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**SOCIETÀ ITALIANA DI OSTEONCOLOGIA**  
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**Biological effects of Cyclin-Dependent Kinase Inhibitors Ribociclib  
on Breast Cancer Bone Enviroment**

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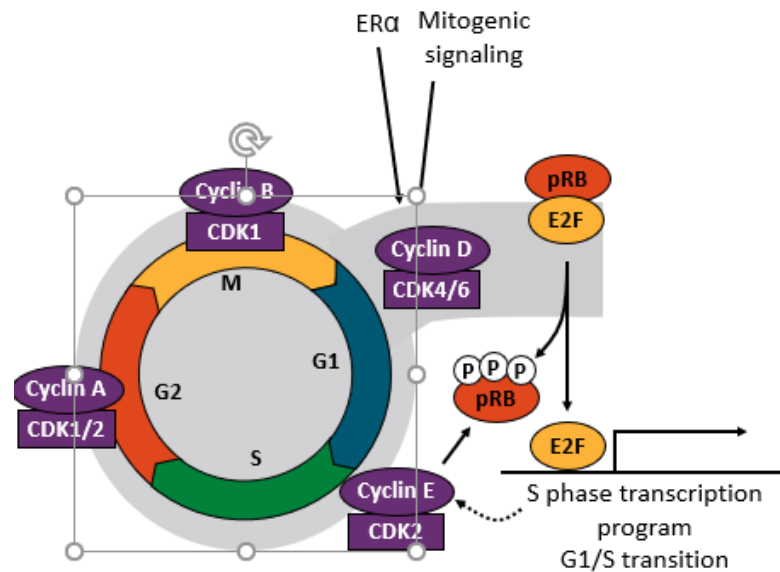
CDK4/6 INHIBITORS...what they are supposed to do ?



Pavia's battle (1525),

*Ci-git Monsieur de La Palice. Si il n'était pas mort, il serait encore en vie"*  
(Qui giace il signore di La Palice. Se non fosse morto, sarebbe ancora in vita)

## THE ROLE OF CDK4/6 IN BREAST CANCER



- Cyclin D–CDK4/6 complexes promote cell proliferation through Rb protein phosphorylation.
- Increased CDK4/6 activity is frequently observed in HR+ breast cancer.
- Activation of the cyclin D–CDK4/6–INK4–Rb pathway has been associated with poor response and resistance to endocrine therapy.

The cyclin-dependent kinase (CDK) 4/6 inhibitors drugs that interrupt proliferation of malignant cells by inhibiting progression through the cell cycle.

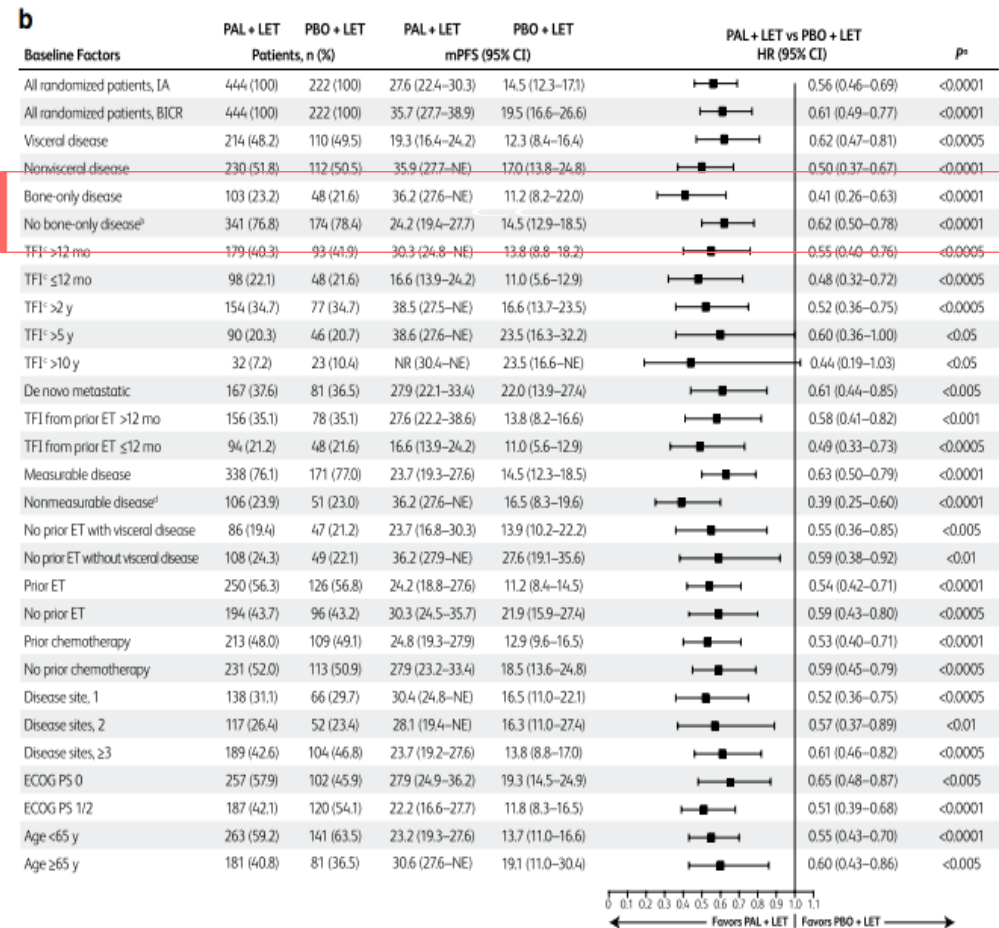


## CDK4/6 Inhibitors: FDA-Approved Indications in HR+/HER2- MBC

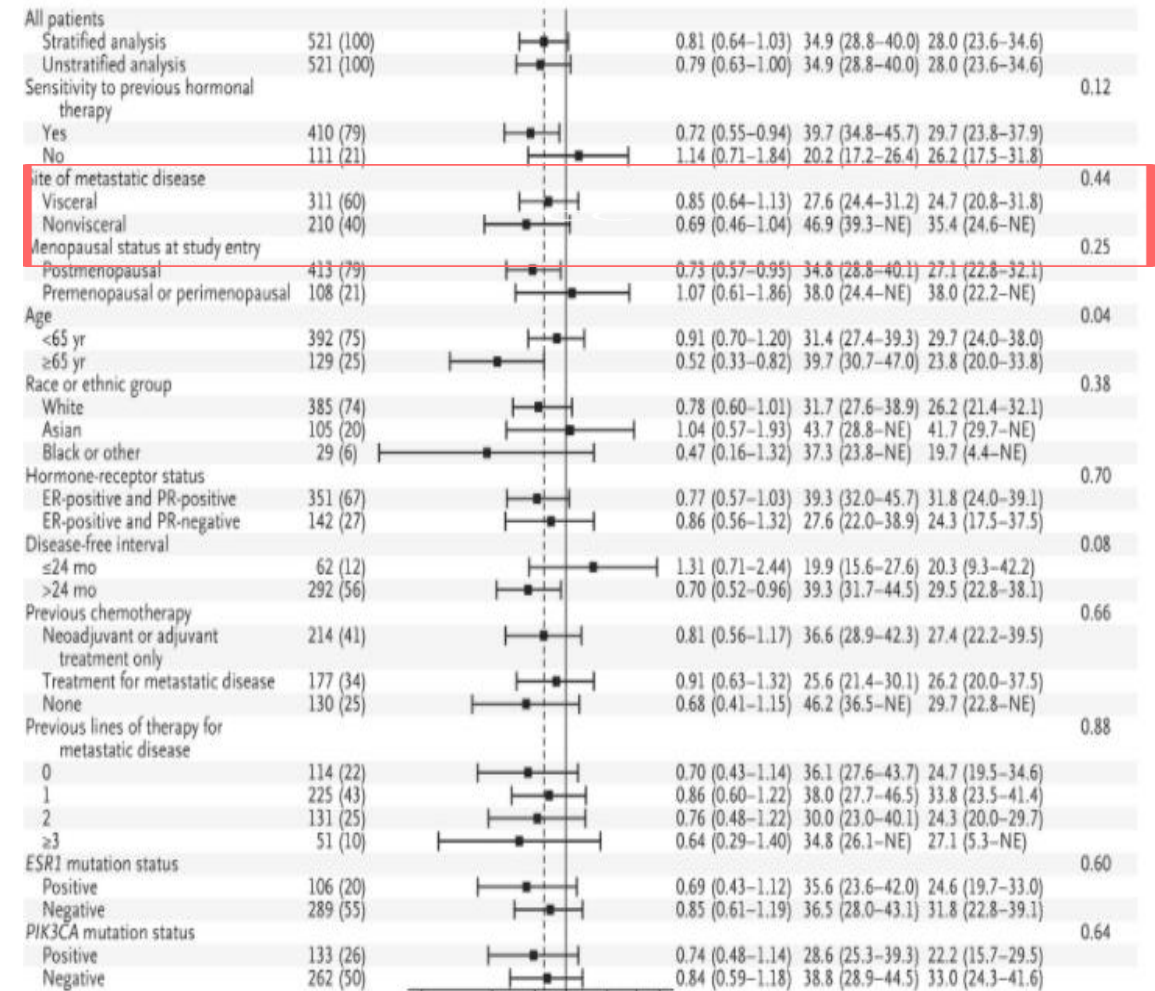
Select Clinical Trials of CDK4/6 Inhibitors for HR+, HER2– ABC <sup>1</sup>		
Ribociclib	Palbociclib	Abemaciclib
<b>MONALEESA-2</b> (First-line RIBO + LET in postmenopausal women)	<b>PALOMA-2</b> (First-line PAL + LET in postmenopausal women)	<b>MONARCH-3</b> (First-line ABE + NSAI in postmenopausal women)
<b>MONALEESA-7</b> (First-line RIBO + ET + OFS in premenopausal women)	<b>PALOMA-1</b> ( <u>Phase 2</u> study of first-line PAL + LET in postmenopausal women)	<b>MONARCH-2</b> (ABE + FUL in patients with ≤1 line of ET for ABC)
<b>ComPLEEment-1</b> (First-line RIBO + LET in an expanded patient population)	<b>PALOMA-3</b> (PAL + FUL with ≤1 line of ET for ABC)	<b>MONARCH-1</b> ( <u>Phase 2</u> study of ABE monotherapy in heavily pretreated patients)
<b>MONALEESA-3</b> (RIBO + FUL in patients with ≤1 line of ET for ABC)		

CDK4/6i and bone ... what we know ?

# PALBOCLIB IN BONE ONLY DISEASE



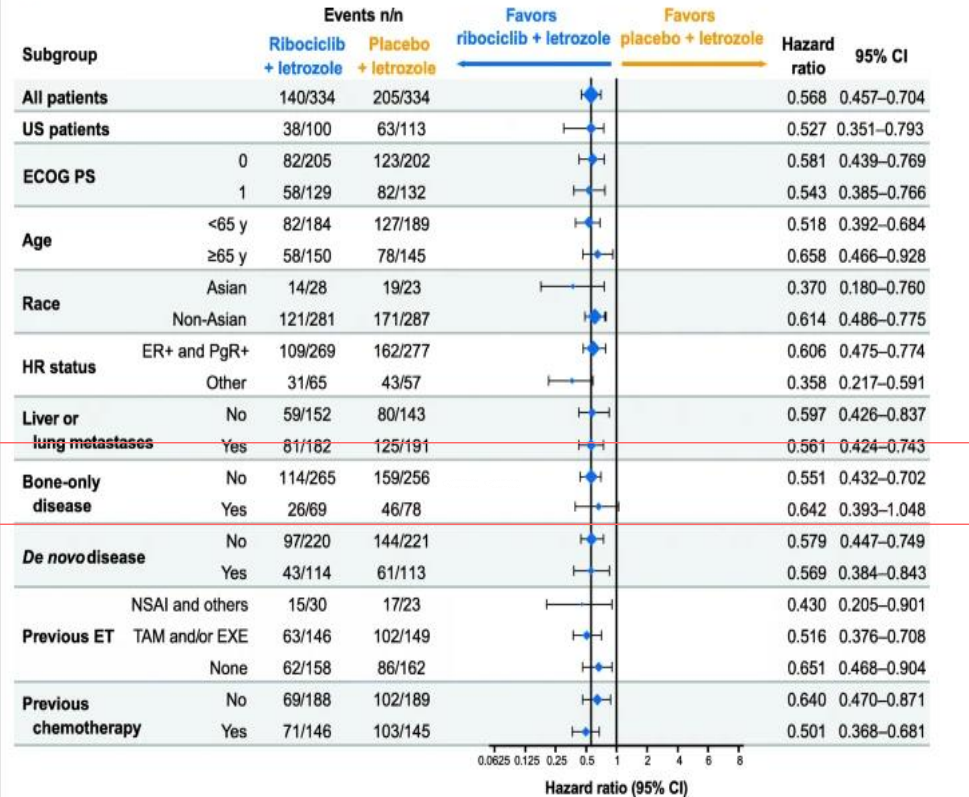
**Paloma2:** Palbociclib+ letrozolo vs placebo+letrozolo  
mPFS NR vs 11,2mo



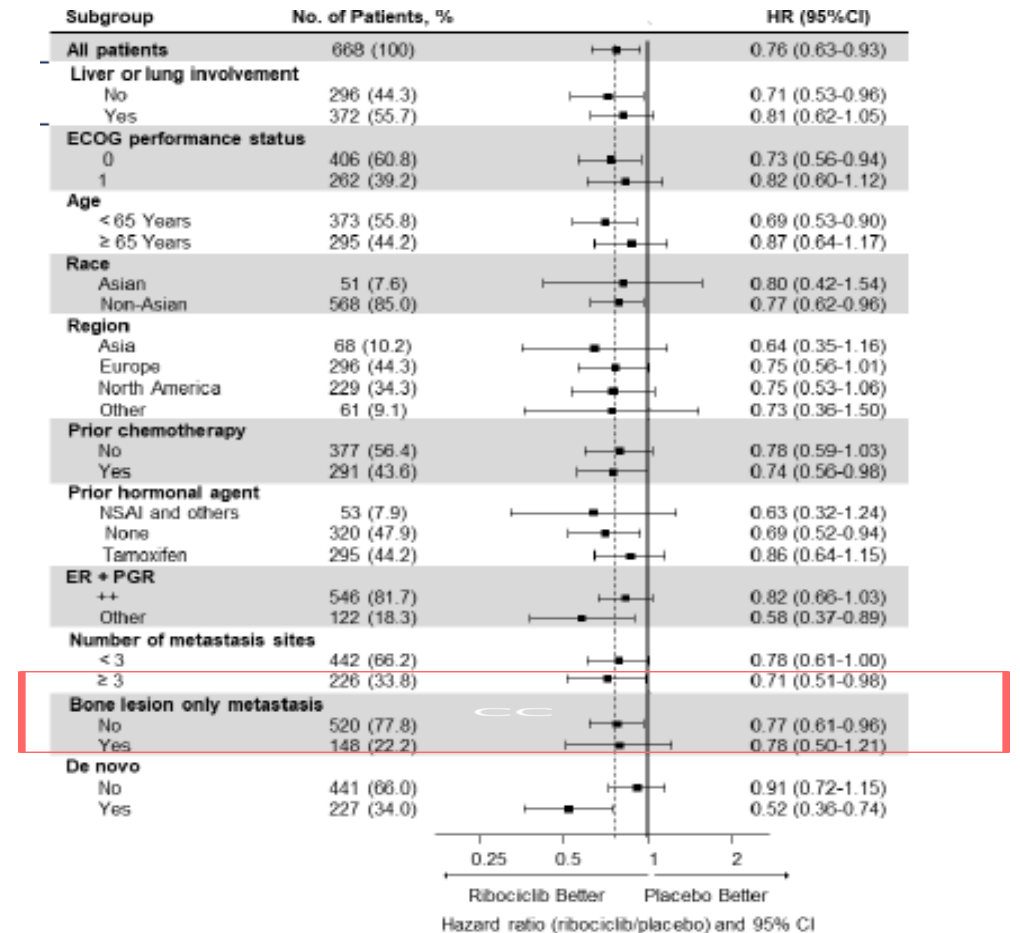
**Paloma3:** Non visceral disease mOS HR0,69 (0,46-1,04)

# RIBOCICLIB IN BONE ONLY DISEASE

Fig. 1

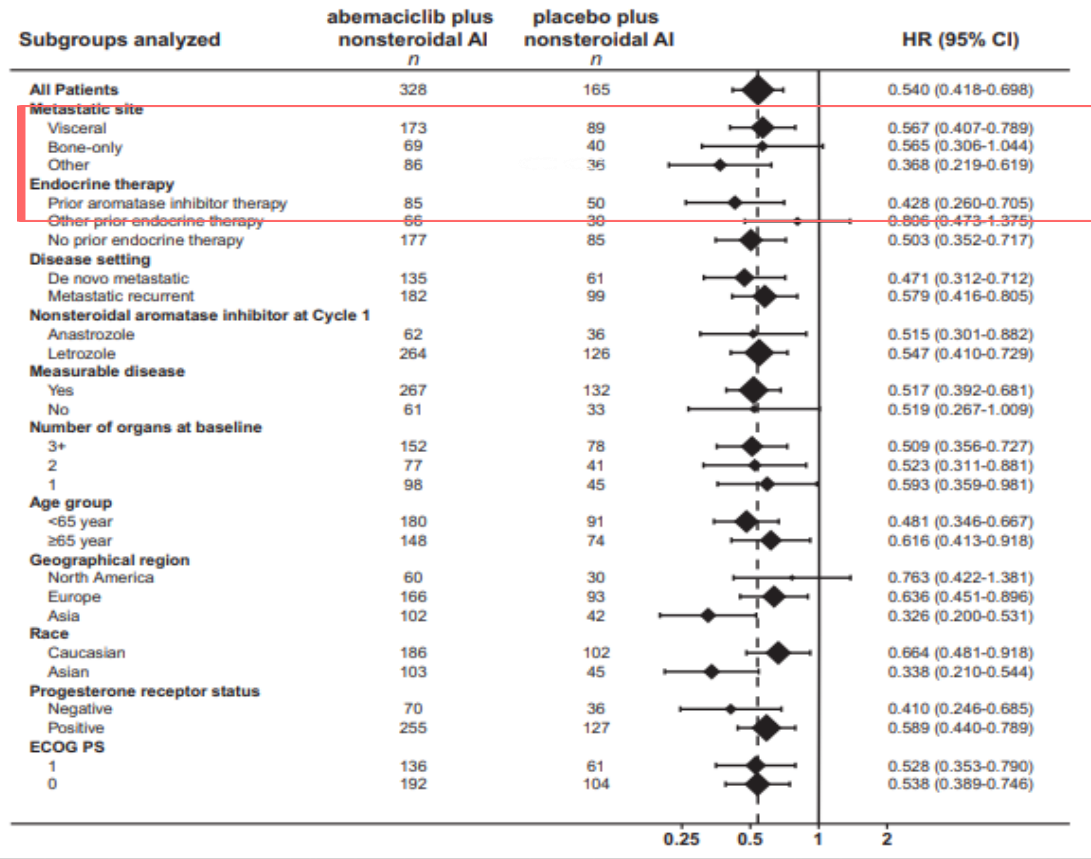


**Monnalisa2:** PFS ribociclib + letrozole group vs placebo + letrozole: NR vs 15.3 mo(HR = 0.690; 95% CI 0.381–1.249)

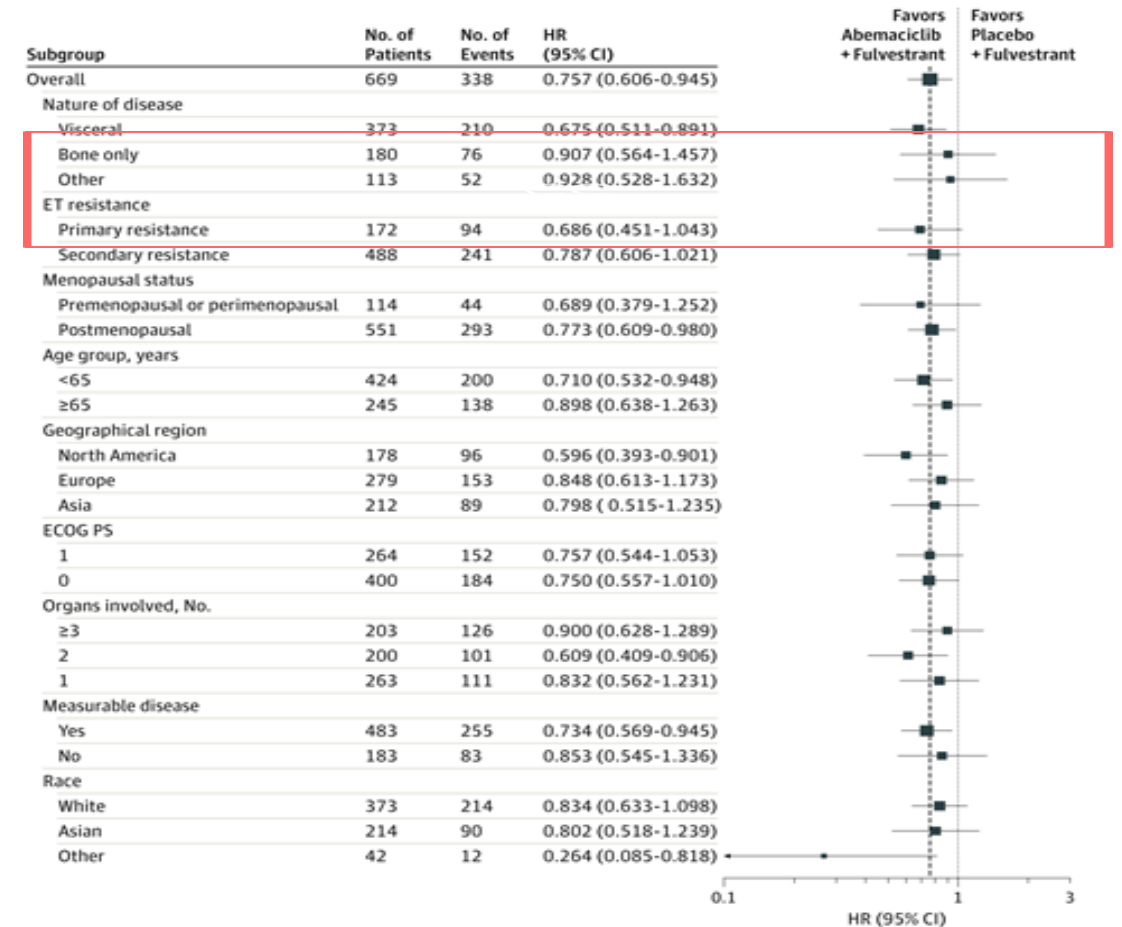


**Monnalisa3:** OS ribociclib + Fulvestrant group vs placebo + fulv: HR 0.78 (0.5–1.21)

# ABEMACICLIB IN BONE ONLY DISEASE











**Monarch3: PFS Abemaciclib +AI vs Placebo +AI:**  
HR 0.565 (0.306-1.044)



**Monarch2 Improvement in OS for Abemaciclib + Fulv:**  
**Bone only NR vs 47,3 mo(HR, 0,907 ; 95% CI, 0,564-1,457)**



# Overall Survival of CDK4/6-Inhibitor–Based Treatments in Clinically Relevant Subgroups of Metastatic Breast Cancer: Systematic Review and Meta-Analysis

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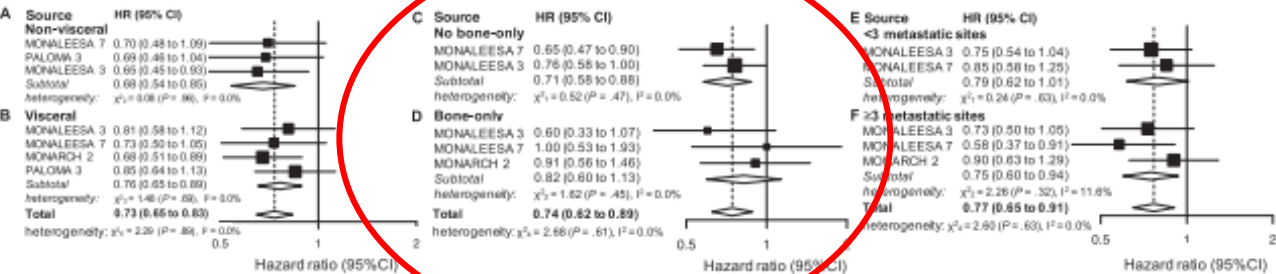
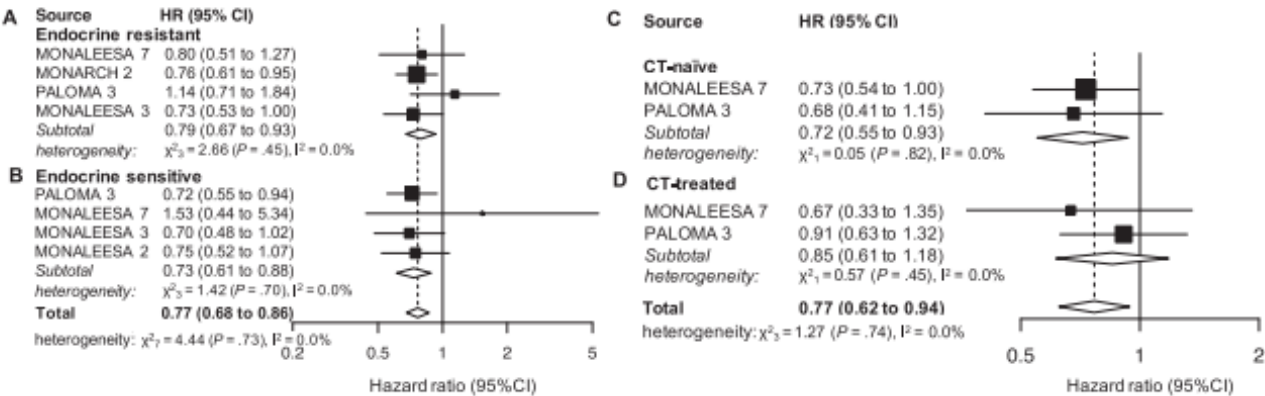


Figure 1. Pooled overall survival (OS) according to metastatic sites and tumor burden. Pooled OS in nonvisceral (A), visceral (B), no bone-only (C), or bone-only (D) disease and in case of less than 3 (E) and 3 or more metastatic sites (F). CI = confidence interval; HR = hazard ratio.



A significant cumulative relative reduction in the risk of death of 24% with cdk4/6 inhibitors + OT vs OT

Reduction of the hazard of dying with CDK4/6 inhibitor of the 29%  
HR 0,74(0,62-0,80)

The OS benefit obtained with CDK4/6-inhibitor–based combinations in **bone-only disease** was not statistically significant BUT... longer follow-up, and more events might be needed to obtain more conclusive results

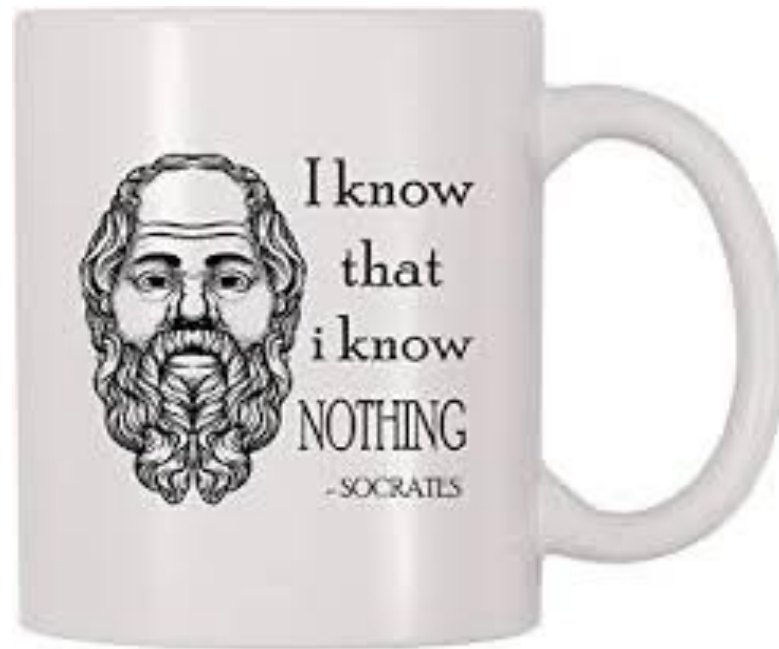
# EFFICACY OF CDK 4/6 INHIBITORS IN BONE METASTASES

The combination of CDK4/6 inhibitors and endocrine therapy represents an effective and well-tolerated approach for the first-line treatment of metastatic breast cancer in the BoD setting.

Bone metastasis increase the risk of skeletal-related events (SREs) in cancer patients. In addition to affecting the quality of life, it also increases the medical costs and mortality risk.

To date, there are no studies that comparatively investigate the impact of CDK4 / 6 inhibitors on bone endpoints (Skeletal Related events and Skeletal progression free survival).

# CDK4/6i and bone ... what we know ?





Article

# Biological Effects of Cyclin-Dependent Kinase Inhibitors Ribociclib, Palbociclib and Abemaciclib on Breast Cancer Bone Microenvironment

Michele Iuliani <sup>1,\*</sup>,<sup>†</sup> , Sonia Simonetti <sup>1,†</sup>, Giulia Ribelli <sup>1</sup>, Andrea Napolitano <sup>1,2</sup> , Umile Giuseppe Longo <sup>3</sup>, Bruno Vincenzi <sup>1</sup>, Paolo Orsaria <sup>4</sup>, Vincenzo Denaro <sup>3</sup>, Giuseppe Tonini <sup>1</sup>, Daniele Santini <sup>1,†</sup> and Pantano Francesco <sup>1,†</sup>

**Abstract:** The CDK4/6 inhibitors (CDKi) palbociclib, ribociclib, and abemaciclib are currently approved in combination with anti-estrogen therapy for the treatment of advanced and/or metastatic hormone receptor-positive/HER2-neu-negative breast cancer patients. Given the high incidence of bone metastases in this population, we investigated and compared the potential effects of palbociclib, ribociclib, and abemaciclib on the breast cancer bone microenvironment. Primary osteoclasts (OCs) and osteoblasts (OBs) were obtained from human monocyte and mesenchymal stem cells, respectively. OC function was evaluated by tartrate-resistant acid phosphatase assay and real-time PCR; OB activity was assessed by an alizarin red assay. OB/breast cancer co-culture models were generated via the seeding of MCF-7 cells on a layer of OBs, and tumor cell proliferation was analyzed using flow cytometry. Here, we showed that ribociclib, palbociclib, and abemaciclib exerted similar inhibitory effects on the OC differentiation and expression of bone resorption markers without affecting OC viability. On the other hand, the three CDKi did not affect the ability of OB to produce bone matrix, even if the higher doses of palbociclib and abemaciclib reduced the OB viability. In OB/MCF-7 co-culture models, palbociclib demonstrated a lower anti-tumor effect than ribociclib and abemaciclib. Overall, our results revealed the direct effects of CDKi on the tumor bone microenvironment, highlighting differences potentially relevant for clinical practice.

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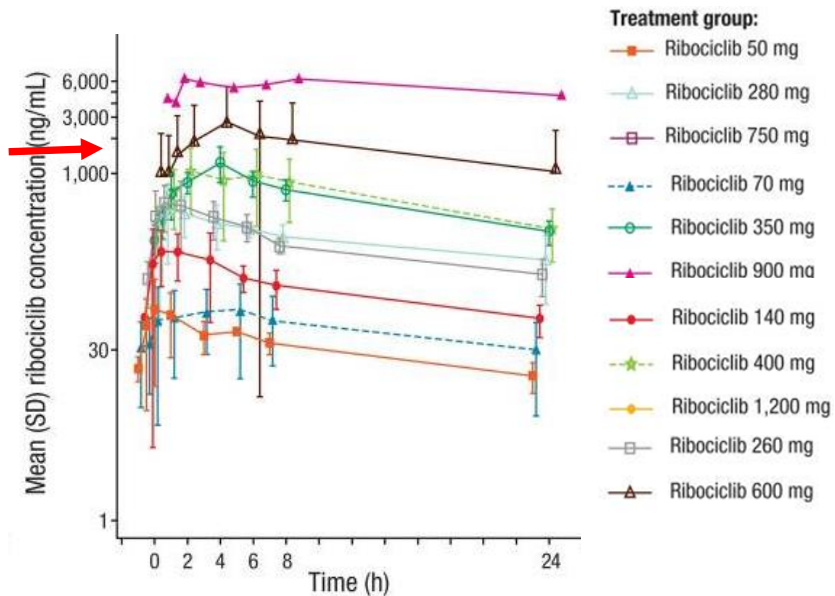
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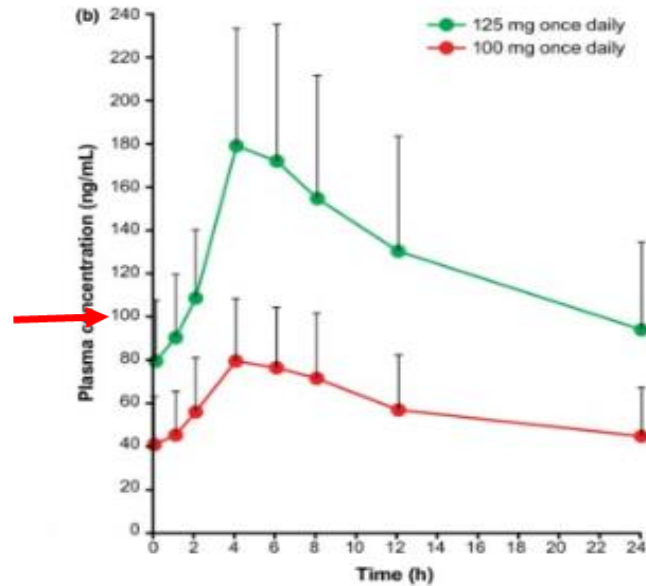
# The choice of cdki drug concentrations: pharmacokinetic considerations

## Ribociclib



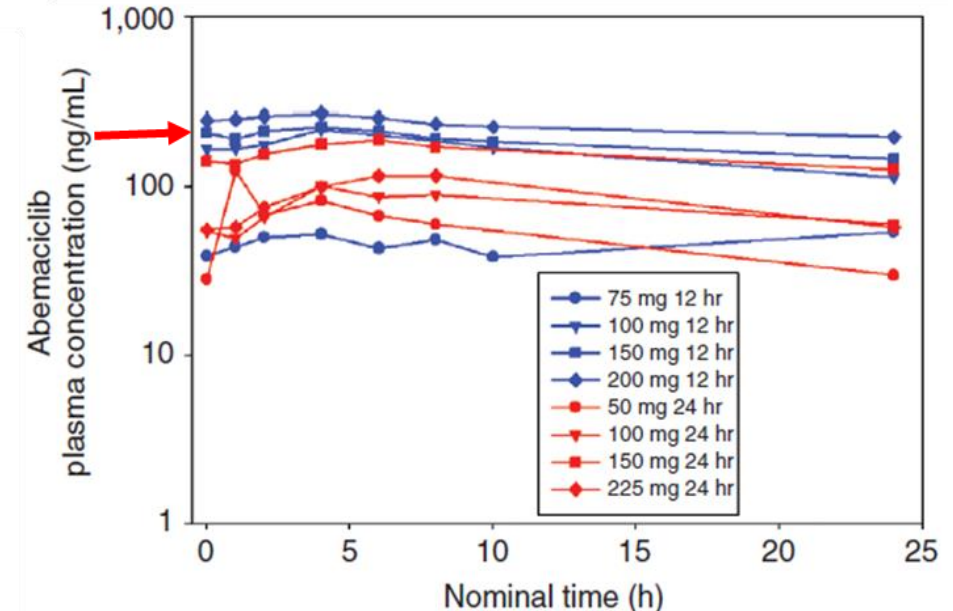
**Ribociclib** Mean plasma concentration  
~ 1500 ng/ml = **3  $\mu$ M**

## Palbociclib



**Palbociclib** Mean Plasma Concentration: ~ 100 ng/ml  
= **0,2  $\mu$ M**

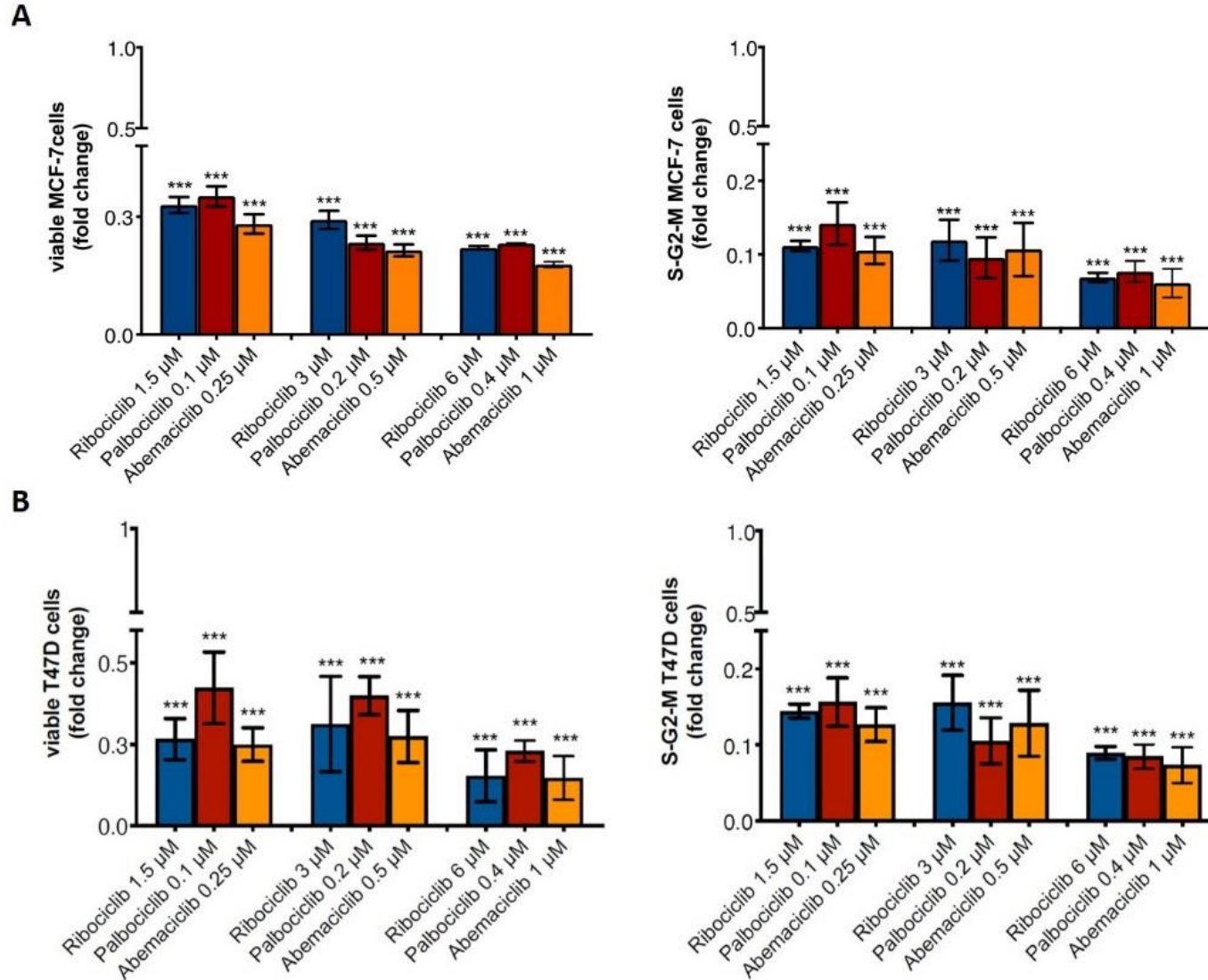
## Abemaciclib



**Abemaciclib** Mean Plasma Concentration:  
~ 300 ng/ml = **0,5  $\mu$ M**

Drug concentrations used in *in vitro* experiments

# The choice of CDKi drug concentrations: in vitro validation



The three CDKi showed a similar anti-tumor effect on the two hormone-sensitive breast cancer cells

**The choice of concentrations was appropriated!!**

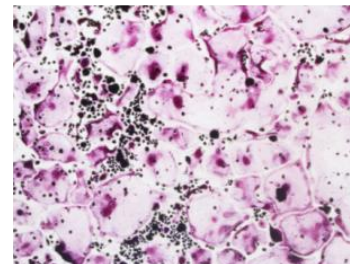
# Human primary in vitro osteoclast generation



## DIFFERENTIATION (TRAP ASSAY)



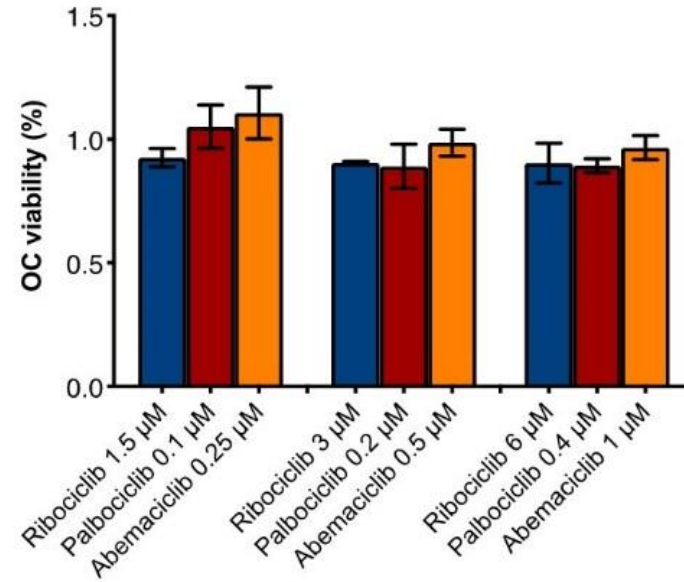
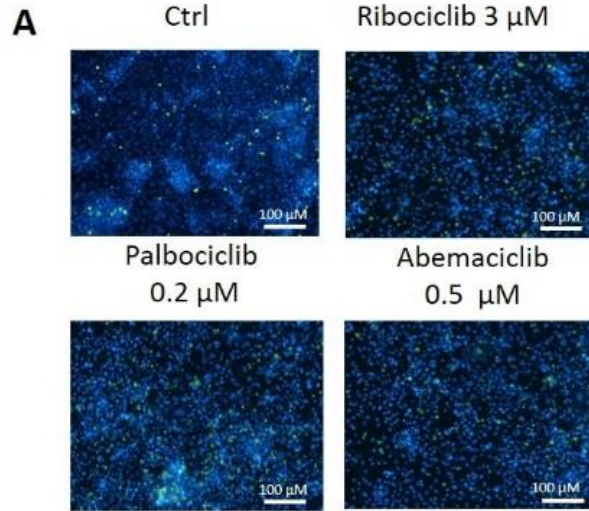
UNDIFFERENTIATED



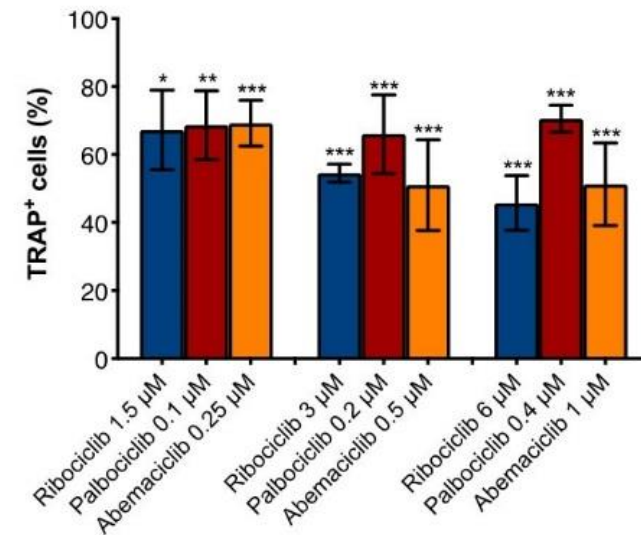
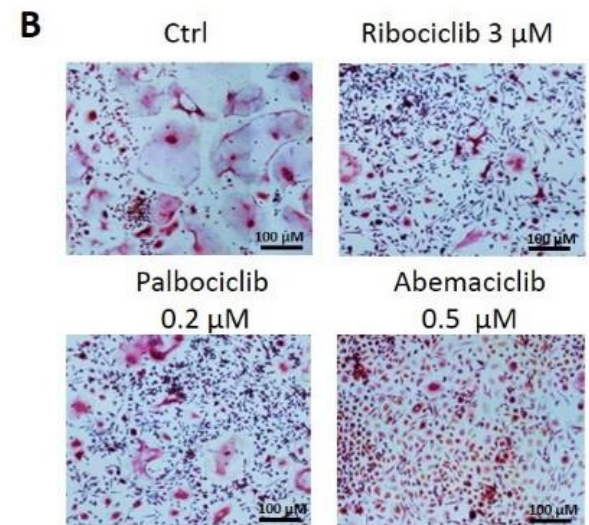
DIFFERENTIATED



## CDKi effects on osteoclast differentiation



The three CDKi inhibited osteoclast differentiation without affecting osteoclast viability

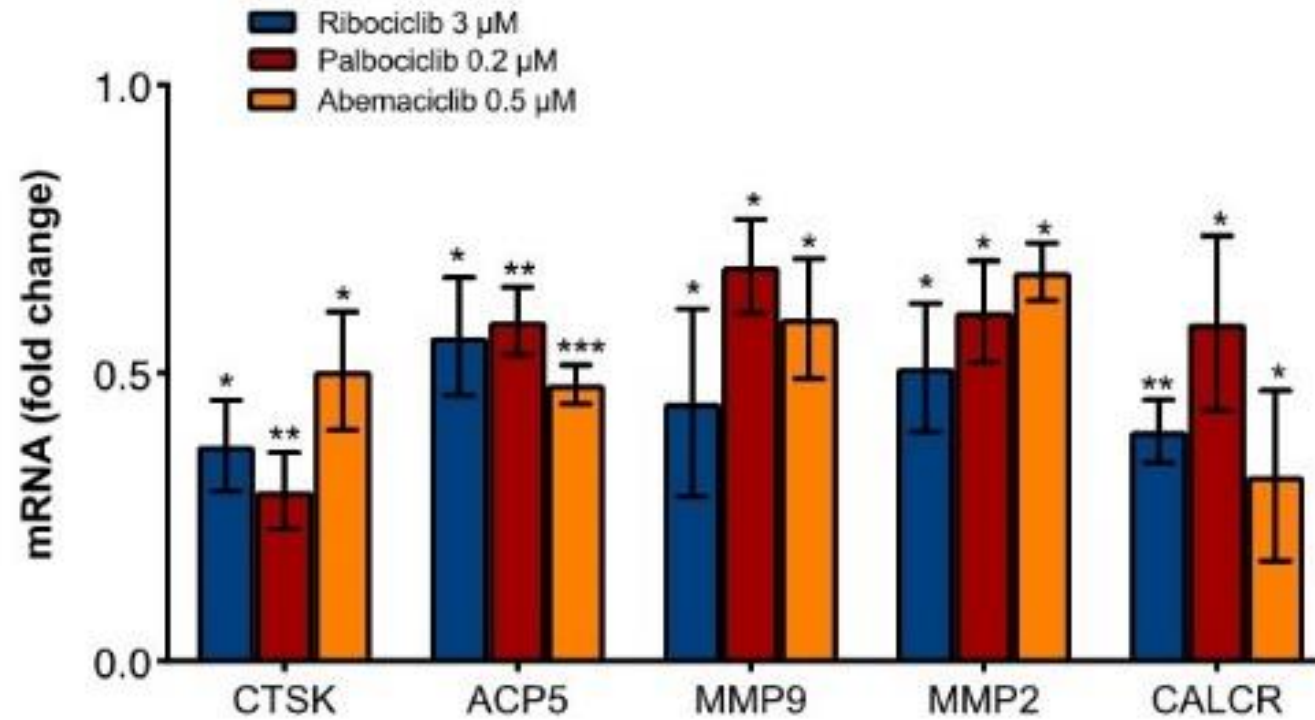


Ribociclib and Abemaciclib exerted an higher osteoclast inhibitory effect compared to Palbociclib

Osteoclasts were cultured in steroid deprived media

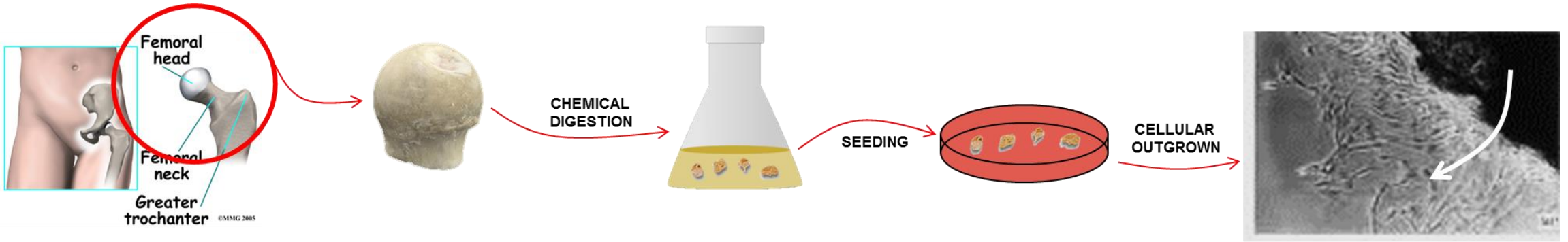


## CDK inhibitors effects on bone resorption



CDK inhibitors reduced the expression of genes involved in bone resorption

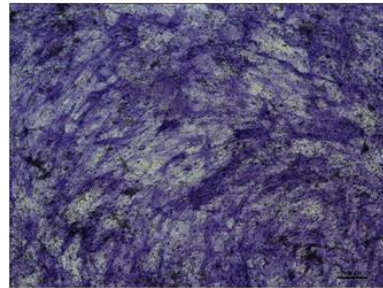
# Human primary in vitro osteoblast generation



## DIFFERENTIATION (ALP ASSAY)

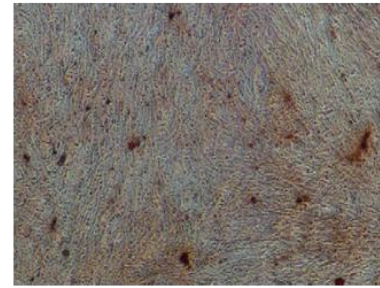


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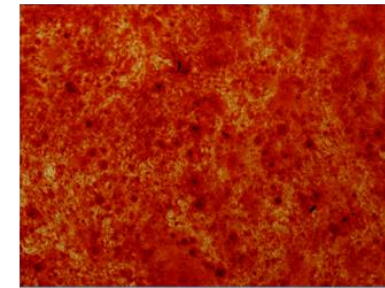


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## ACTIVITY (ALIZARIN RED ASSAY)

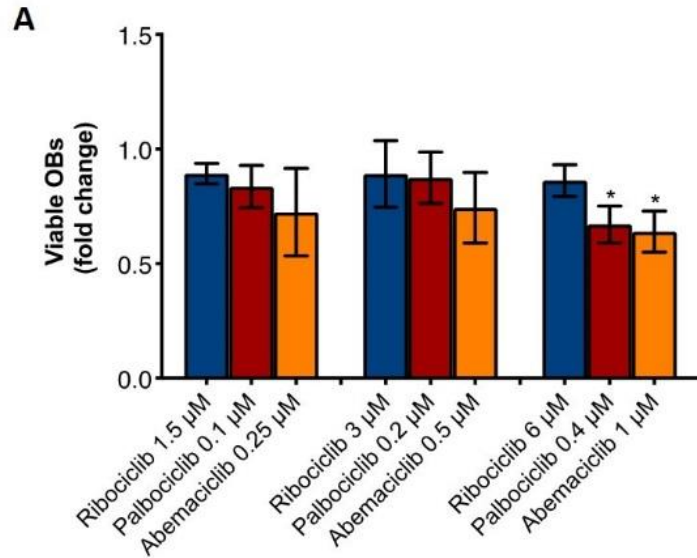


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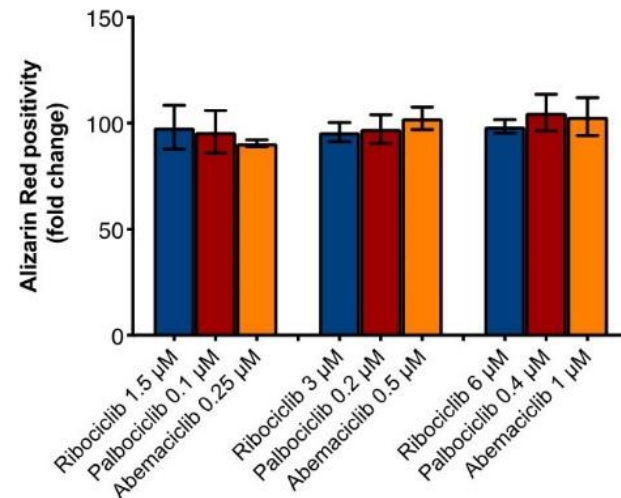
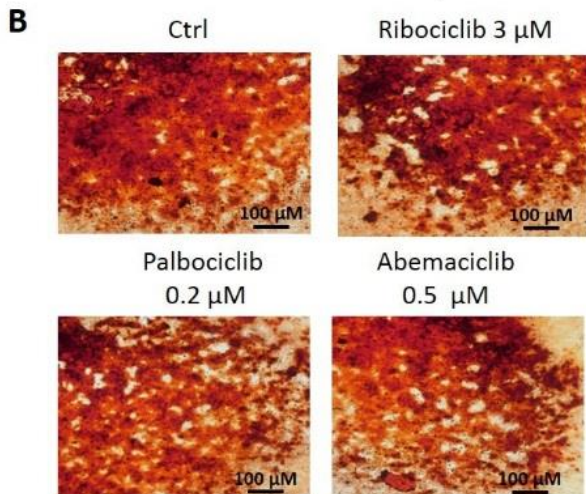


DIFFERENTIATED

## CDKi effects on osteoblast differentiation



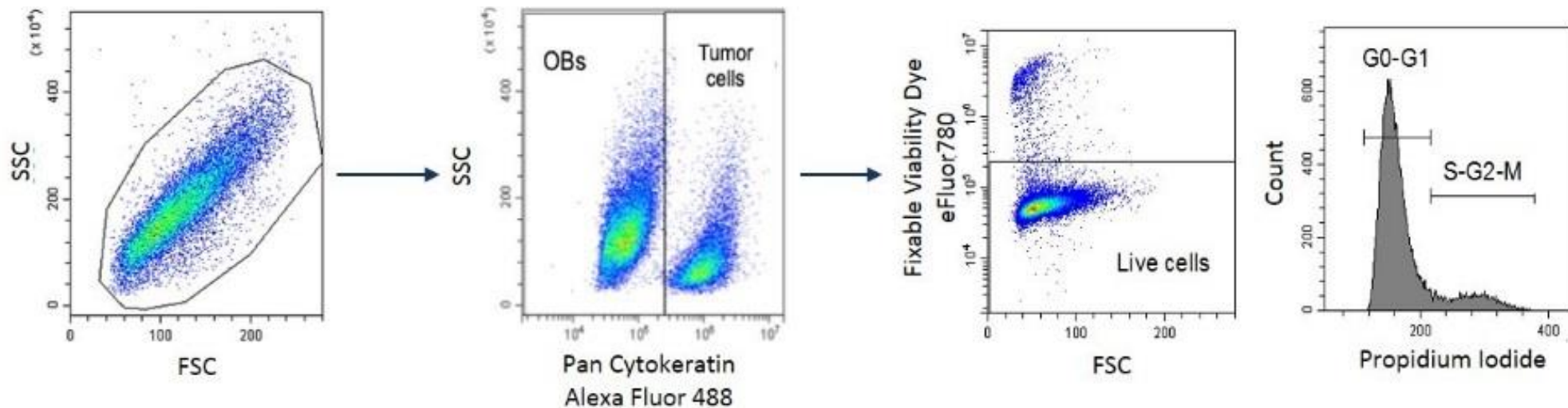
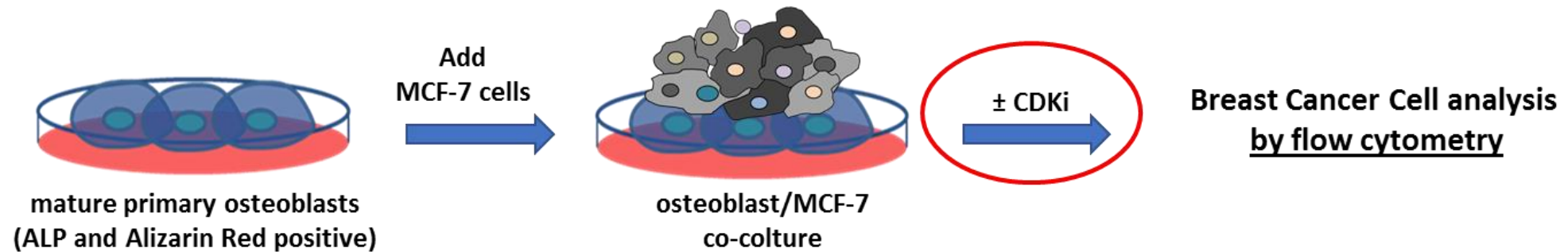
The higher concentrations of palbociclib and abemaciclib reduced osteoblast viability



CDKi did not affect the ability of osteoblasts to produce bone matrix

Osteoblasts were cultured in steroid deprived media

# Breast cancer bone metastatic model co-culture generation and flow cytometry analysis

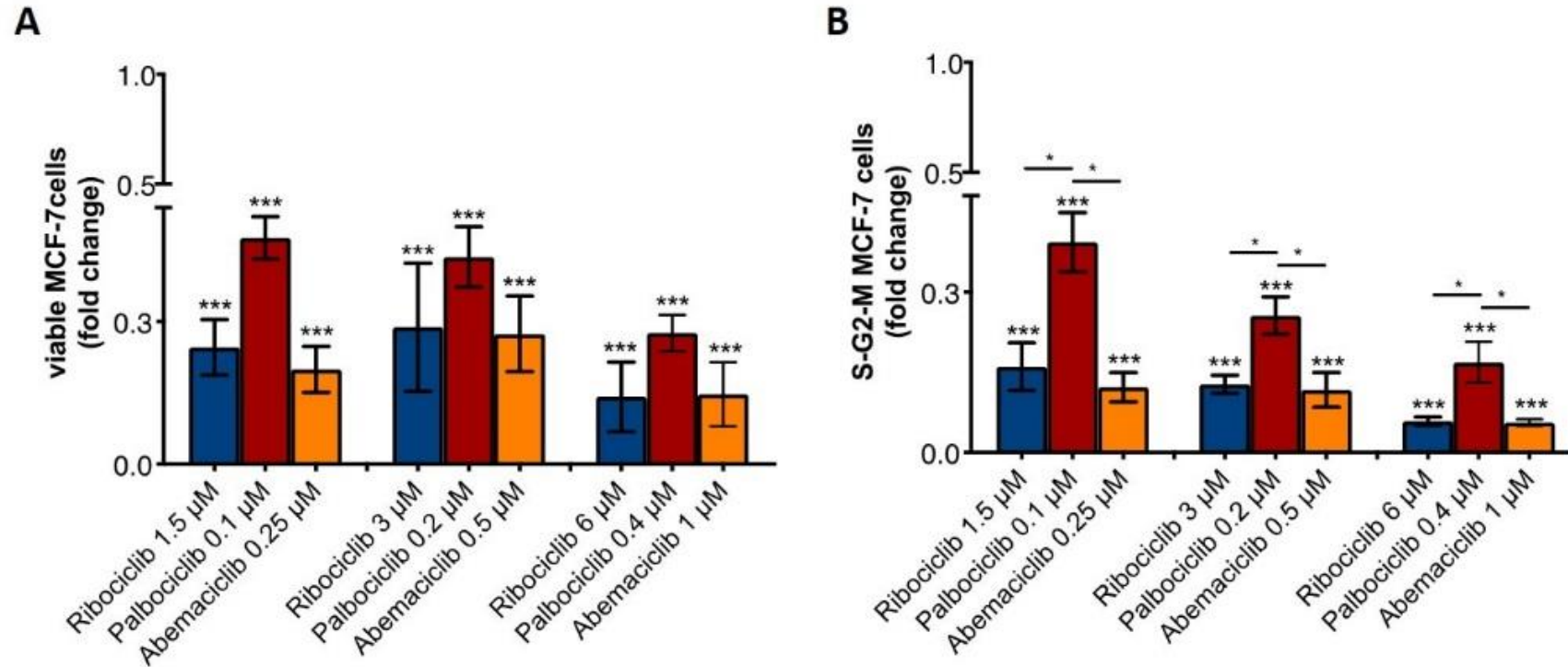


Pan-Cytokeratin staining allows to discriminate between breast cancer cells and osteoblasts (Pan-Cytokeratin negative)

Breast cancer/osteoblasts were cultured in steroid deprived media



# CDK inhibitors effect on breast cancer cells in bone microenvironment



The three CDKi reduced cell viability and cancer cell proliferation in breast cancer/osteoblasts co-culture models

Ribociclib and abemaciclib exerted a higher anti-tumor effect compared to Palbociclib

# Conclusions

## **Osteoclasts:**

- The three CDKi inhibited osteoclast differentiation and activity
- The comparative analysis shows that Ribociclib and Abemaciclib exerted an higher osteoclast inhibitory effect compared to Palbociclib

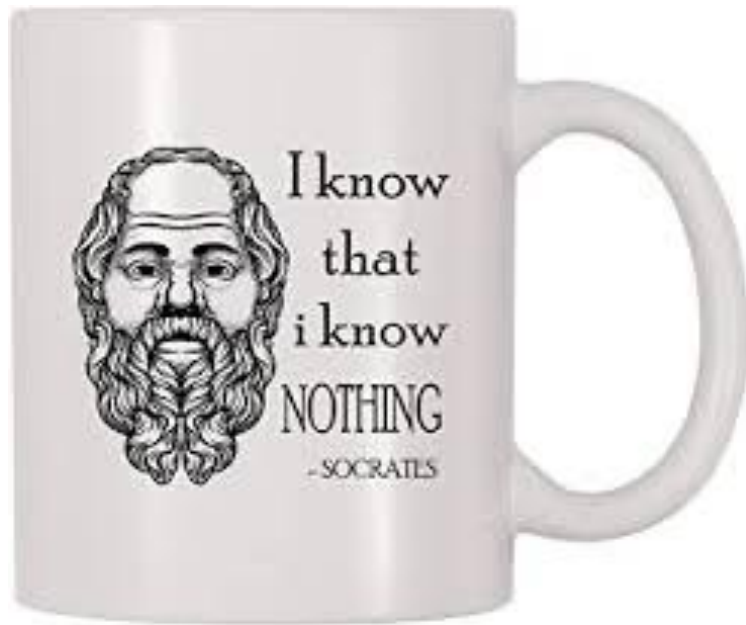
## **Osteoblasts:**

- The three CDKi did not affect the ability of **osteoblasts** to produce bone matrix
- The comparative analysis shows that the higher concentrations of palbociclib and abemaciclib reduced **osteoblast** viability

## **Breast cancer bone metastatic models:**

- The three CDKi reduced cell viability and cancer cell proliferation in breast cancer/osteoblasts co-culture models
- The comparative analysis shows that Ribociclib and abemaciclib exerted a higher anti-tumor effect compared to Palbociclib

**The three CDKi exert a direct effect on the tumor bone microenvironment, but with differences potentially relevant for clinical practice**



- **Bone health in CDK4/6 inhibitors: real world experience.**
- Studio retrospettivo osservazionale multicentrico di real life di Palbociclib, Ribociclib e Abemaciclib in combinazione con inibitori dell'aromatasi o Fulvestrant in pazienti con ca mammella HR+/HER2- e metastasi ossee.