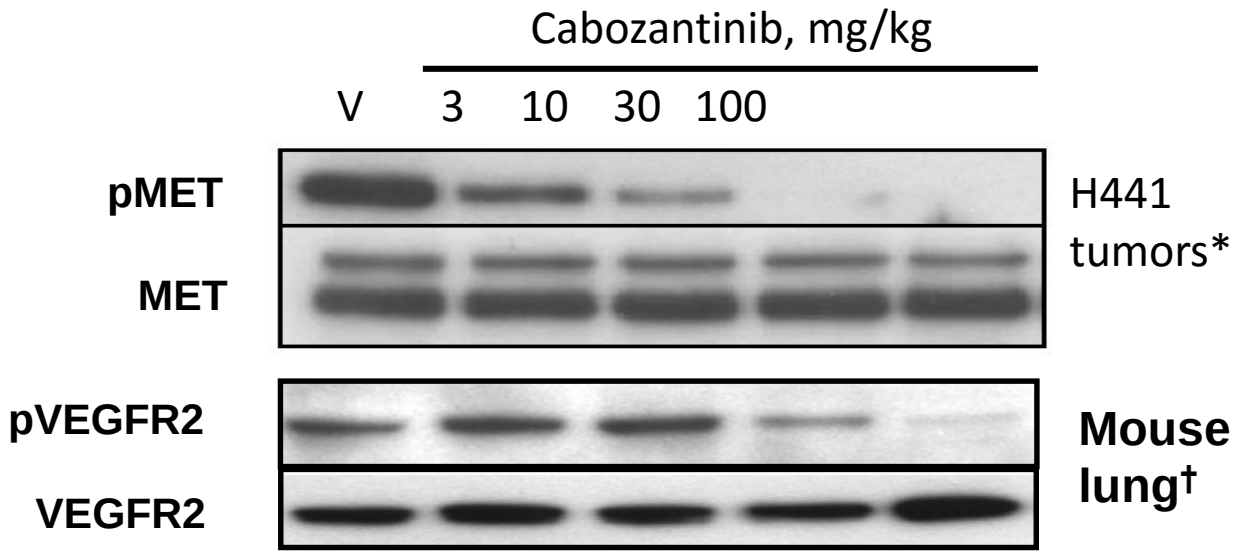
Cabozantinib (XL184): Target Profile

" New therapeutic targets in the bone microenvironment for treatment of bone metastasis"

Kinase	IC_{50}, nM
MET	1.8
VEGFR2	0.035
RET	5.2
KIT	4.6
AXL	7.0
TIE2	14
FLT3	14
S/T Ks (47)	>200

ATP competitive, reversible

RTK	Cellular IC_{50}, nM, Autophosphorylation
MET	8
VEGFR2	4

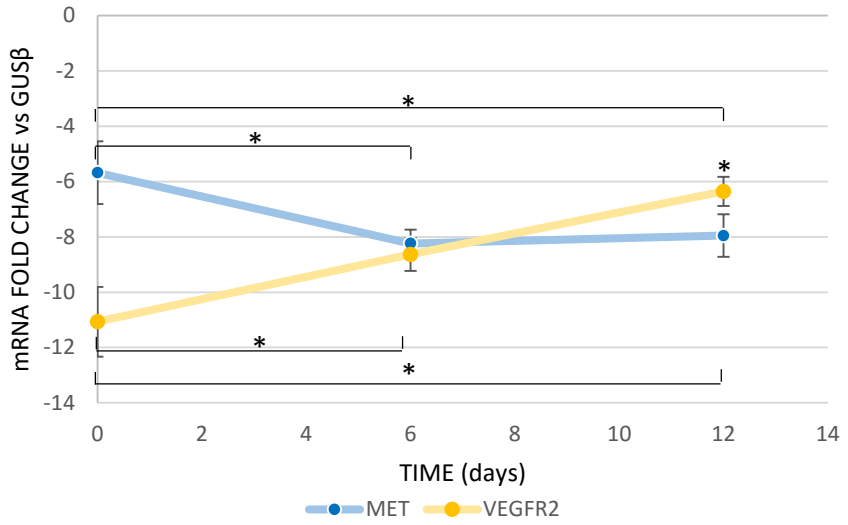


*No growth factor stimulation.
†VEGF-A administered 30 min prior to harvest.

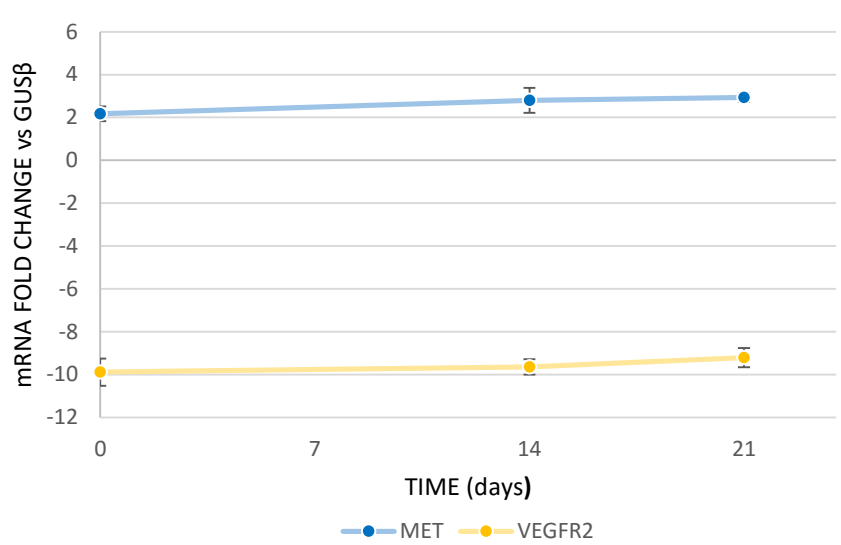
Data courtesy of Ron Weitzman and Dana Aftab.

Molecular characterization of cabozantinib targets in human primary osteoclast/osteoblast

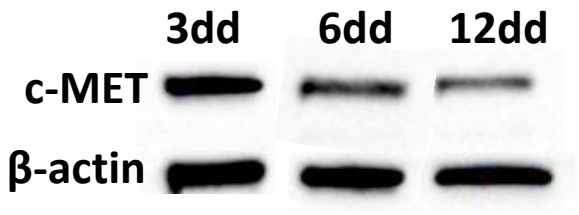
A



B



C



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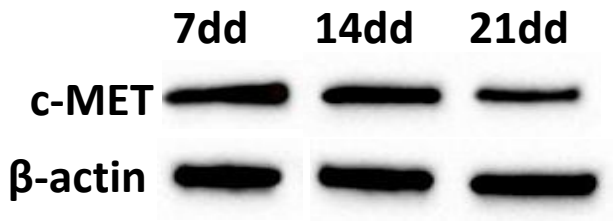
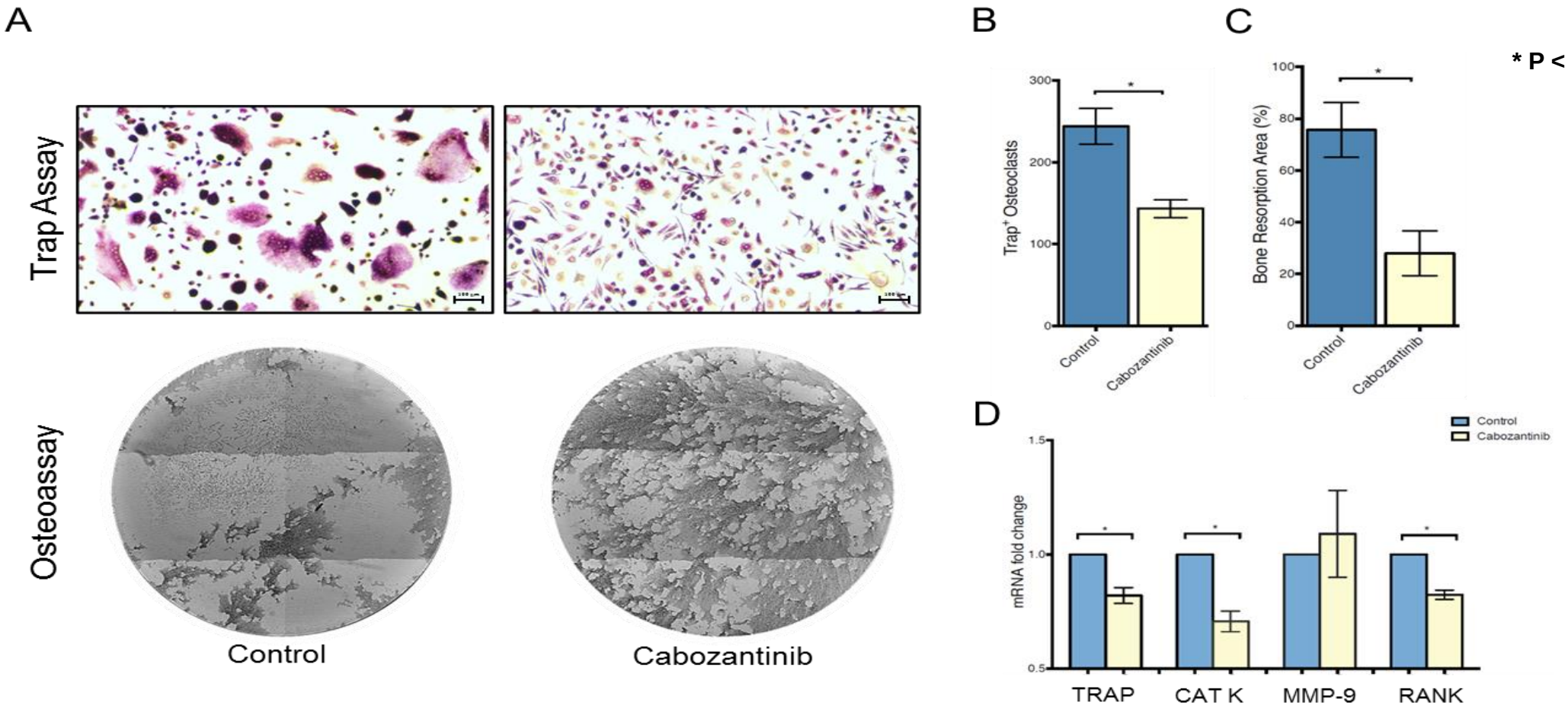


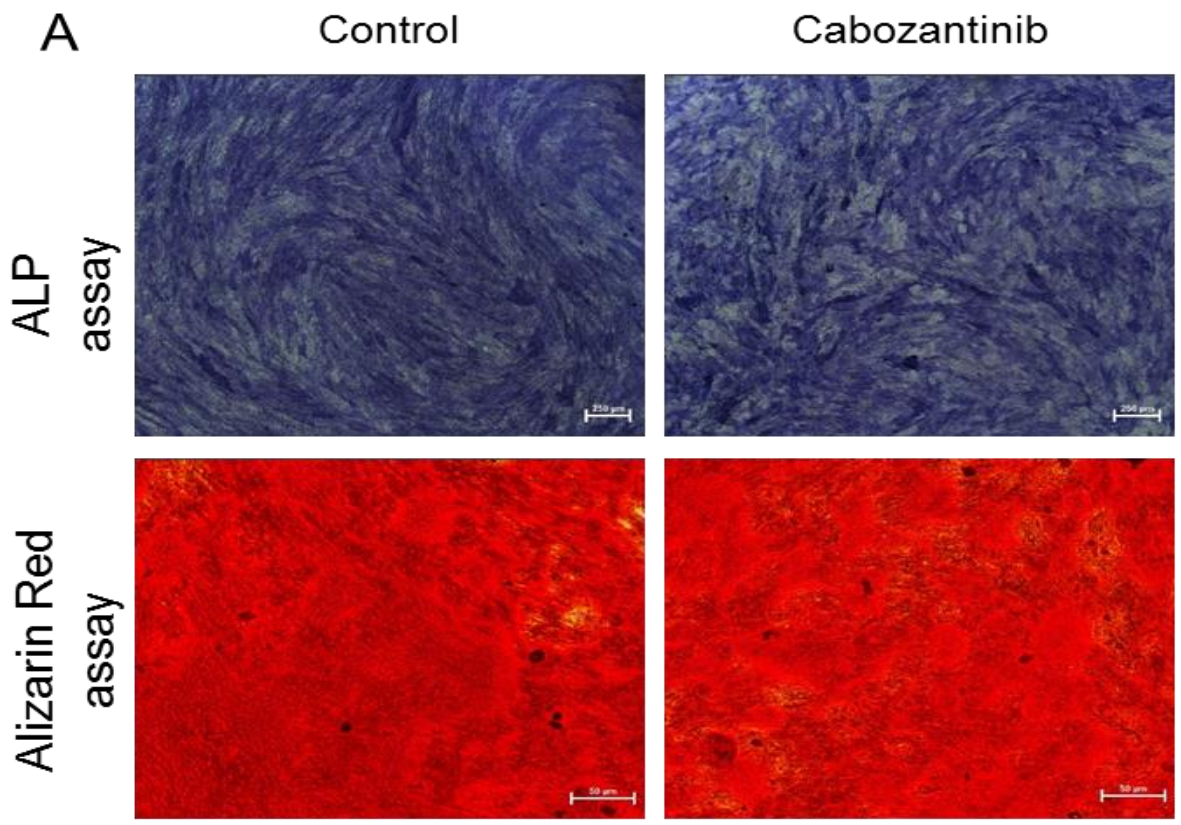
Under Review on Scientific Reports

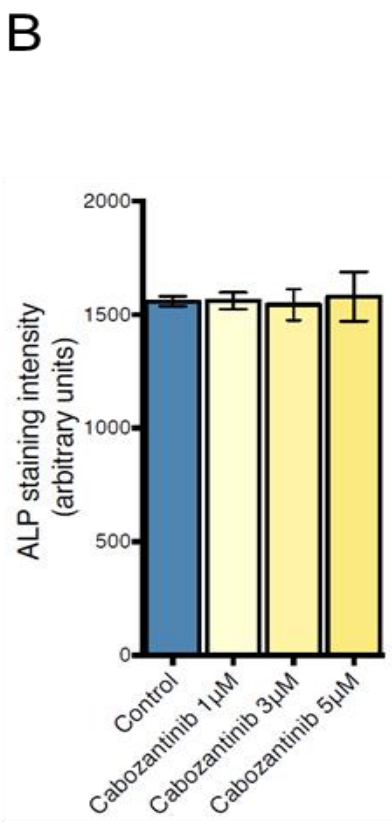
Cabozantinib inhibits osteoclast differentiation and activity

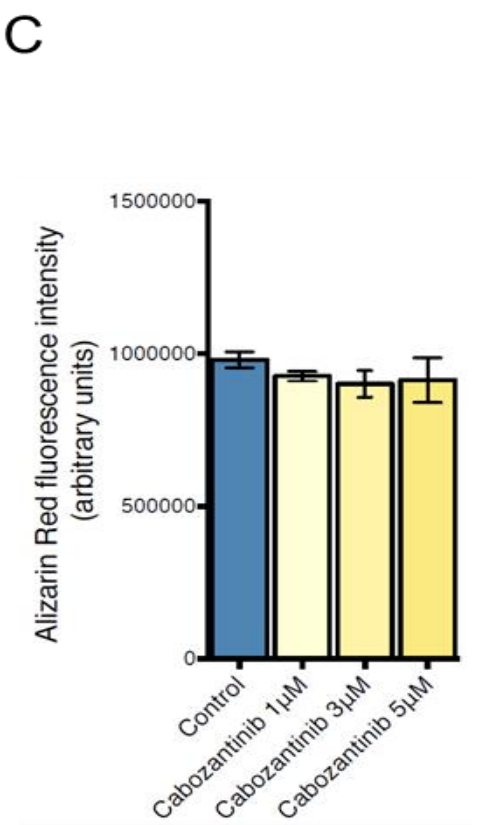


Under Review on Scientific Reports

Cabozantinib does not affect osteoblast differentiation and activity

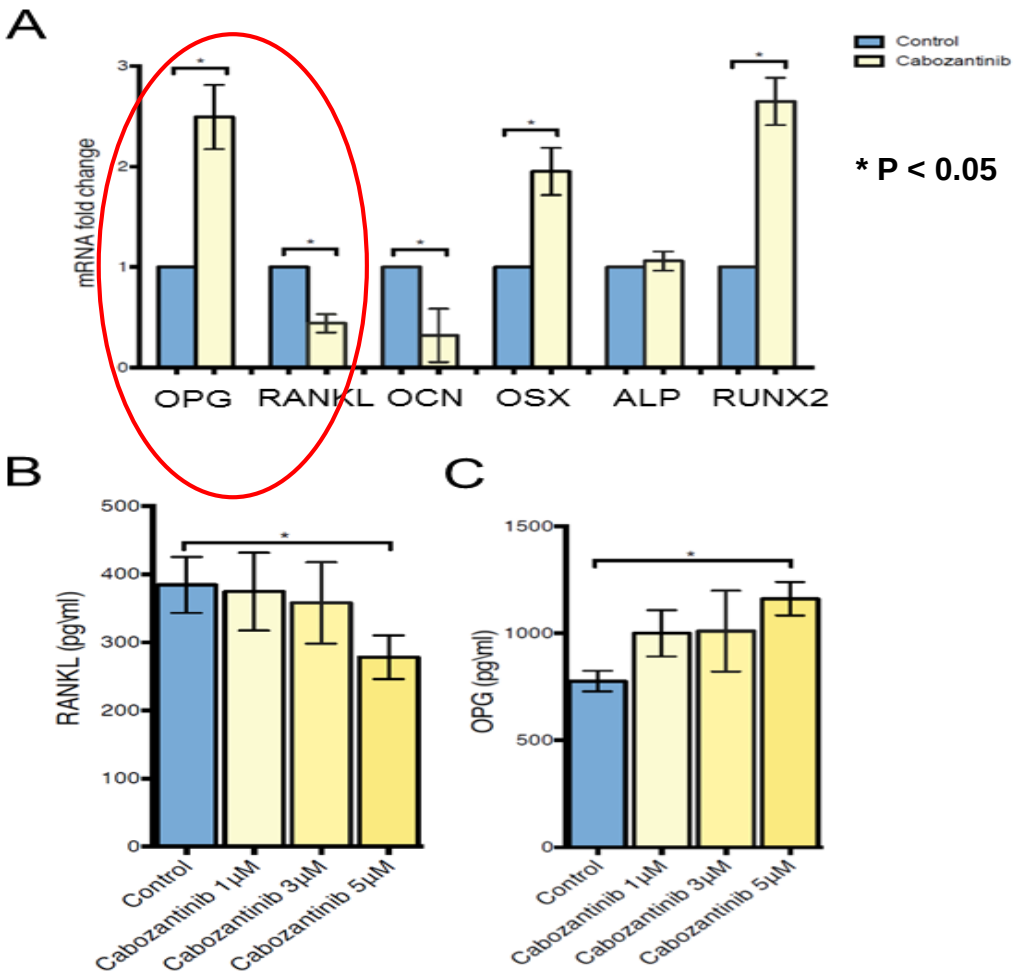






Under Review on Scientific Reports

Cabozantinib treatment up-regulates OPG gene/protein sectretion and down-modulates RANKL gene/protein secretion altering RANKL/OPG balance



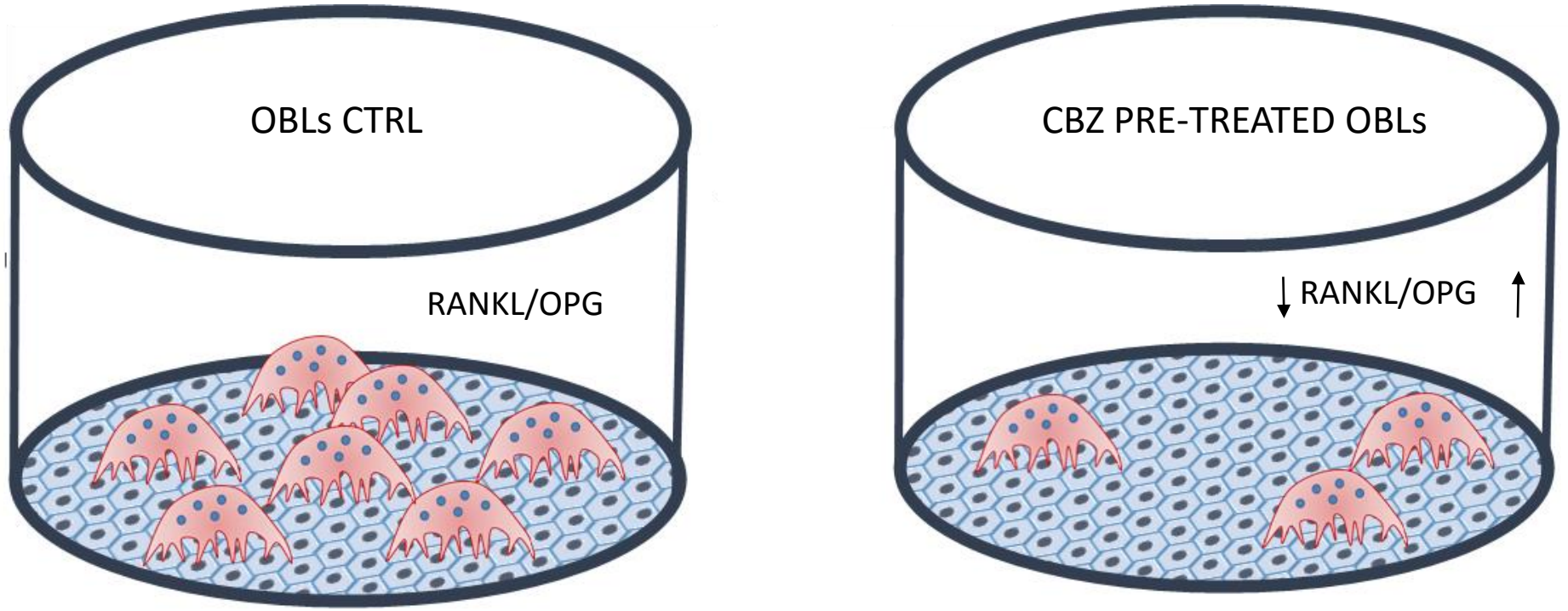
* P < 0.05

mRNA

Proteins

Under Review on Scientific Reports

Cabozantinib pre-treated osteoblasts influence osteoclasts differentiation?

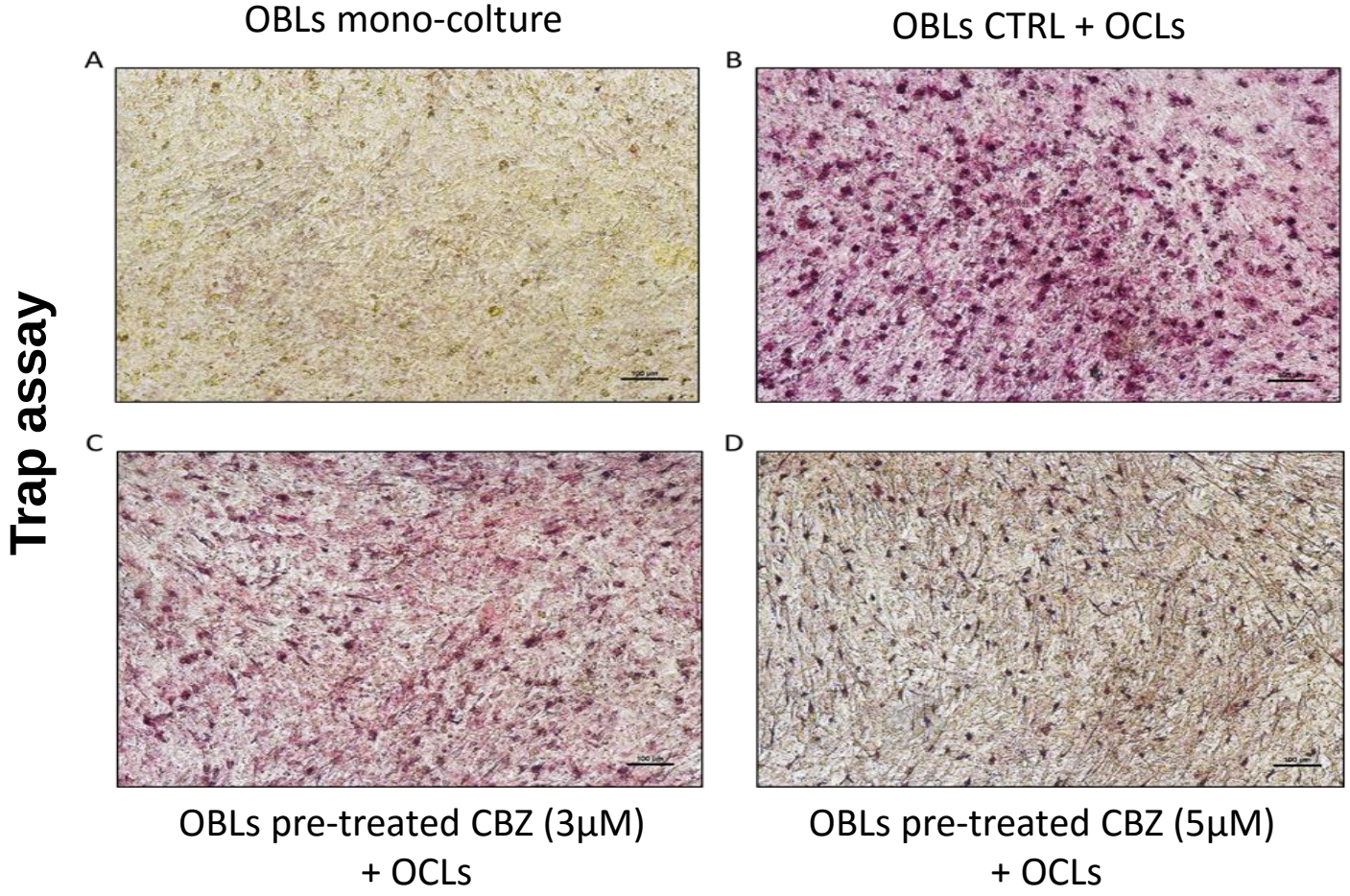


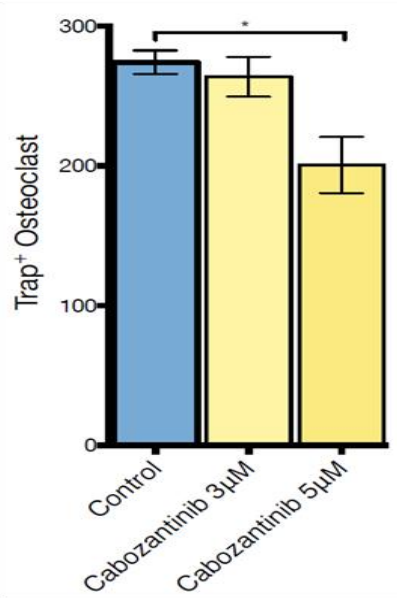
COCOLTURE OSTEOBLAST/OSTEOCLAST "CELL-TO-CELL CONTACT"

Experimental methodology:

- Osteoblasts were differentiated in presence/absence of CABO ZANTINIB
- Osteoclasts were seeded and differentiated directly on osteoblast layer (un/treated with CABO) without exogenous RANKL supplement
- At day 12 the number of cathepsin k positive cells (identified as osteoclasts) was evaluated

Cabozantinib pre-treated osteoblasts reduced osteoclast differentiation compared to untreated osteoblast





* P < 0.05

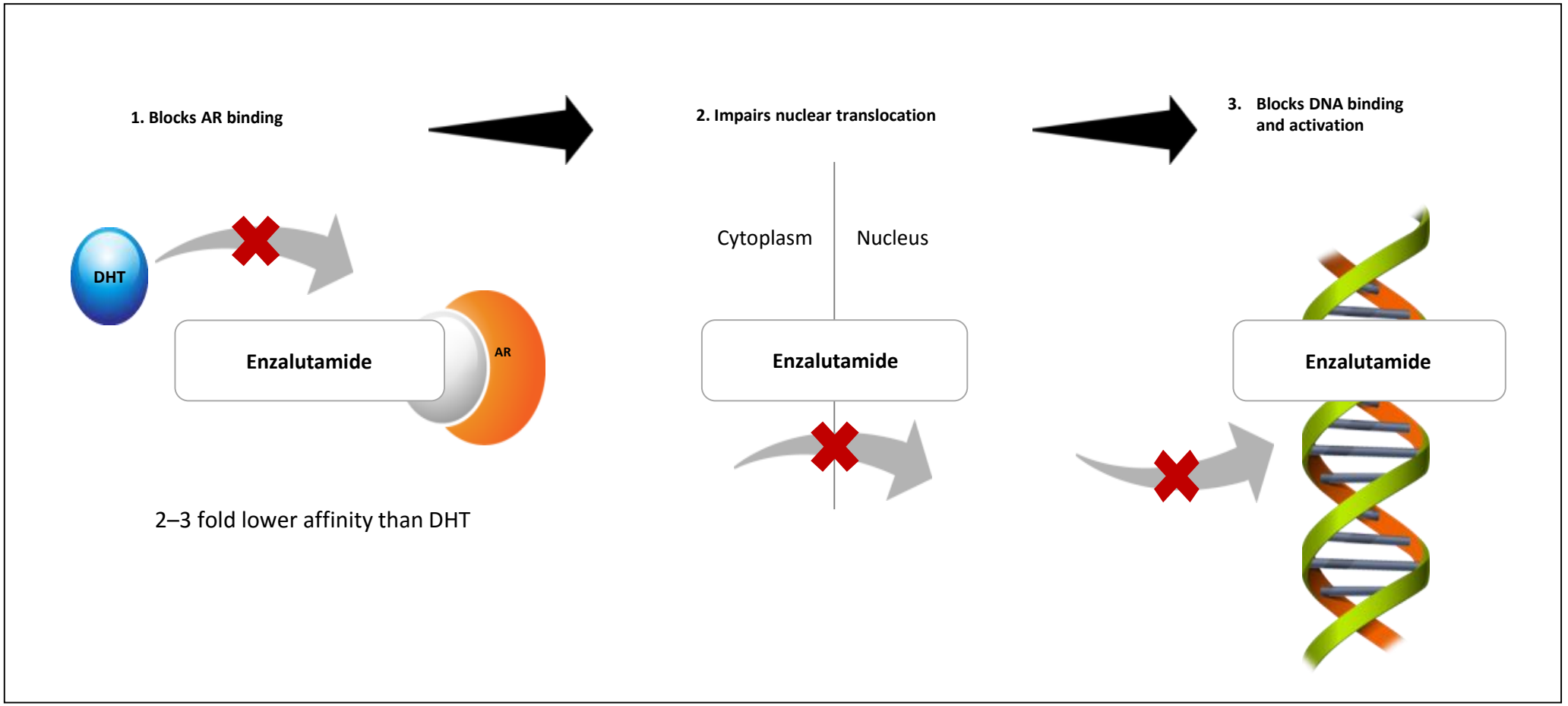
Under Review on Scientific Reports

Enzalutamide and bone microenvironment

Starting from preclinical and clinical evidence we designed an *in vitro*/translational study in order to investigate a direct effect of ENZA in our models of primary human OCLs/OBLs

Enzalutamide: mechanism of action

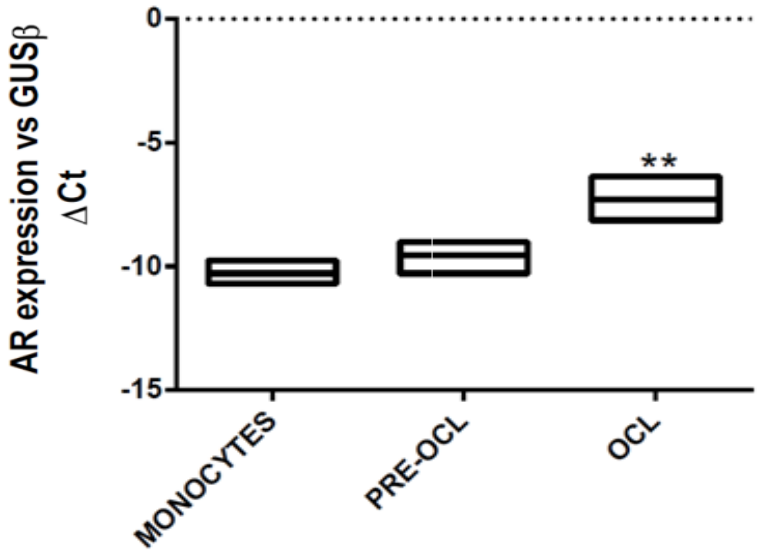
" New therapeutic targets in the bone microenvironment for treatment of bone metastasis"



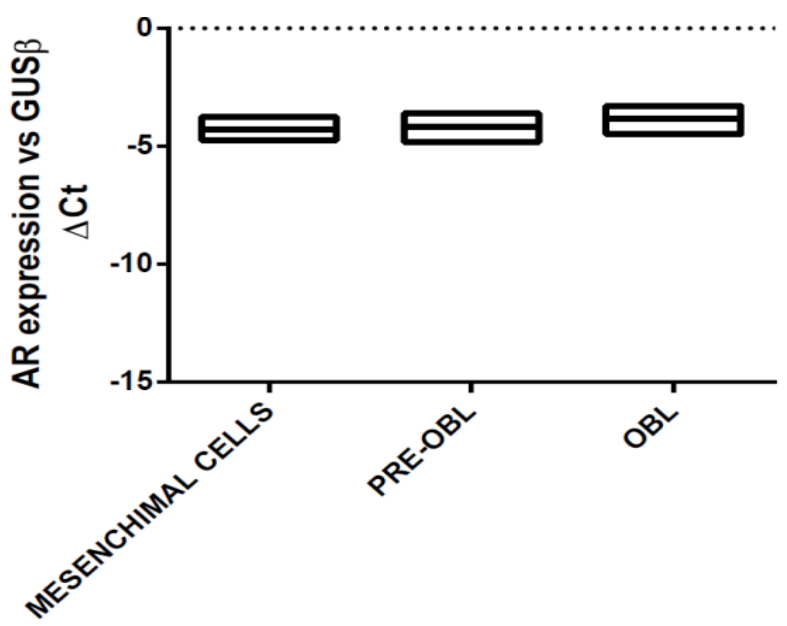
Molecular characterization of enzalutamide targets in human primary osteoclast/osteoblast

REAL TIME PCR

Osteoclasts

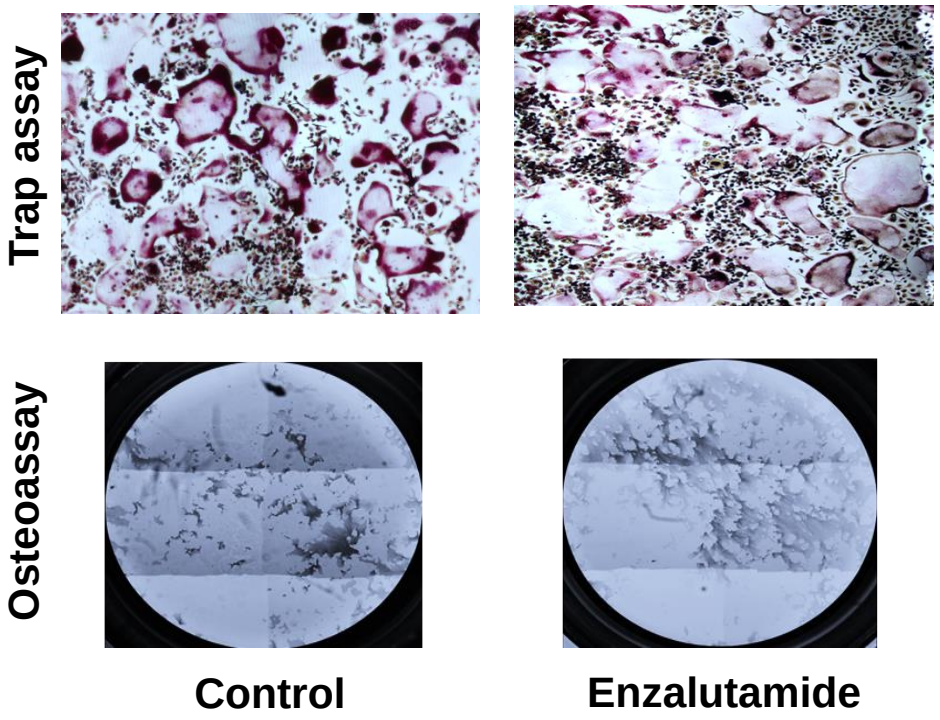


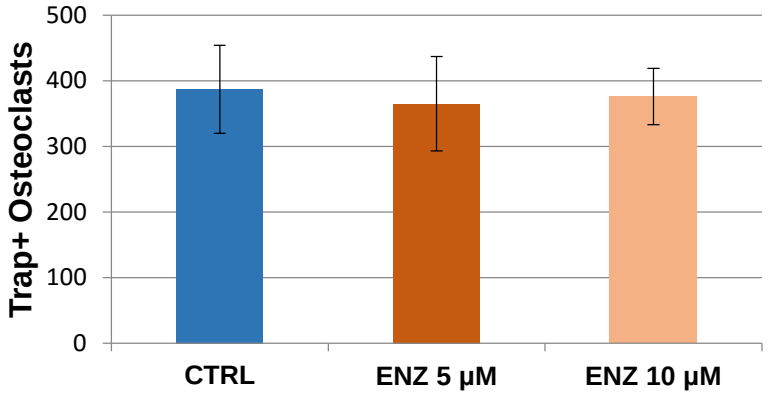
Osteoblasts

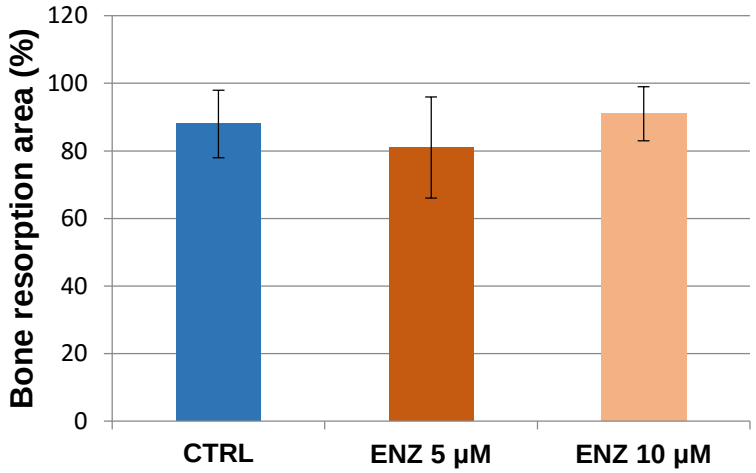


AR is expressed in primary human osteoclast/osteoblast

Enzalutamide does not affect osteoclast differentiation and activity

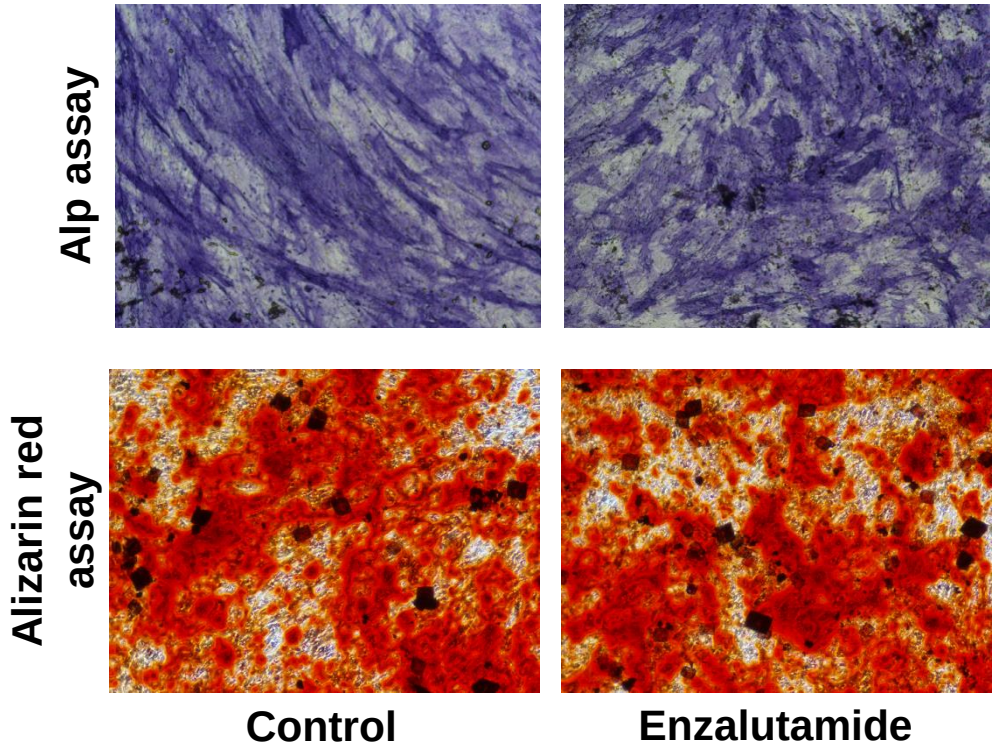


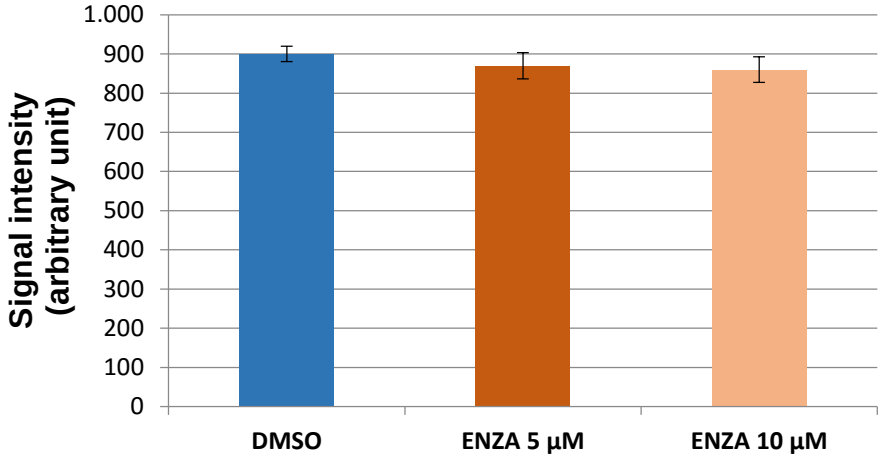


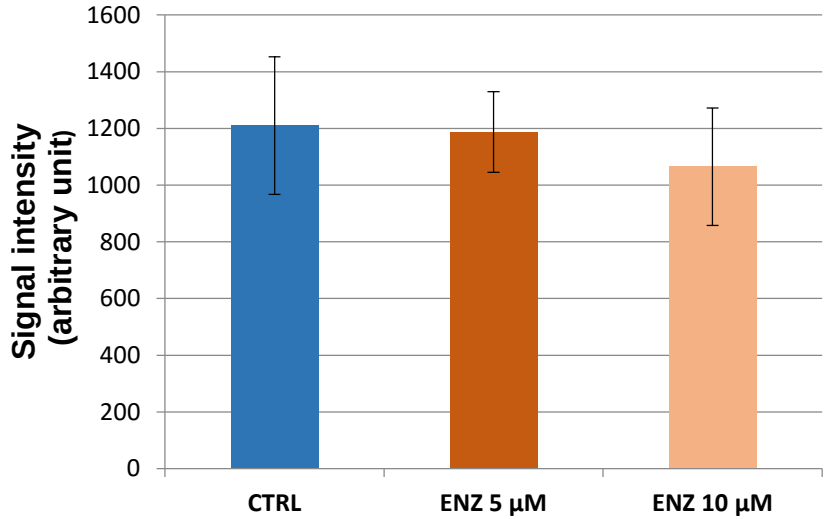


Unpublished data

Enzalutamide does not affect osteoblast differentiation and activity



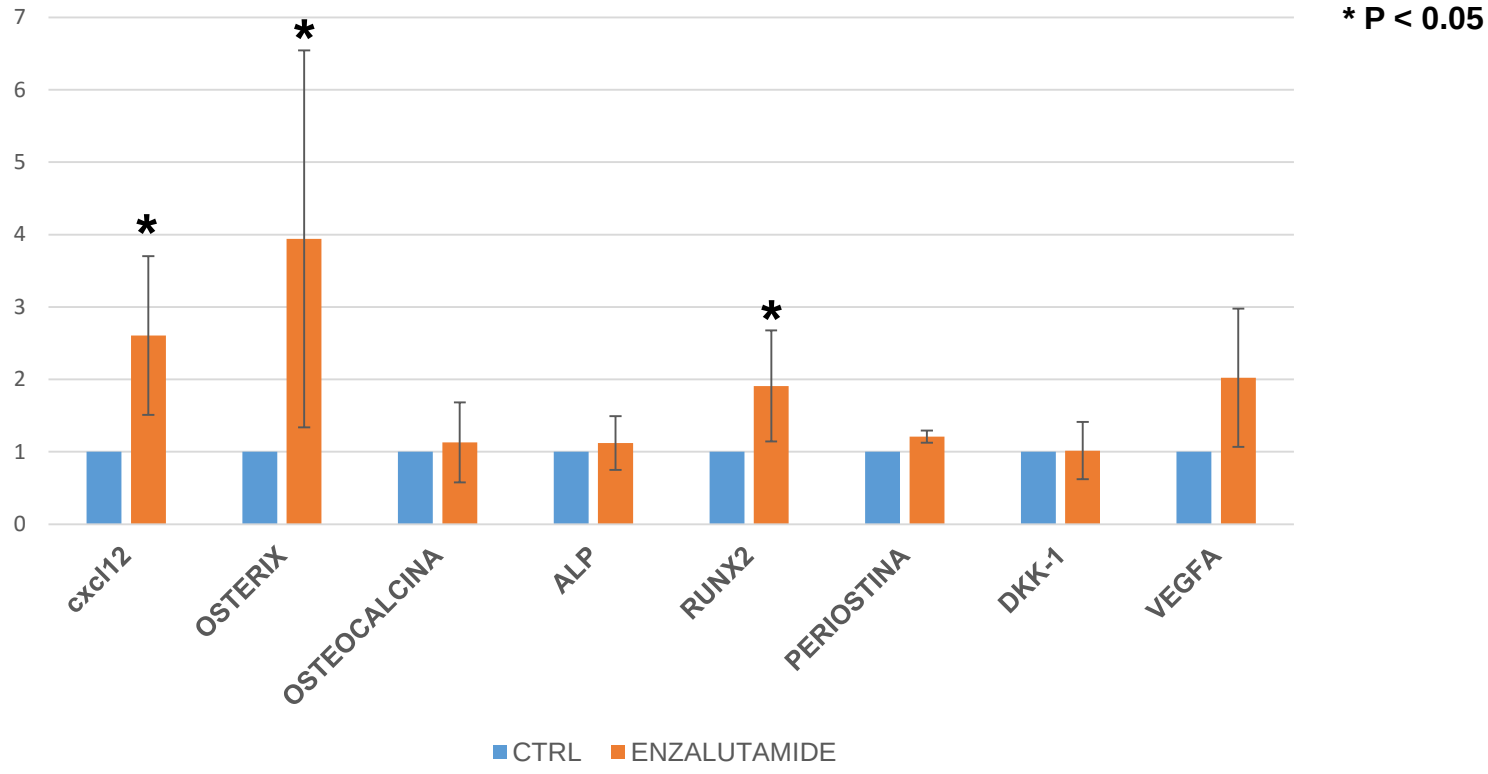




Unpublished data

Enzalutamide treatment up-regulates some osteoblastic marker genes: CXCL12, OSTERIX and RUNX2

mRNA

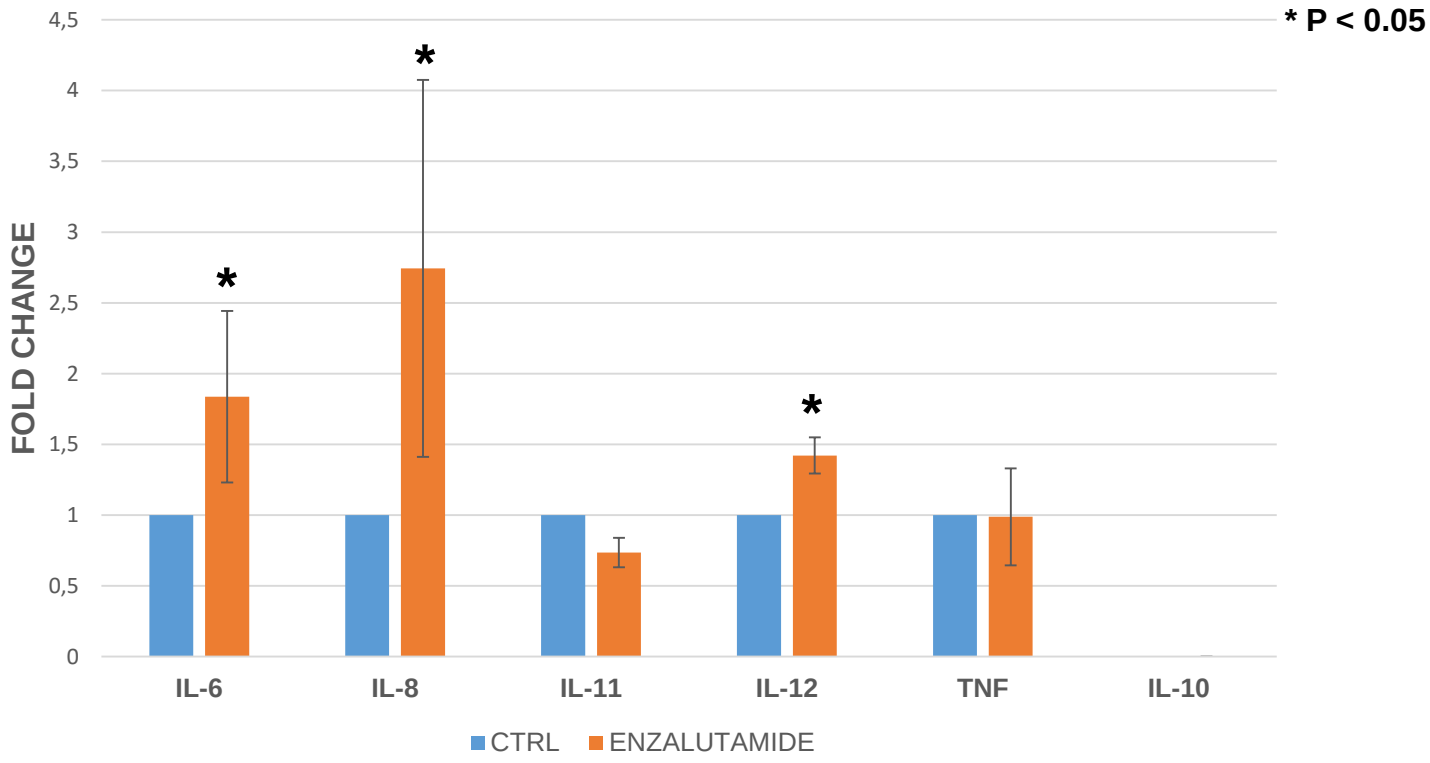


* P < 0.05

Unpublished data

Enzalutamide treatment up-regulates pro-inflammatory genes IL-6, IL-8, IL-12

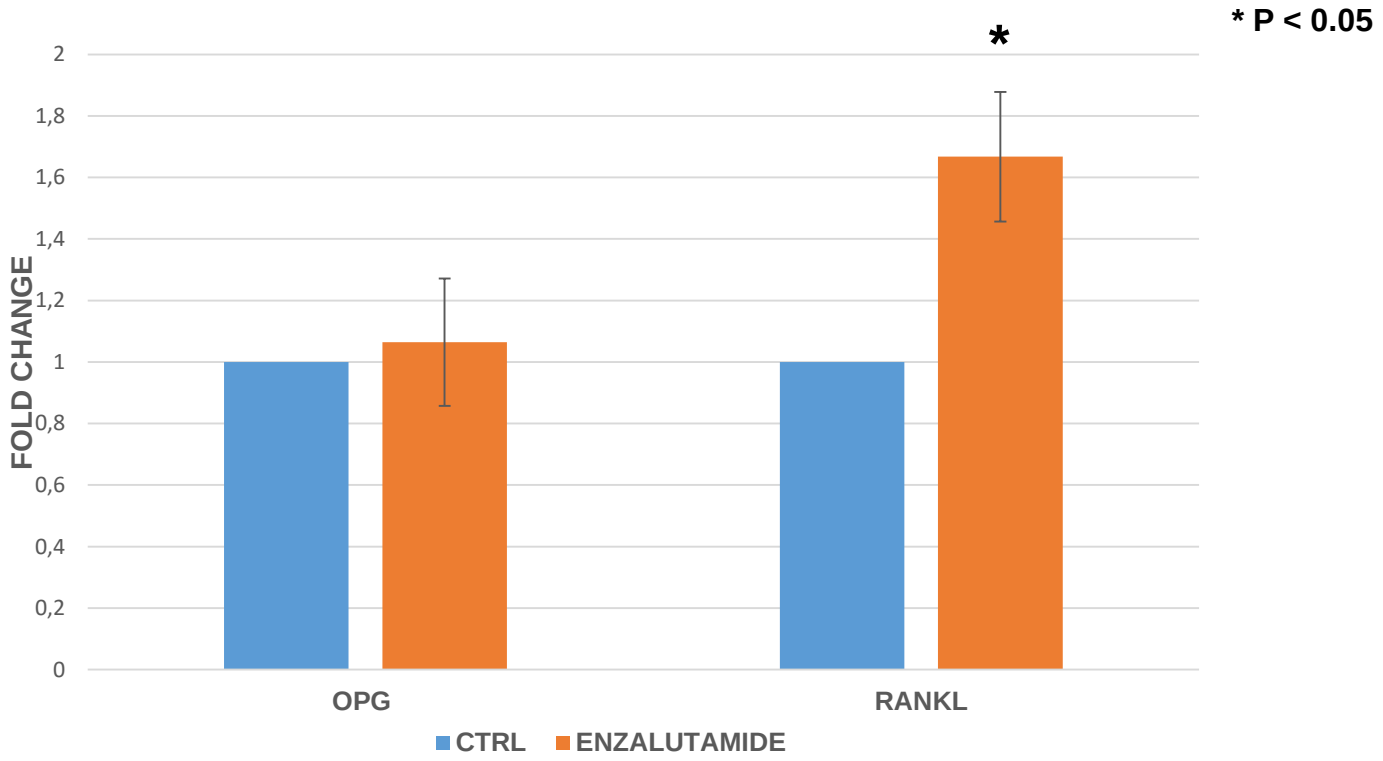
mRNA



Unpublished data

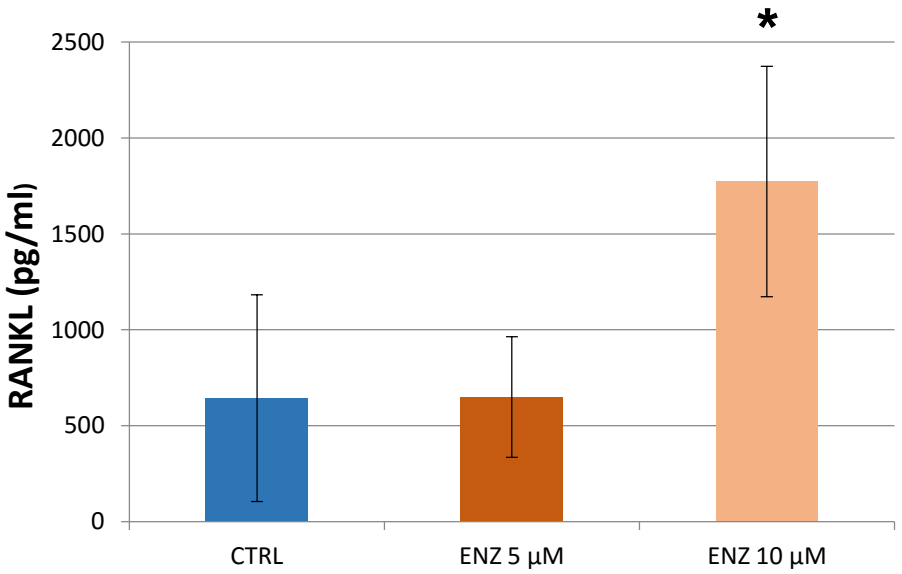
Enzalutamide treatment up-regulates RANKL gene expression

mRNA



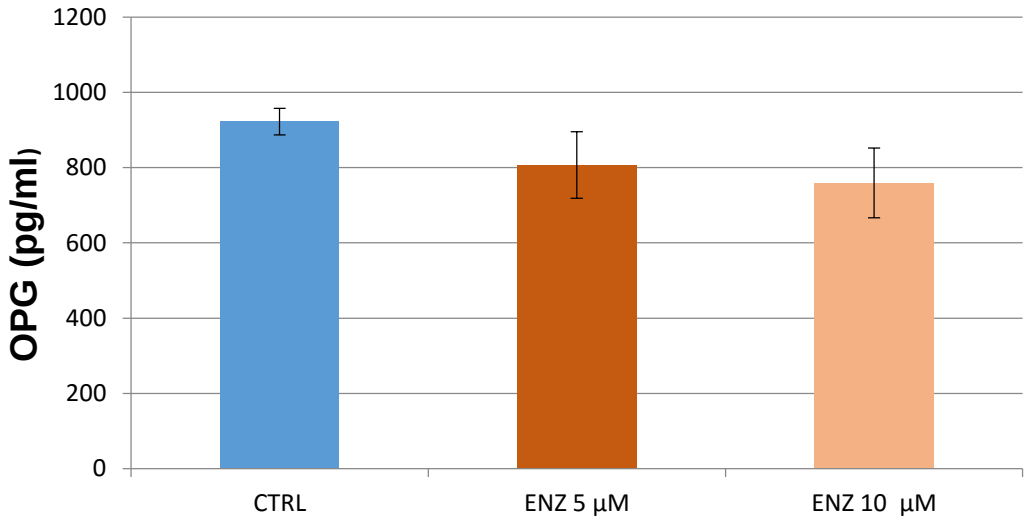
Unpublished data

Enzalutamide treatment up-regulates RANKL protein secretion



Proteins

* P < 0.05

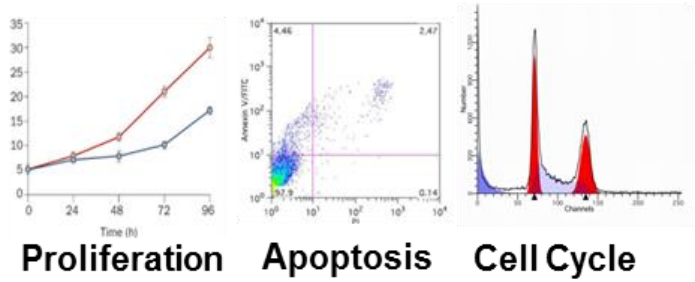


Proteins

Unpublished data

Future perspectives: Abiraterone and Enzalutamide

- ✓ To investigate the “indirect” osteoblast-mediated effects of Abiraterone and Enzalutamide on the modulation of main biological parameters such as proliferation, apoptosis and cell cycle of CRPC exposed to pre-treated OBL conditioned media



- ✓ To investigate the “indirect” osteoblast-mediated effects of Abiraterone and Enzalutamide on the androgen receptor activation of CRPC exposed to pre-treated OBL conditioned media

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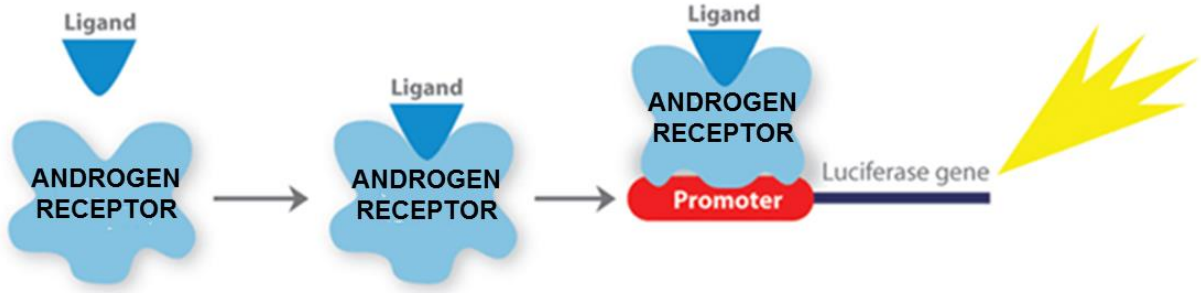
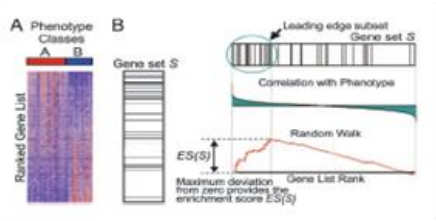


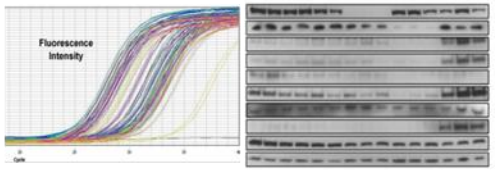
Future perspectives: Abiraterone and Enzalutamide

- ✓ To investigate the “indirect” osteoblast-mediated effects of Abiraterone and Enzalutamide in terms of gene expression modulation of CRPC exposed to pre-treated OBL conditioned media using an high-throughput approach



Trascriptomic and bioinformatic analysis

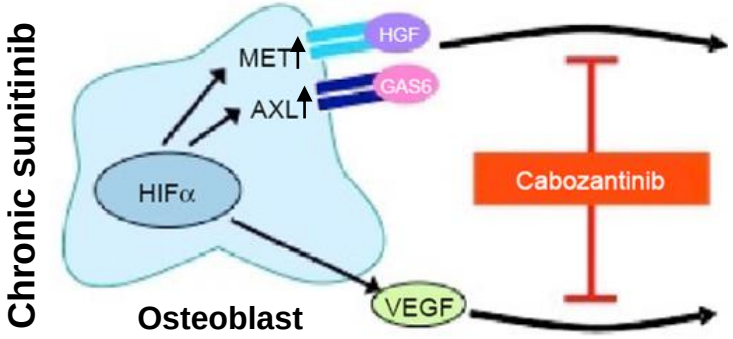
- ✓ To investigate the “indirect” osteoblast-mediated effects of Abiraterone and Enzalutamide on the activation of specific signaling pathways in CRPC exposed to pre-treated OBL conditioned media in order to identify new potential mechanisms of activity and resistance to anti-androgen therapy

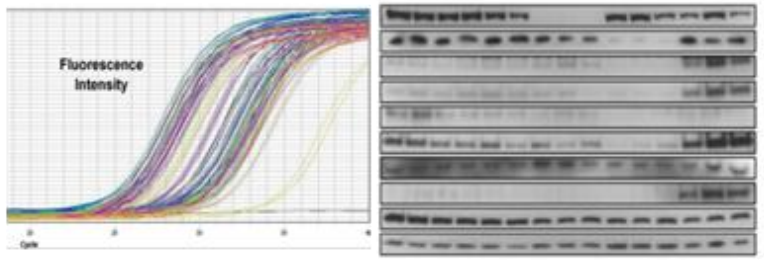


Gene expression, protein and phosphoproteomic analysis of specific signalling pathways identified by trascriptomic analysis

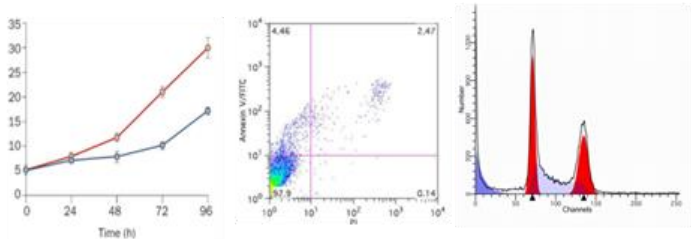
Future perspectives: Cabozantinib

- ✓ To investigate the AXL and c-MET activation status in osteoblasts/osteoclasts previously treated with sunitinib and the following modulation of cells in terms of gene expression and protein analyses after cabozantinib administration.





- ✓ To investigate a potential “indirect” antitumor effect of cabozantinib mediated by osteoblasts on renal carcinoma (RCC) cells evaluating the modulation of main biological parameters such as proliferation, apoptosis and cell cycle of cancer cells



Proliferation Apoptosis Cell Cycle

Thank you very much for the Attention

Paolo Manca Michele Iuliani Francesco Pantano Marco Fioramonti Giulia Ribelli