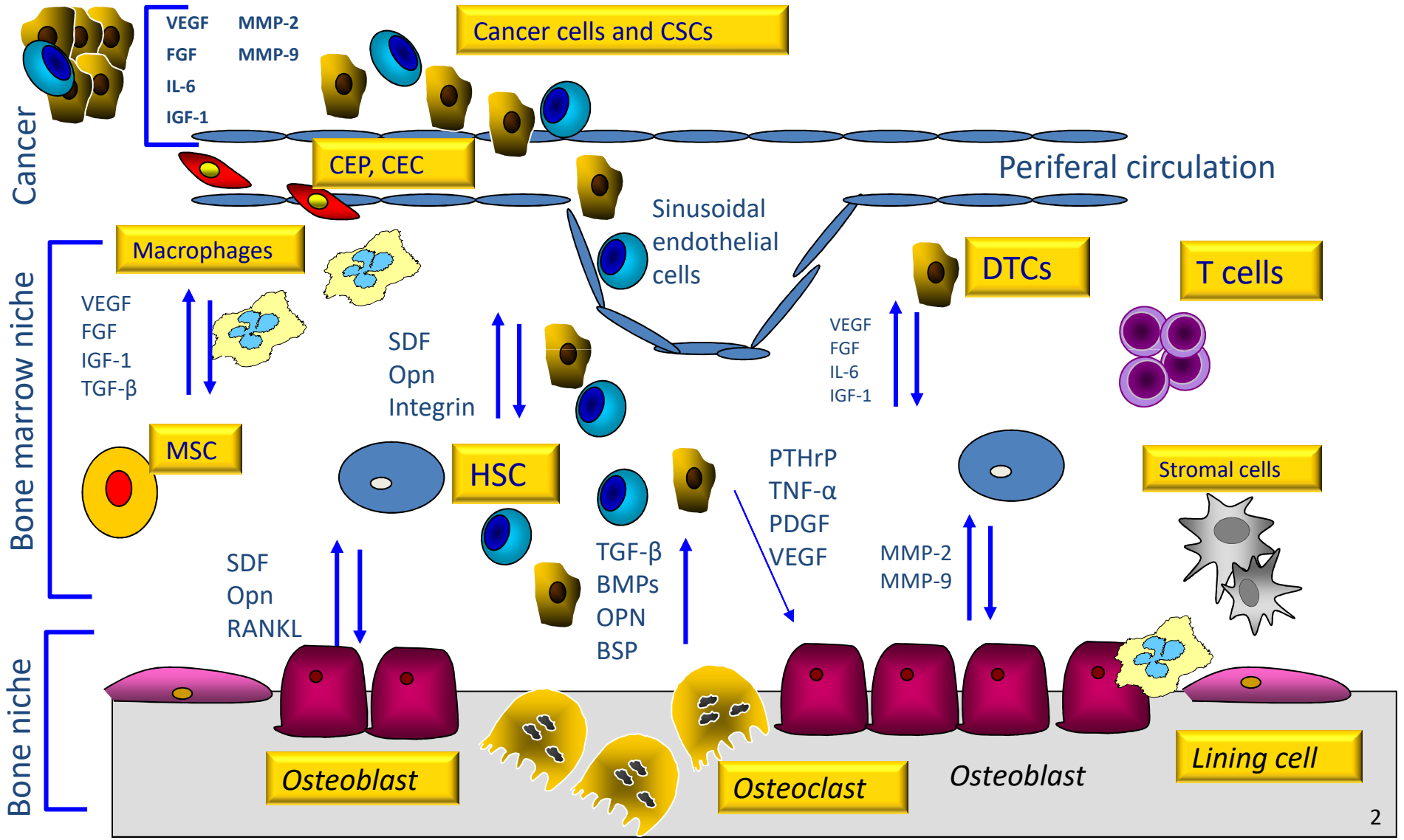

# Effetto sul microambiente osseo di Abiraterone, Enzalutamide e Cabozantinib

**Francesco Pantano**

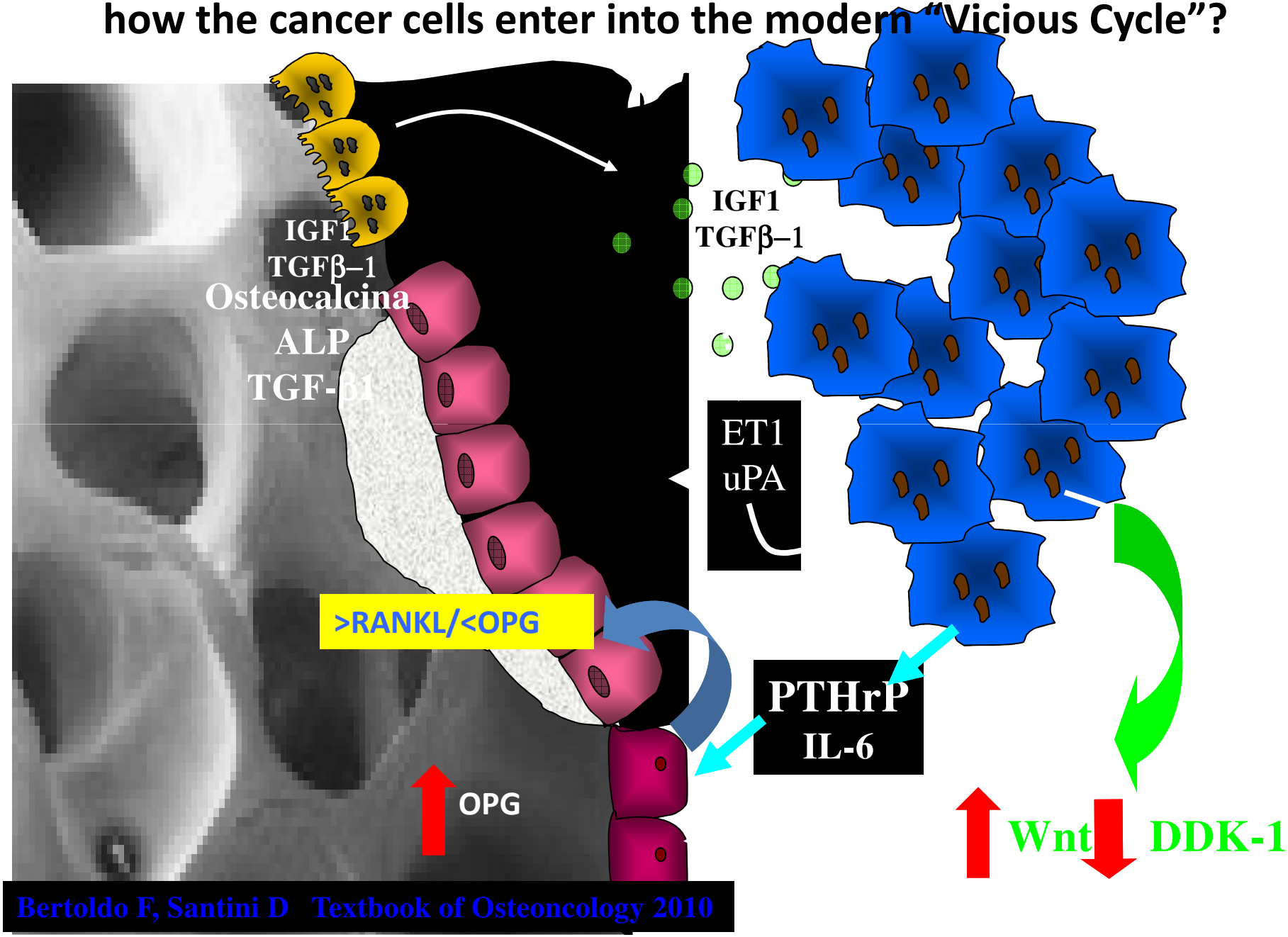
**Università Campus Bio-Medico di Roma**

# Interactions between cancer cells and bone microenvironment cells

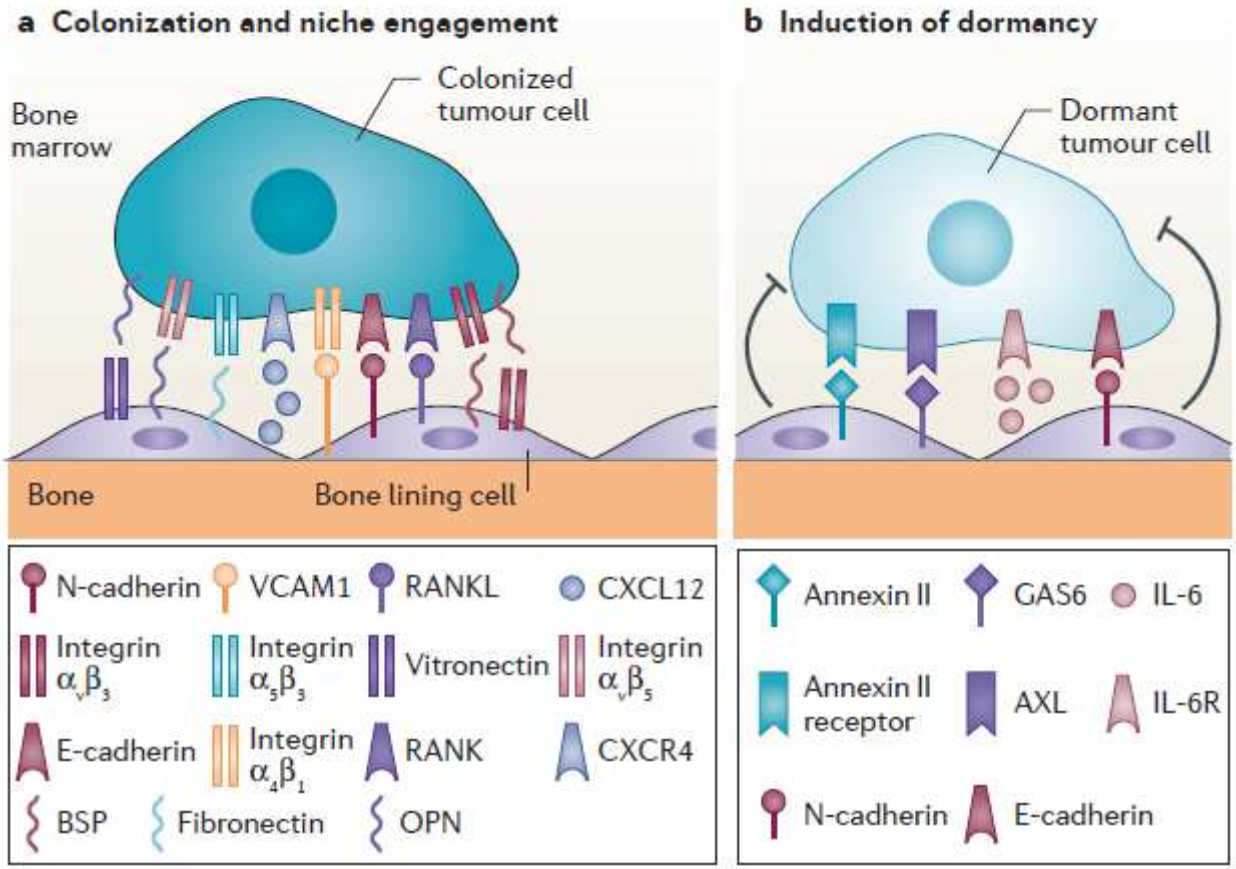
Modified from F. Bertoldo



# The fourth question is:
# how the cancer cells enter into the modern "Vicious Cycle"?

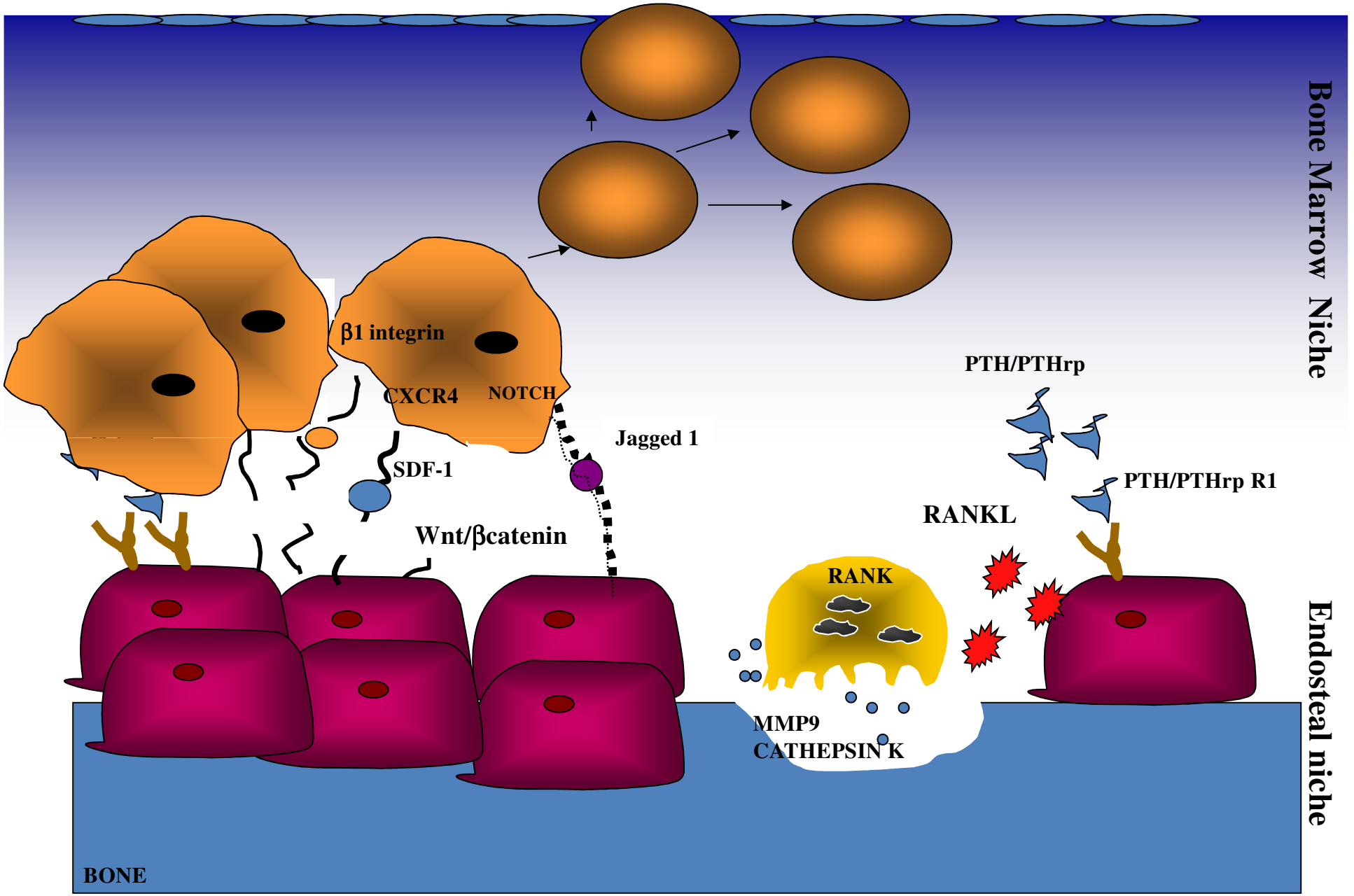


# Osteoblasts regulate the cancer cell *fatus* in the preneoplastic niche




*Bone metastasis: the importance of the neighbourhood. NATURE REVIEWS. 2016*

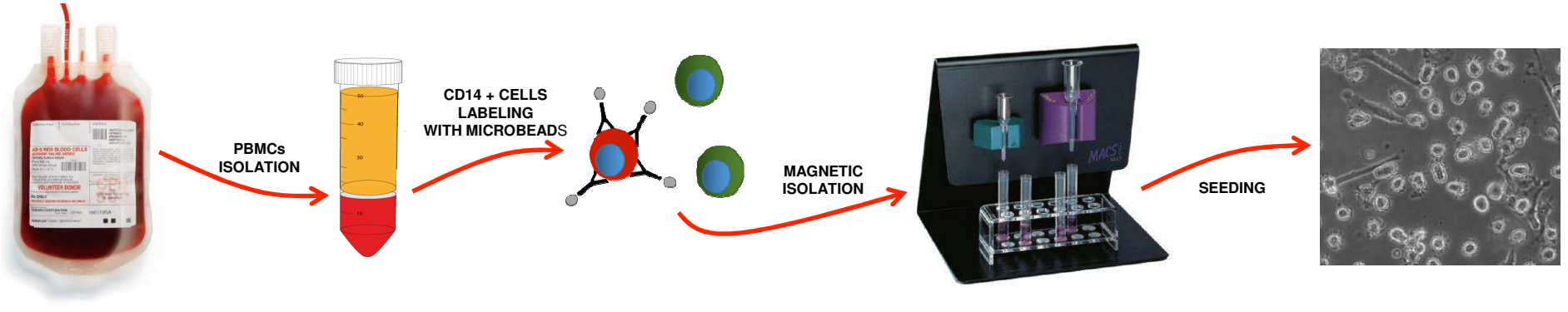
# The third question is: how the cancer cells go away?



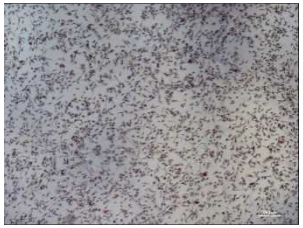

Ratajczak MZ Leukemia 2010; Kollet Ot Nature 2006; Calvi LM Ann NY Acad Sci 2006

# *IN VITRO* MODELS OF BONE CELLS

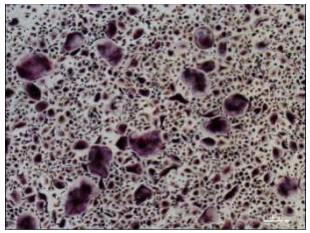
## PRIMARY HUMAN OSTEOCLASTS




## DIFFERENTIATION (TRAP ASSAY)



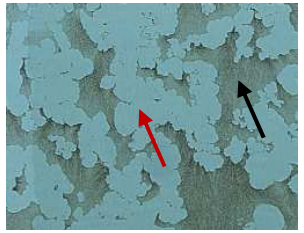
UNDIFFERENTIATED



DIFFERENTIATED

## ACTIVITY (OSTEOASSAY)

UNDIFFERENTIATED

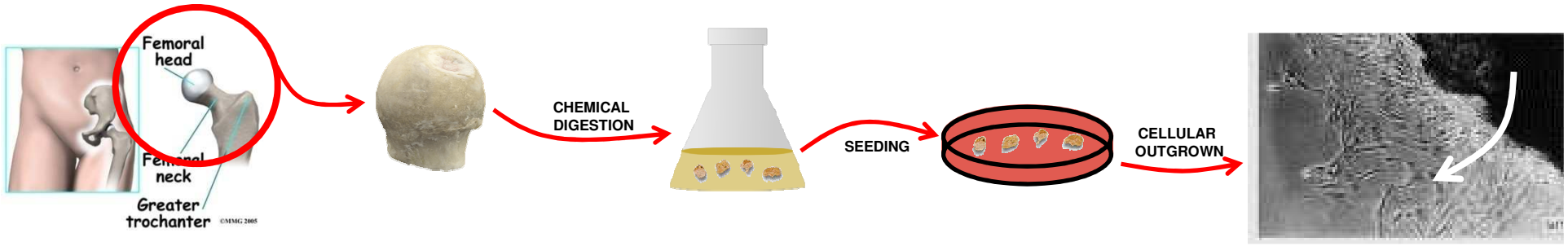


DIFFERENTIATED

BONE LAYER
RESORBED AREA

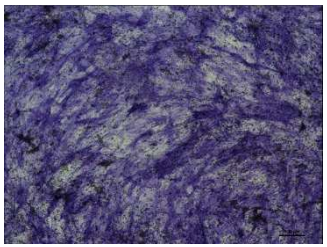
# *IN VITRO* MODELS OF BONE CELLS

## PRIMARY HUMAN OSTEOBLASTS




## DIFFERENTIATION (ALP ASSAY)

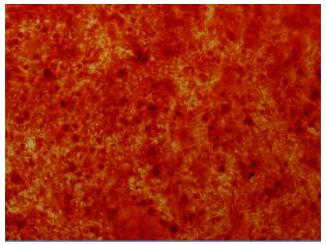
UNDIFFERENTIATED



DIFFERENTIATED

## ACTIVITY (ALIZARIN RED ASSAY)

UNDIFFERENTIATED

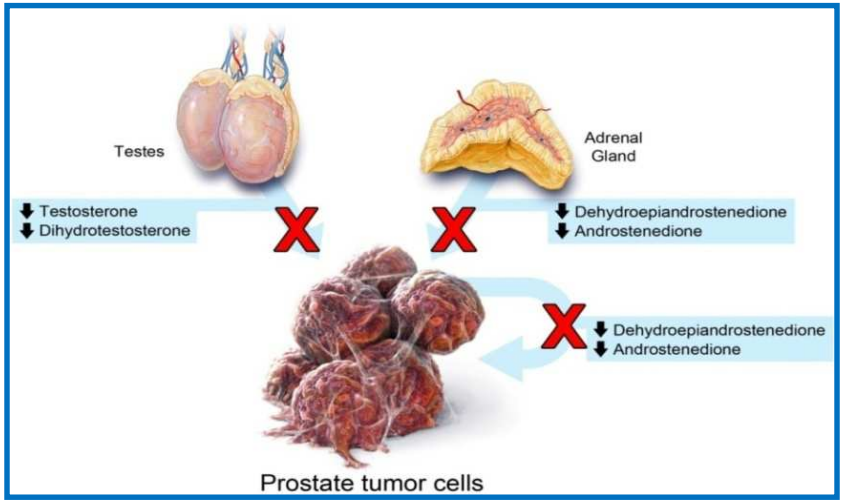


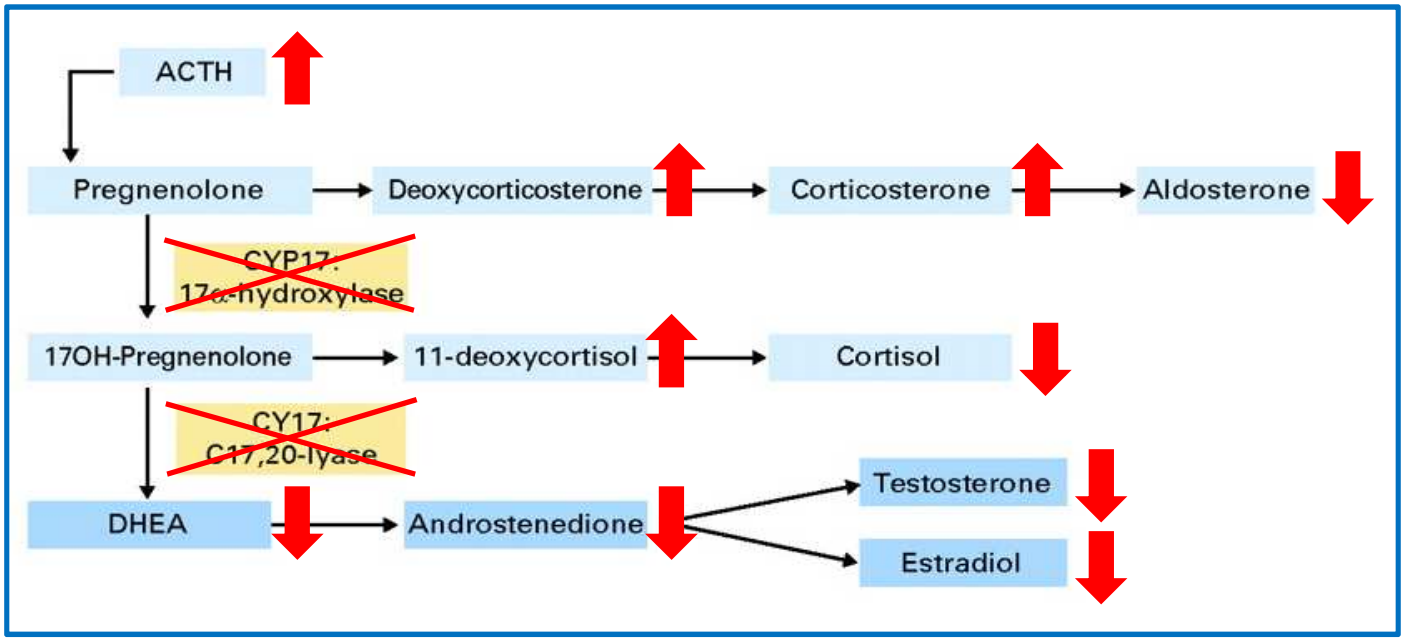
DIFFERENTIATED

# Abiraterone Inhibits Androgen Biosynthesis Through CYP17: 17α-Hydroxylase/17,20-lyase

**Abiraterone inhibits biosynthesis of androgen produced at 3 critical sites:**

- Testes
- Adrenal Gland
- Prostate Tumor Cell




Modified from Attard G. et al. J Clin Oncol 2008

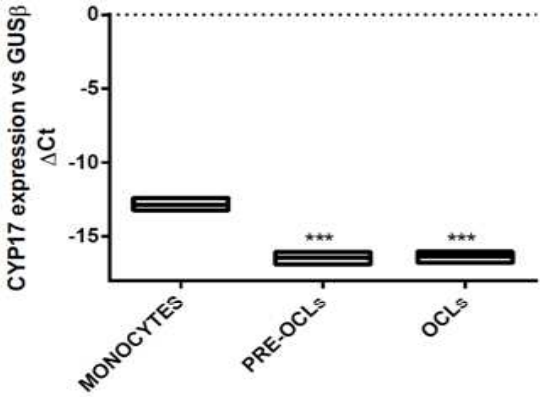
www.impactjournals.com/oncotarget/ Oncotarget, Advance Publications 2015

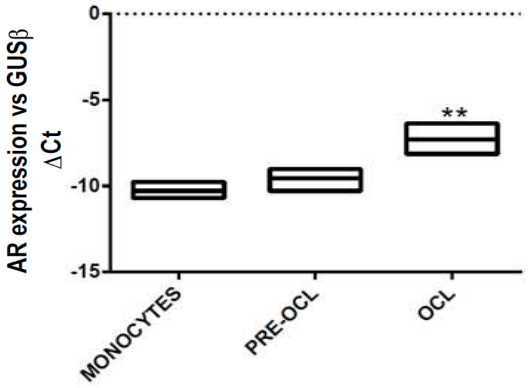
# Biological and clinical effects of abiraterone on anti-resorptive and anabolic activity in bone microenvironment

**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]**

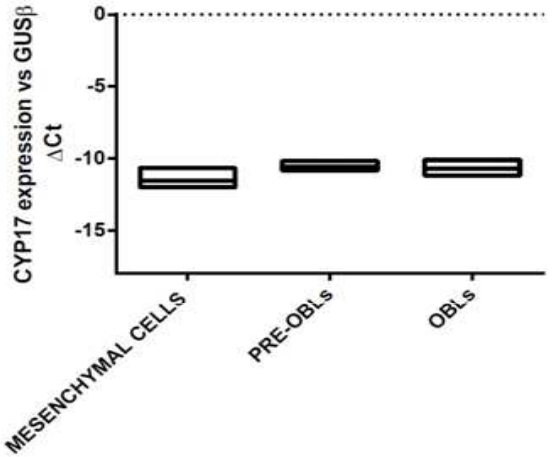
# **CYP17A1** and **ANDROGEN RECEPTOR** are both expressed in our *in vitro* models

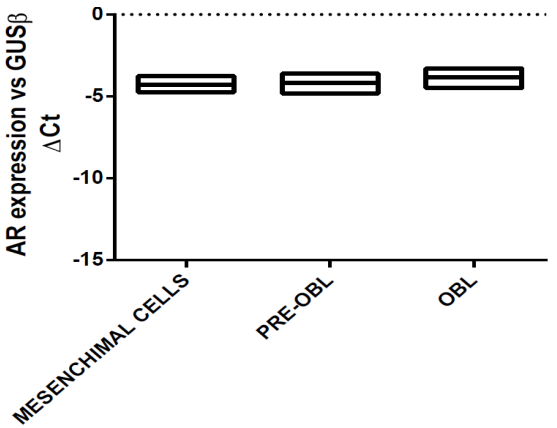
**osteoclast**




**osteoblast**

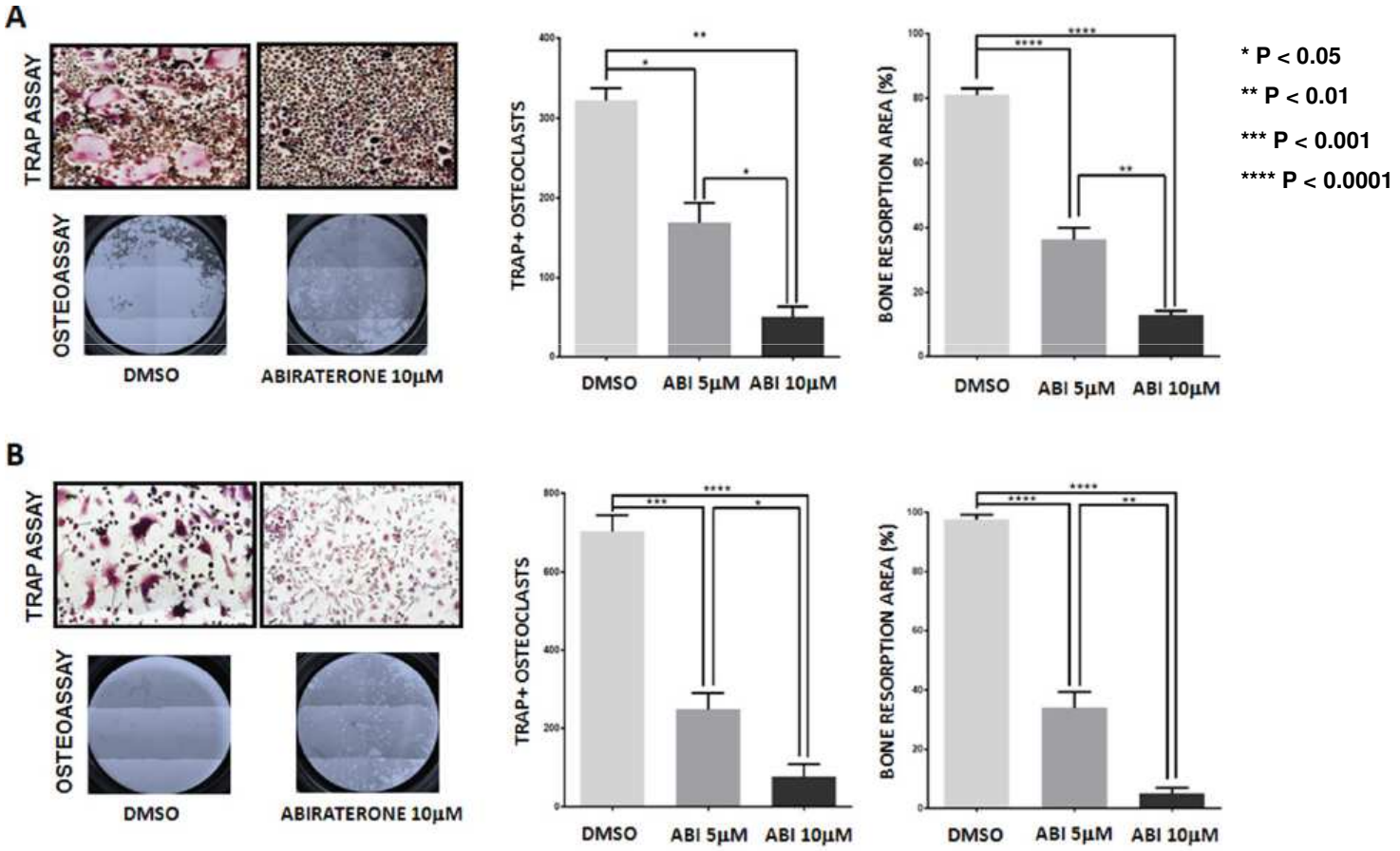


# Abiraterone treatment inhibits osteoclast differentiation and activity both in presence and absence of steroids

Steroids

NO Steroids




* P < 0.05

** P < 0.01

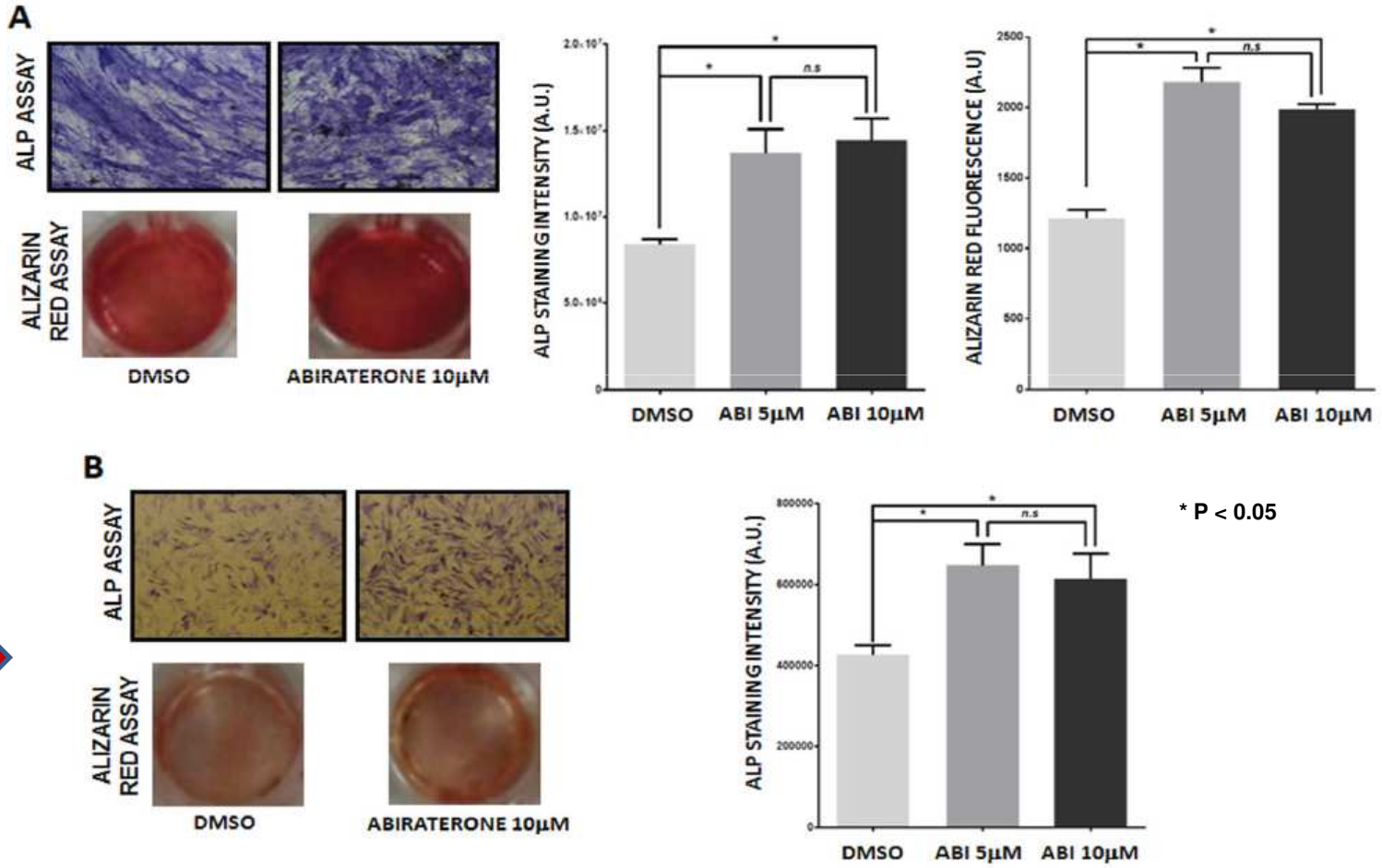
*** P < 0.001

**** P < 0.0001

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

Steroids

NO Steroids

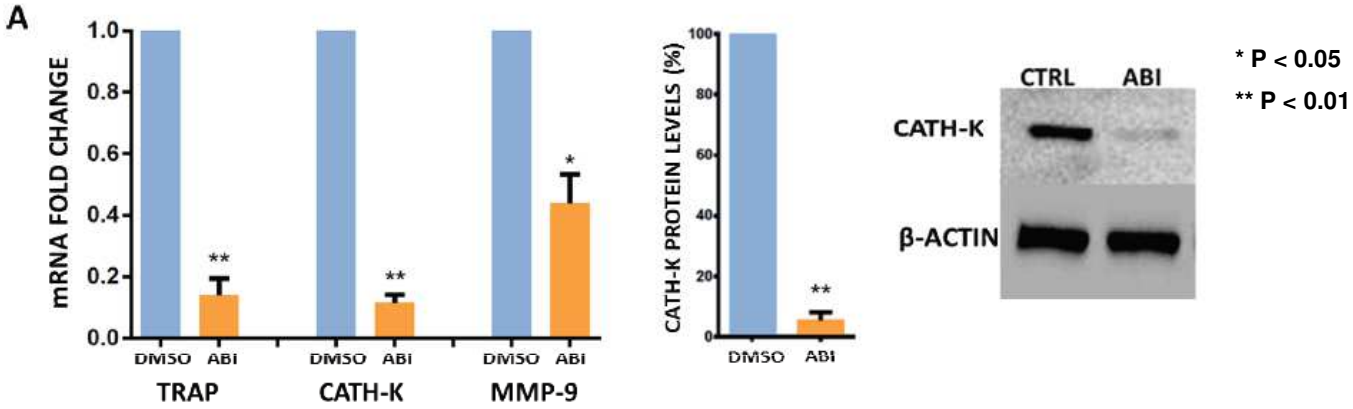



* P < 0.05

# Abiraterone treatment modulates gene expression in osteoclast and osteoblast

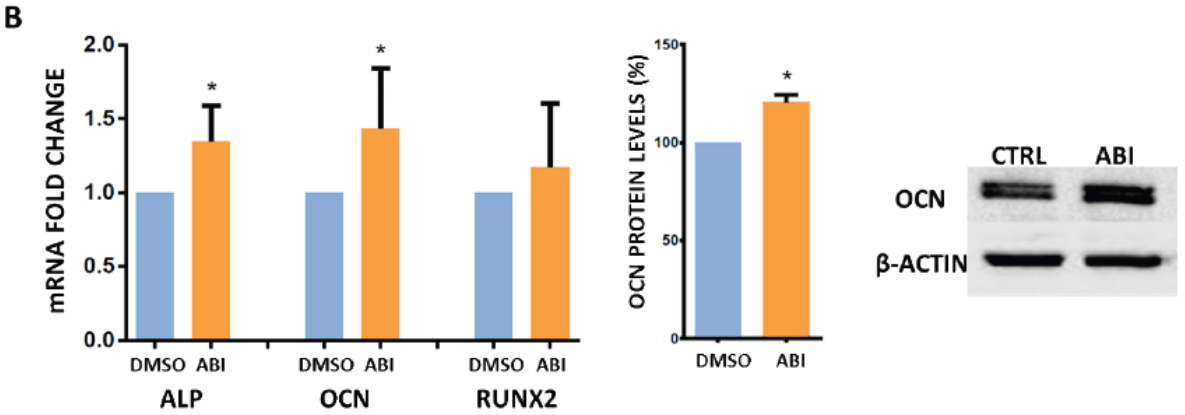
**Osteoclastic gene markers:**
TRAP
Cathepsin K (Cath-k)
Metalloproteinase-9 (MMP-9)

## Abiraterone down-modulates osteoclasts marker genes




**Osteoblastic gene markers:**
ALP
Osteocalcin (OCN)
Runx2

## Abiraterone up-regulates osteoblasts marker genes

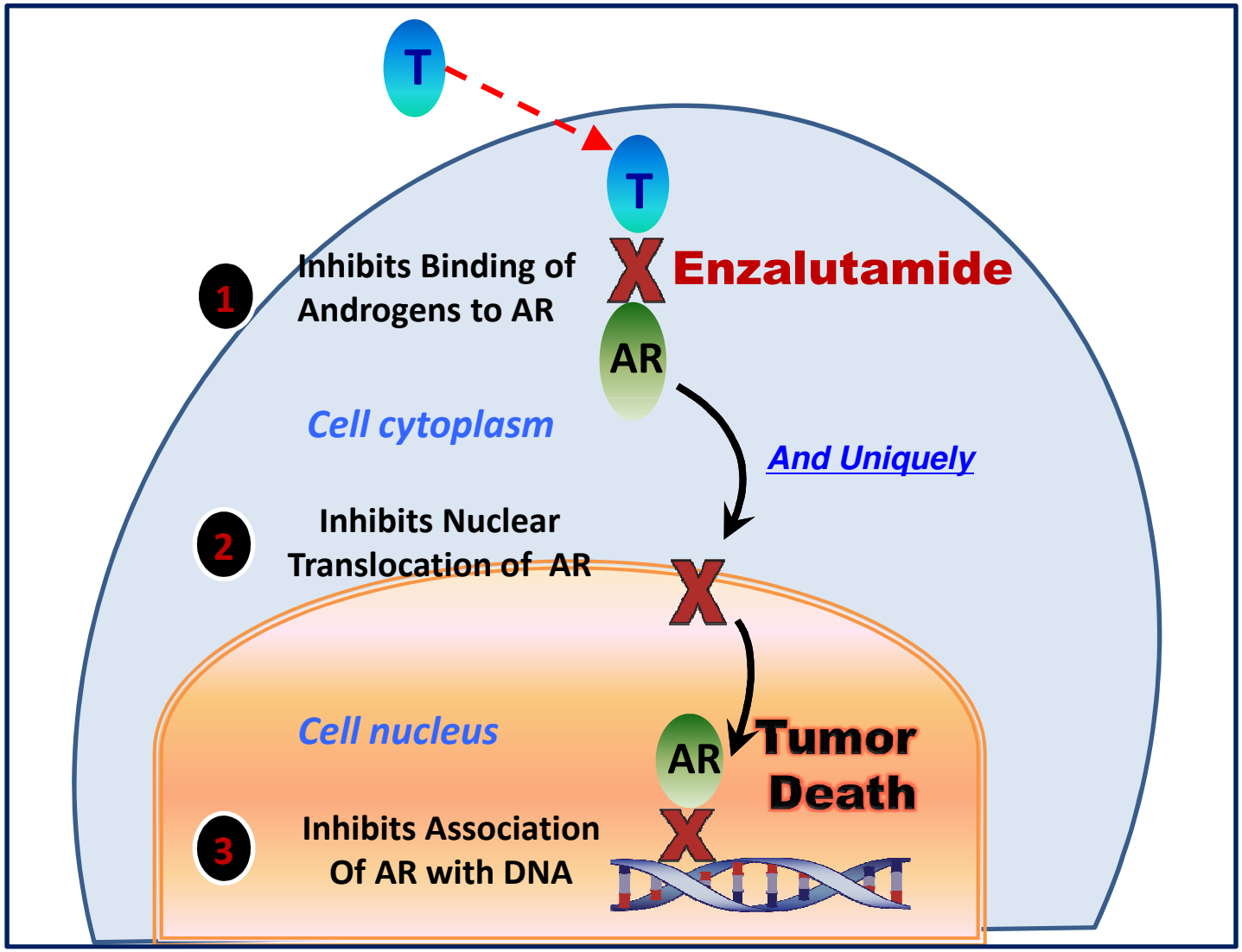


# A significant decrease of CTX values and an increase of ALP was found in serum of 49 mCRPC patients treated with Abiraterone

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

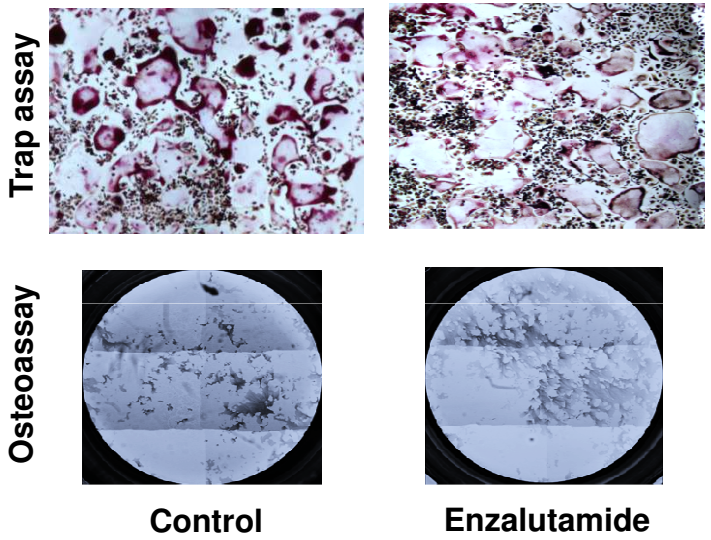
| **CTX** | **Baseline ng/mL** | **Three months ng/mL** | **Six months ng/mL** | **Nine months ng/mL** |
|---|---|---|---|---|
| Median, 95% IC | 0.86, (0.84-1.25) | 0.78, ( 0.67-1.01) | 0.61, (0.73-1.19) | 0.66, (0.38-0.71) |
| p (compare to baseline) | | p=0.077 | p=0.027 | p=0.006 |
| ALP | Baseline 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 |

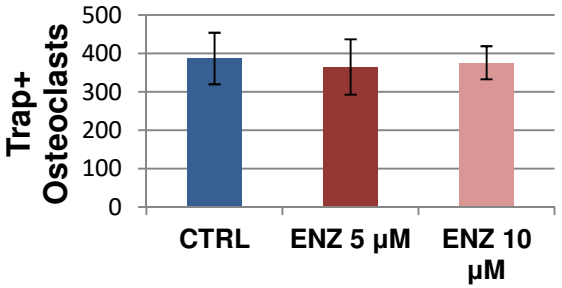
# Enzalutamide impacts multiple steps in AR signaling pathway

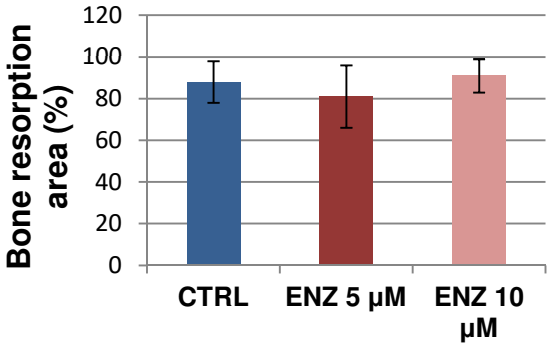



Modified from Tran et al. Science 2009

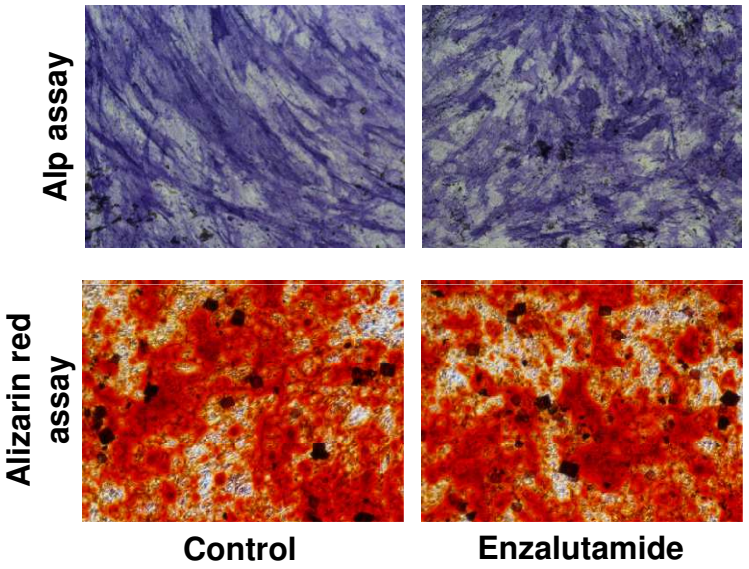
# Enzalutamide does not affect osteoclast differentiation and activity

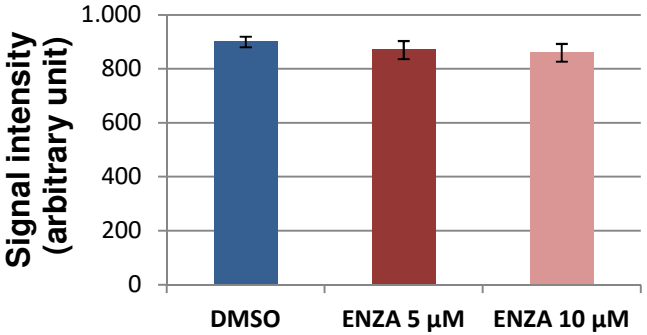


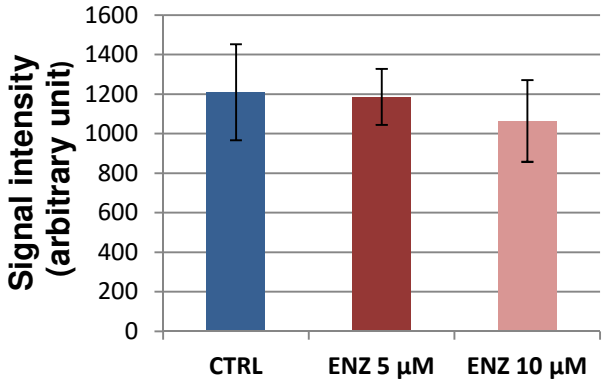



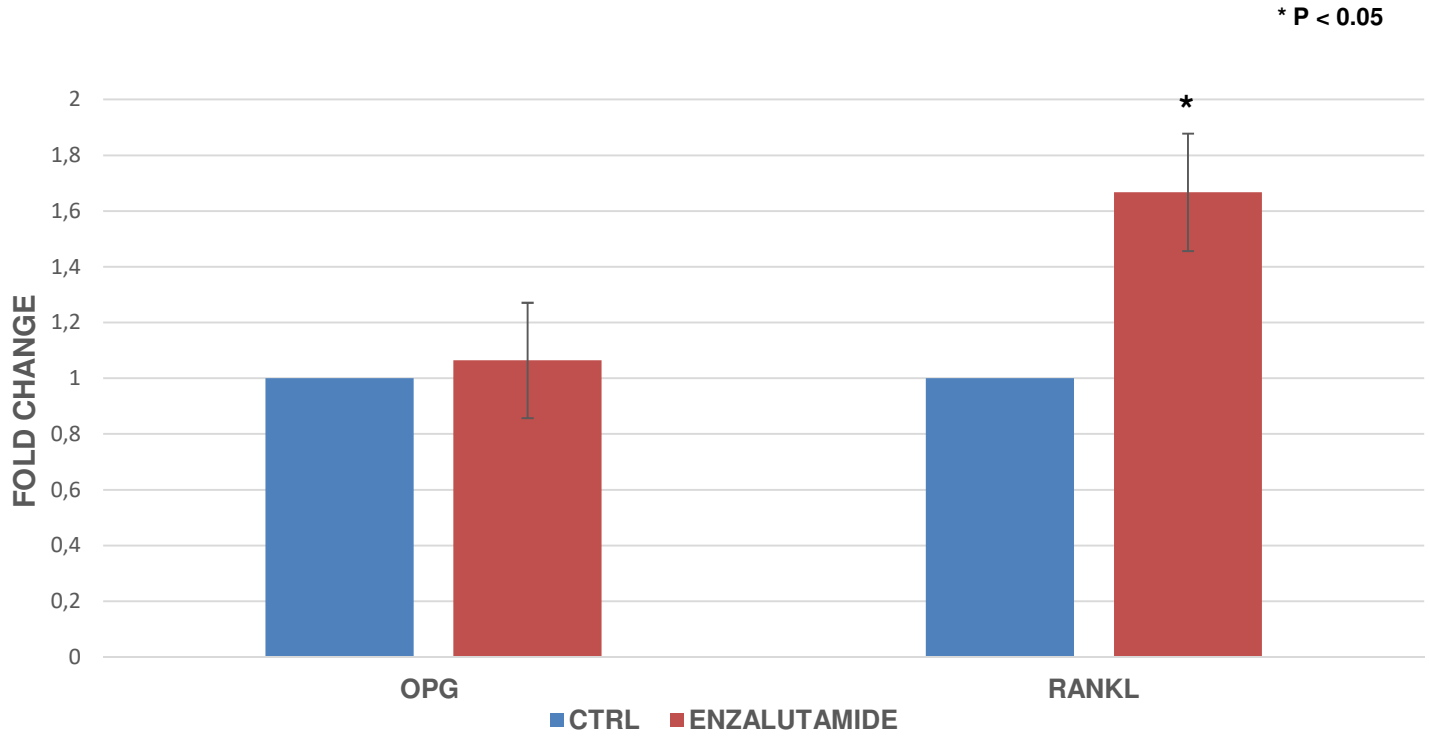
# Enzalutamide does not affect osteoblast differentiation and activity



# Enzalutamide treatment up-regulates RANKL gene expression




**This result was not confirmed by ELISA assay**

# Conclusions

## Abiraterone treatment:

- inhibits osteoclast differentiation and activity down-regulating osteoclasts marker genes
- increases osteoblast differentiation and activity up-modulating osteoblasts marker genes
- decrease CTX values and increase of ALP in mCRPC patients

## Enzalutamide treatment:

- does not affect osteoclast differentiation and activity
- does not affect osteoblast differentiation and activity
- up-regulates RANKL gene expression, but not at protein levels

# Cabozantinib: a novel MET and VEGFR2 inhibitor

- MET and its ligand, HGF, drive tumor cell invasion and metastasis
- MET and VEGFR2 synergize to promote angiogenesis
- Bone metastases are associated with high levels of MET expression

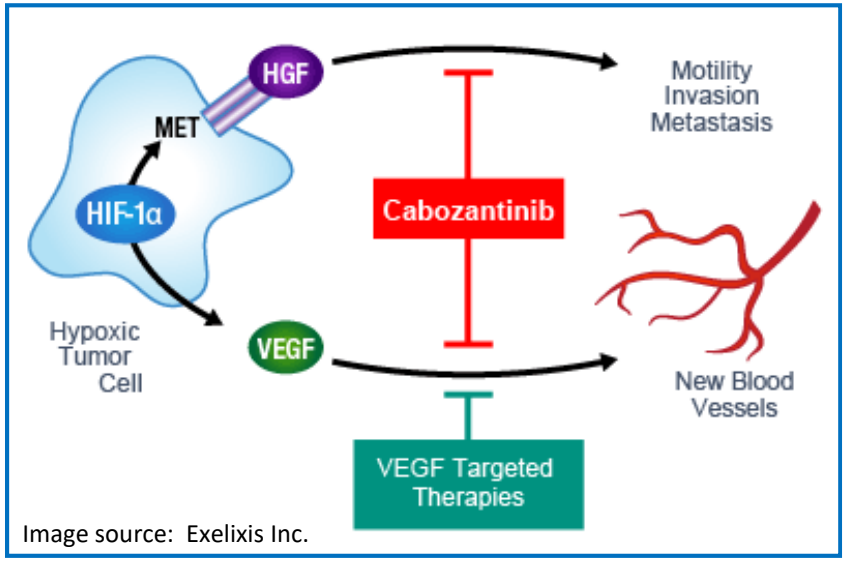


Image source: Exelixis Inc.

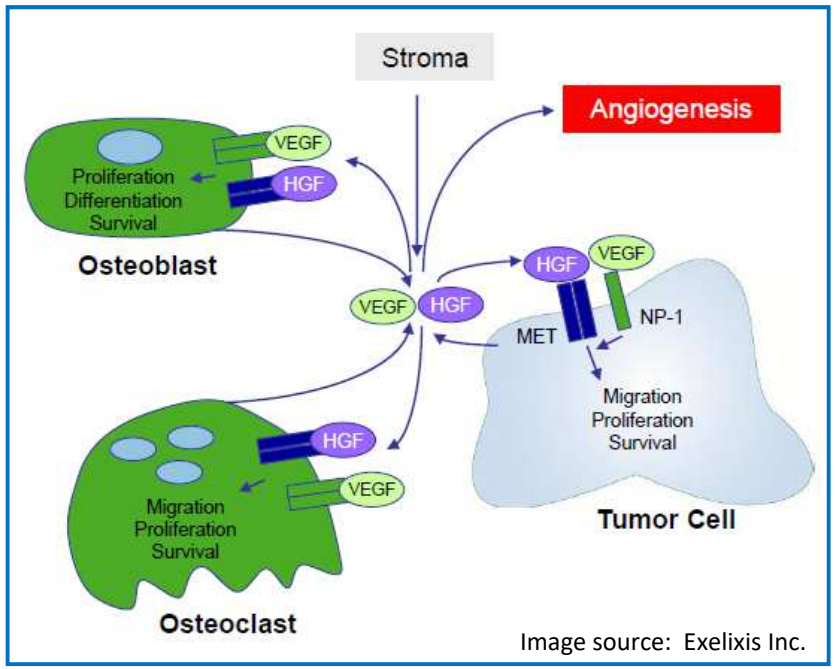


Image source: Exelixis Inc.

- Osteoblast and osteoclast express MET and VEGFR2 and respond to HGF and VEGF

**Cabozantinib could regulate the tumor cell/bone cells cross-talk**

www.impactjournals.com/oncotarget/ Oncotarget, 2017, Vol. 8, (No. 12), pp: 20113-20121

Research Paper

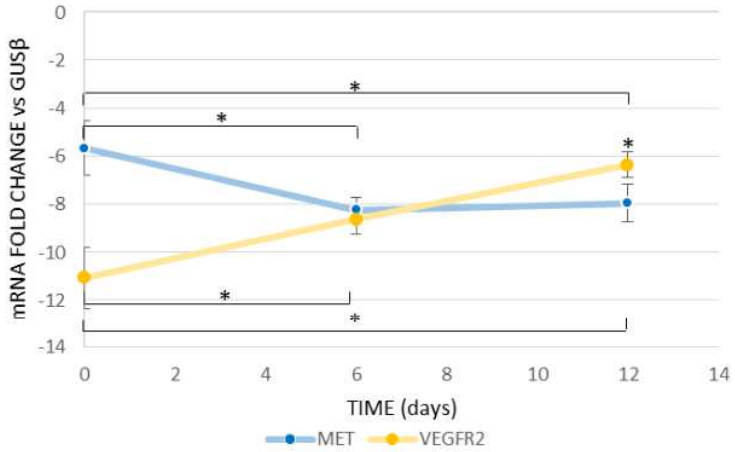
# Cabozantinib targets bone microenvironment modulating human osteoclast and osteoblast functions

Marco Fioramonti[1,*], Daniele Santini[1,*], Michele Iuliani[1], Giulia Ribelli[1], Paolo Manca[1], Nicola Papapietro[2], Filippo Spiezia[2], Bruno Vincenzi[1], Vincenzo Denaro[2], Antonio Russo[3], Giuseppe Tonini[1], Francesco Pantano[1]

# c-MET is strongly expressed in our model

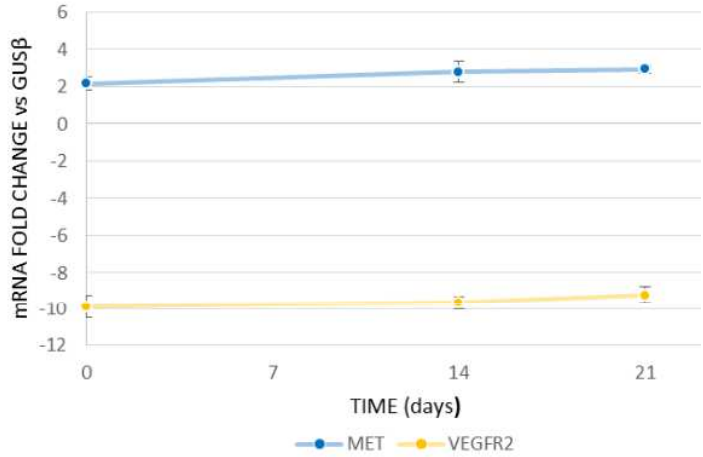
## osteoclast

A

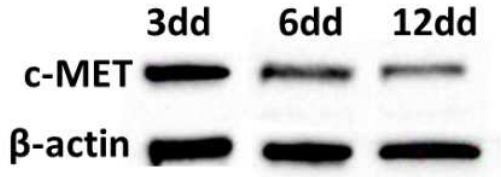



## osteoblast

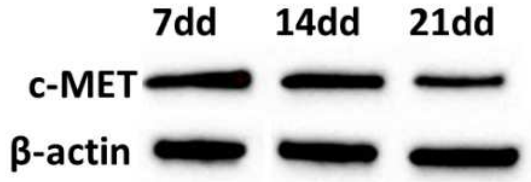
B



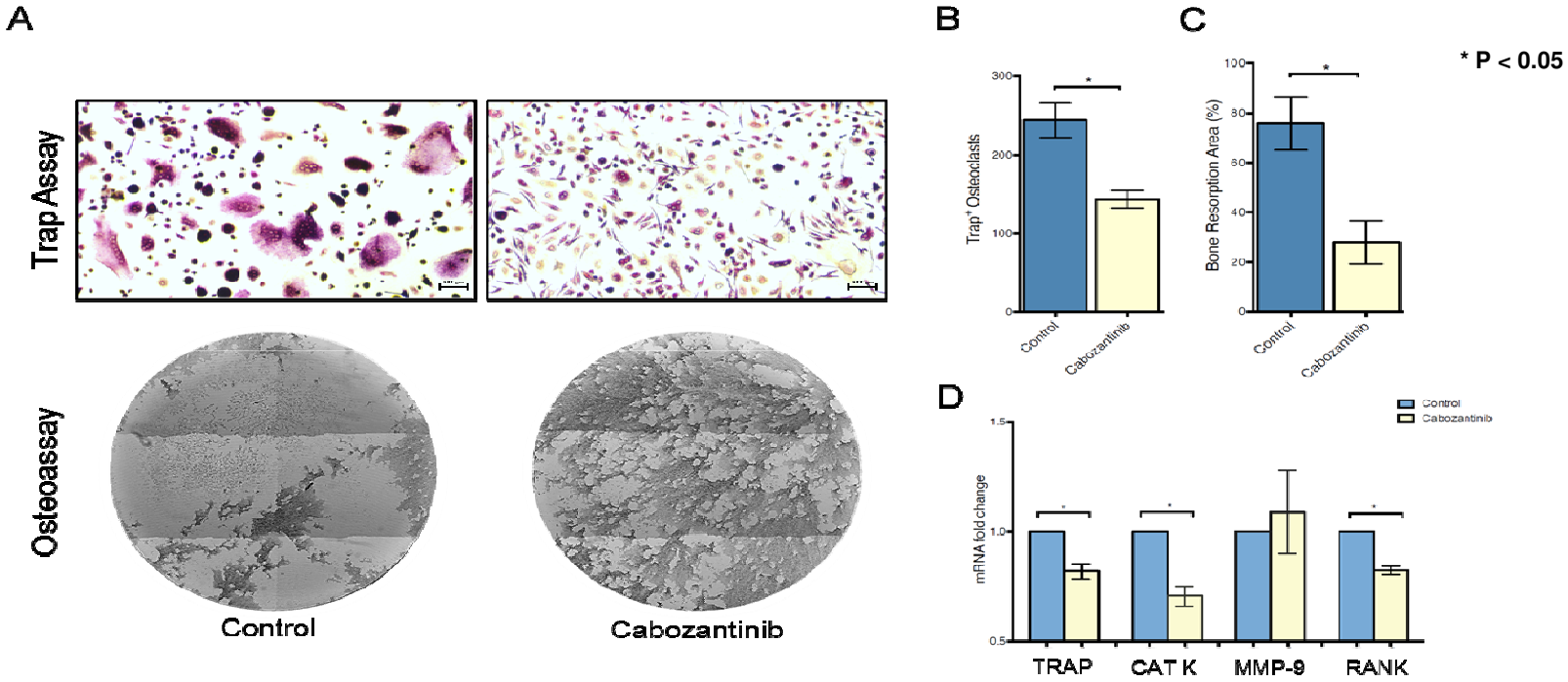

C



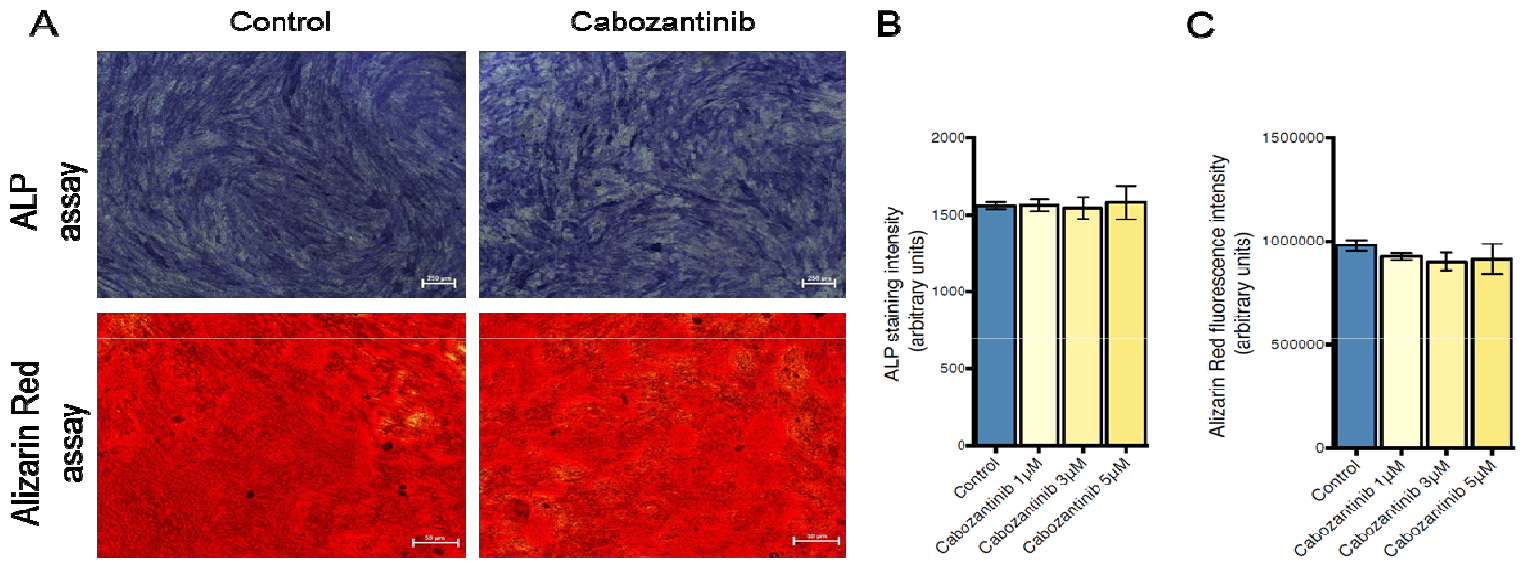

D



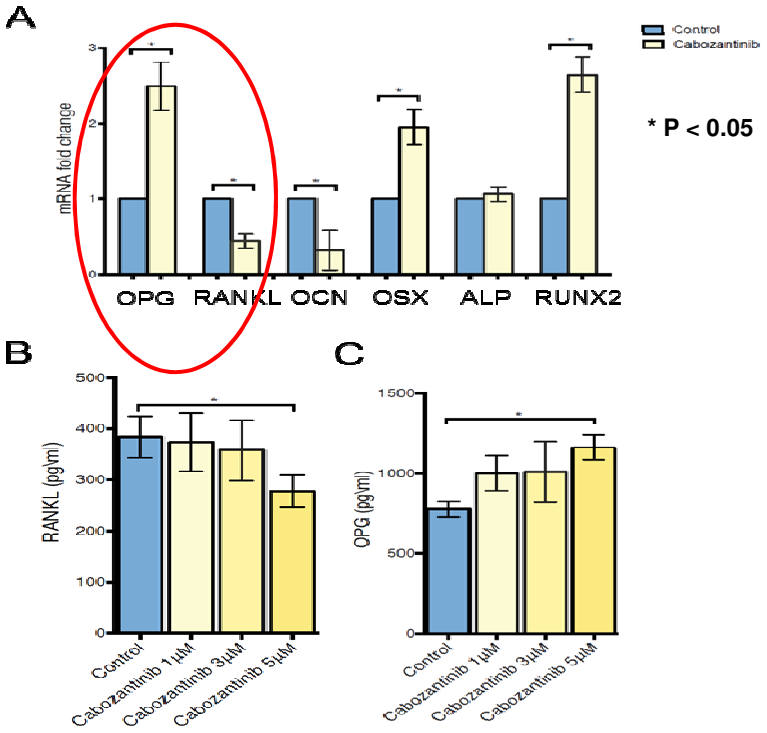
# Cabozantinib inhibits osteoclast differentiation and activity



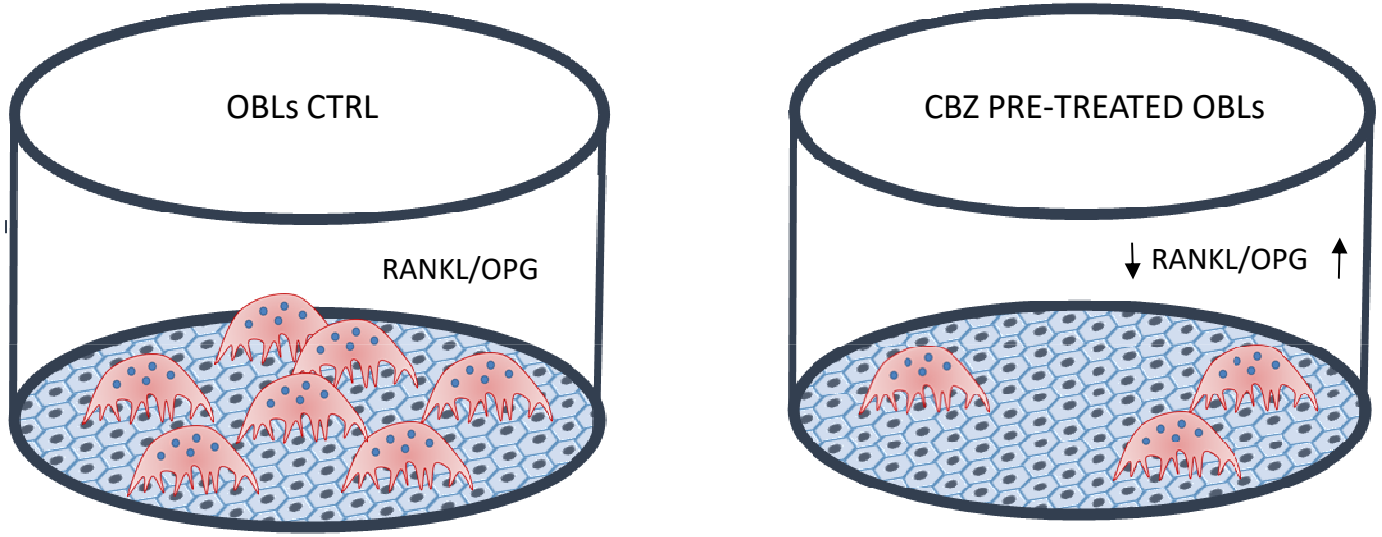
# Cabozantinib does not affect osteoblast differentiation and activity



# Cabozantinib treatment up-regulates OPG gene/protein secretion and down-modulates RANKL gene/protein secretion

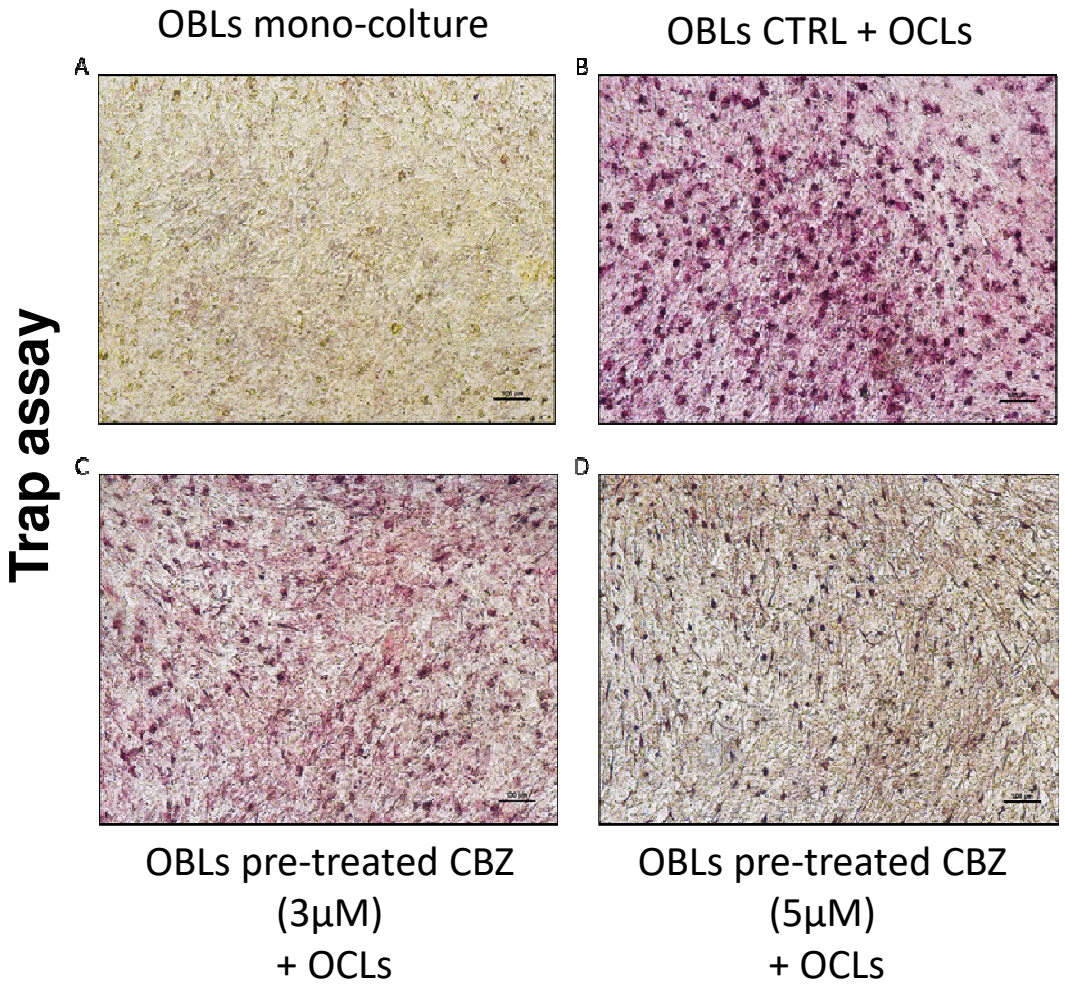


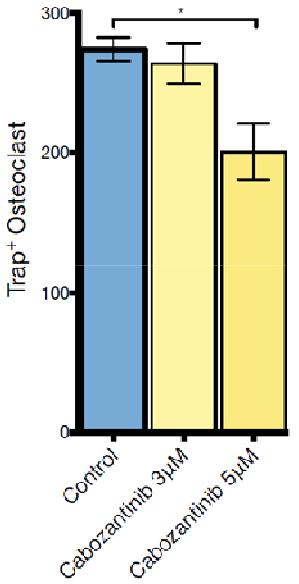
# Cabozantinib pre-treated osteoblasts influence osteoclasts differentiation?



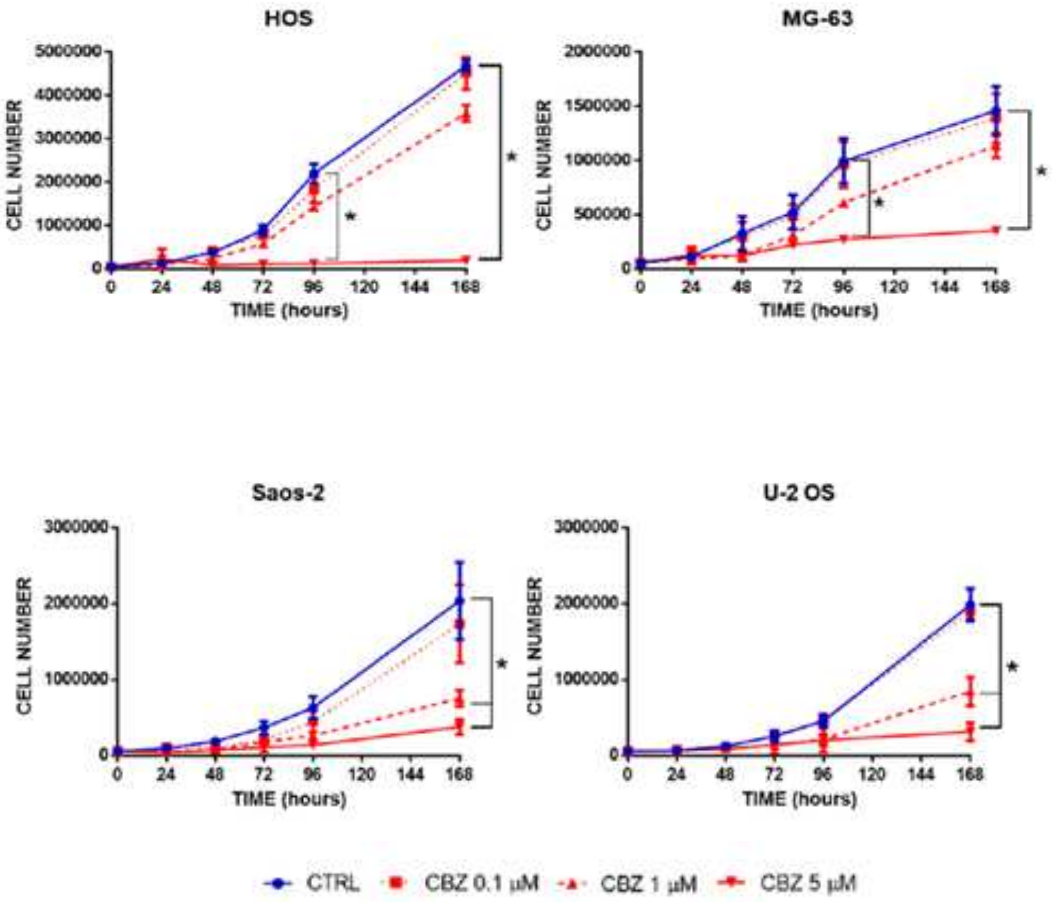

**COCOLTURE OSTEOBLAST/OSTEOCLAST "CELL-TO-CELL CONTACT"**

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



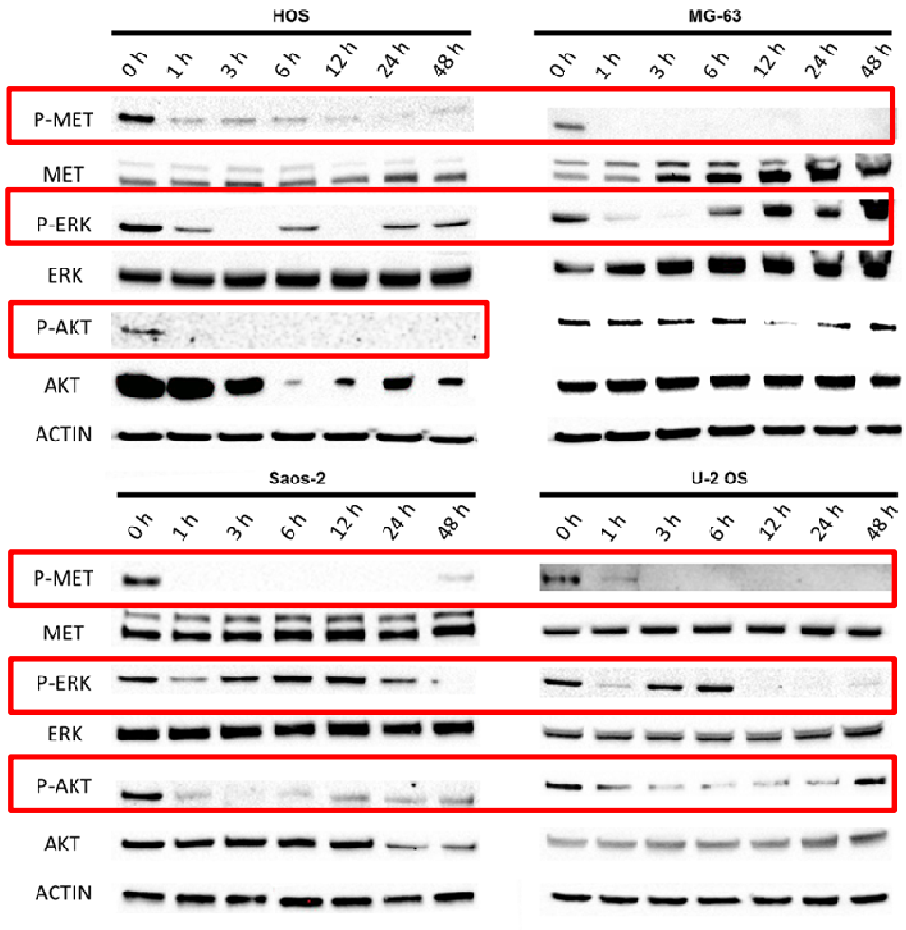



# Cabozantinib reduces cell proliferation in four OSTEOSARCOMA cell lines



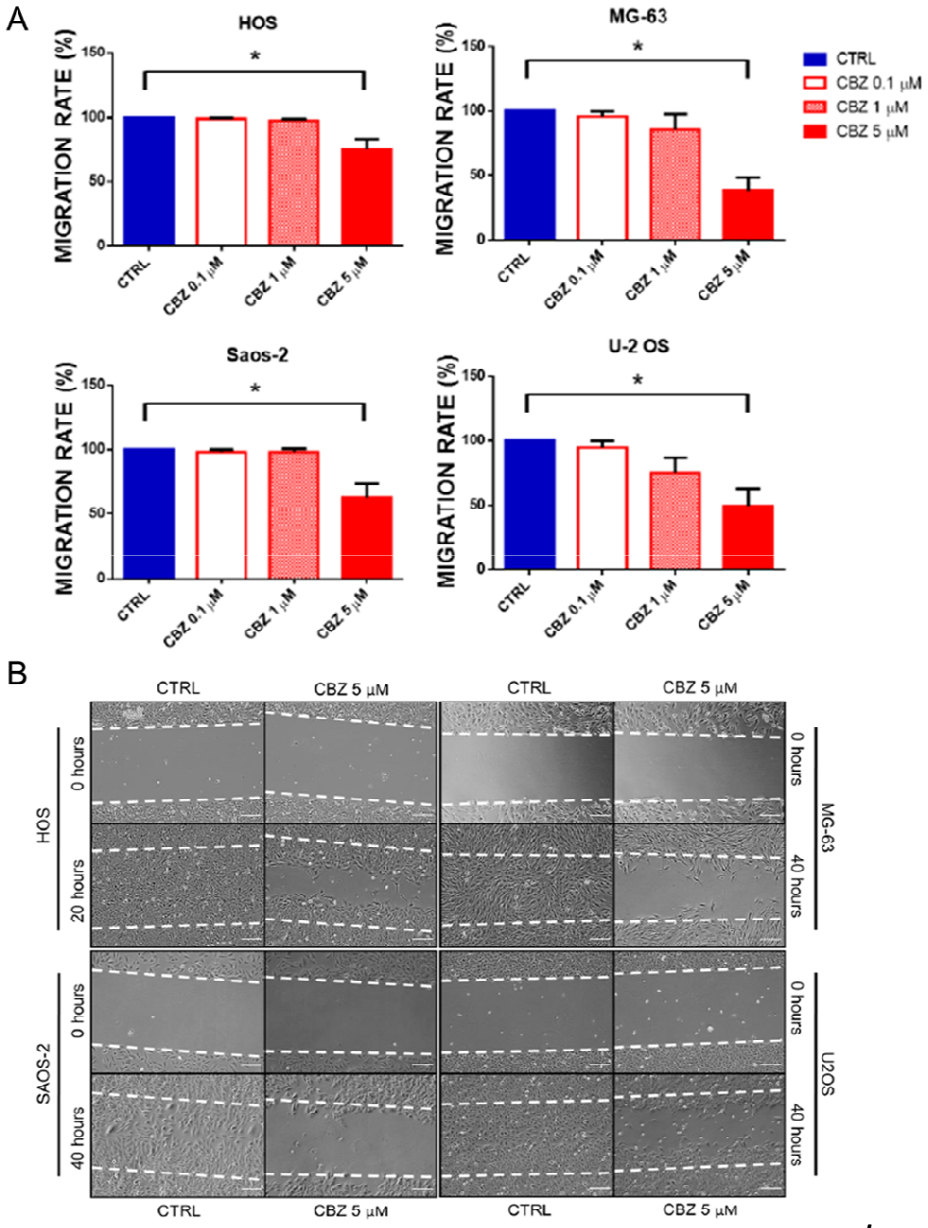

*In press. Fioramonti et al, SCI REP 2017*

# Cabozantinib inhibits the phosphorilation of proliferative signals pathways MET, ERK and AKT



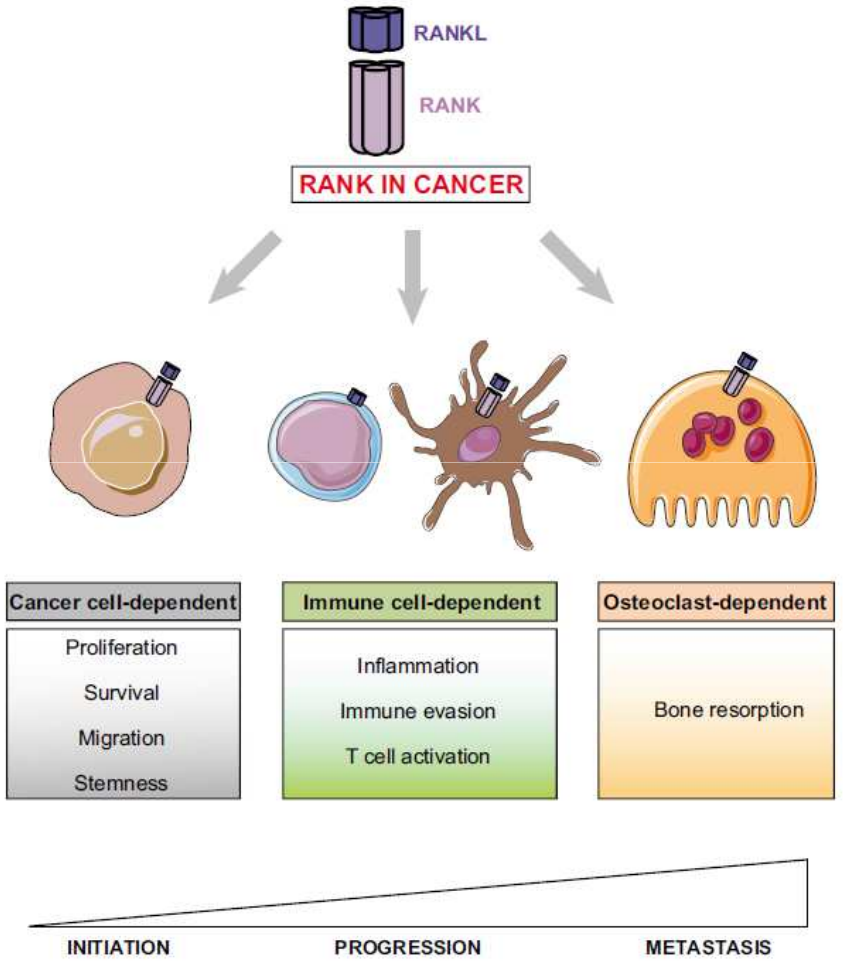

*In press. Fioramonti et al, SCI REP 2017*

# Cabozantinib reduces cell migration in four OSTEOSARCOMA cell lines




*In press. Fioramonti et al, SCI REP 2017*

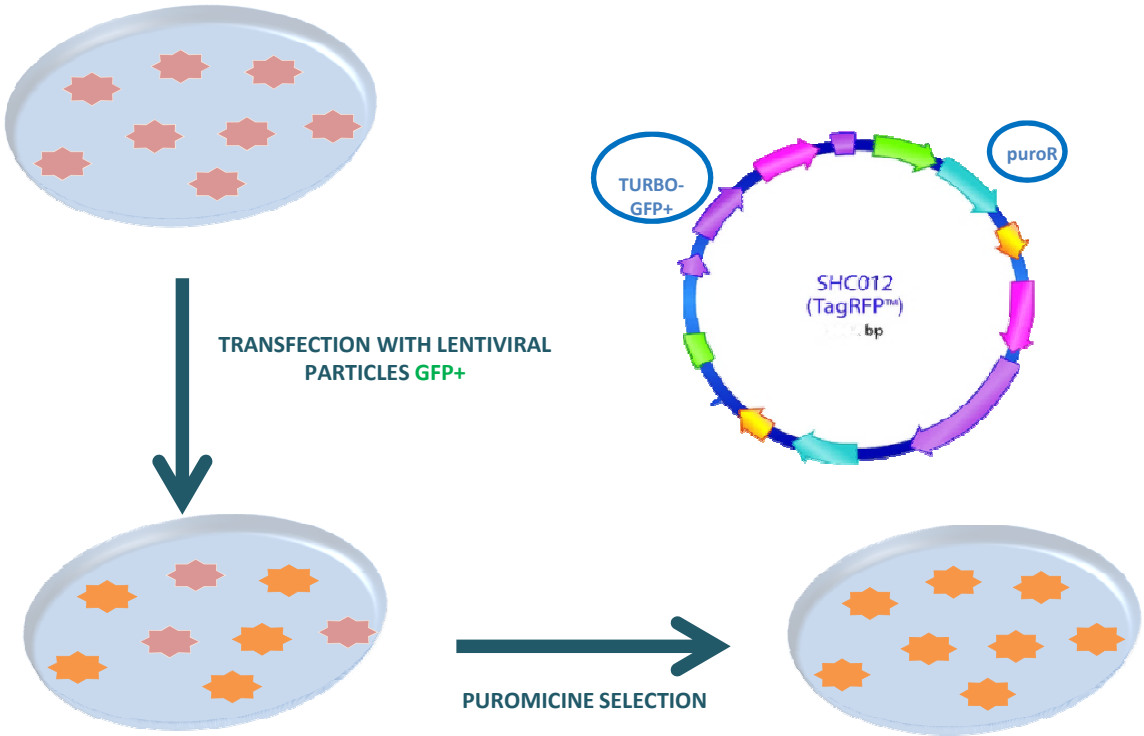
# Pleiotropic effects of the RANK pathway in cancer




*Gonzalez-Suarez E. et al., The FEBS Journal 2016*

# MATERIAL AND METHODS

## Generation of stable GFP+ cell line

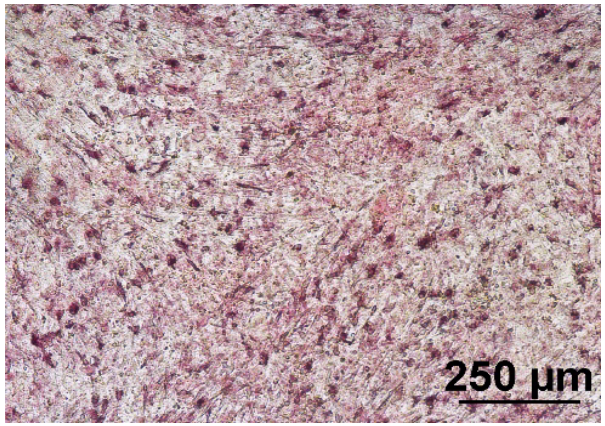


# *IN-VITRO* COCOLTURE SYSTEMS

**Direct cocultures:**

- Osteoblasts

Osteoclasts
Osteoblasts




- Osteoblasts/ Cancer cells

cancer cell
Osteoblasts