

# Scaffold biomimetici come modelli *in vitro* per lo studio della metastasi ossea da carcinoma della mammella

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CLAUDIA B.  
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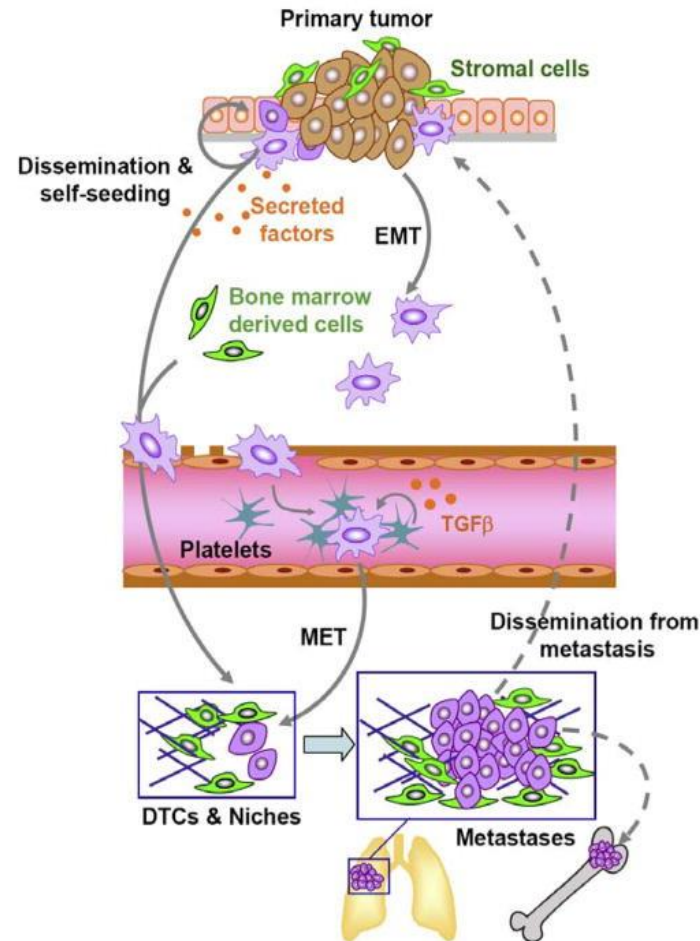


ALESSIA A.  
Familiare



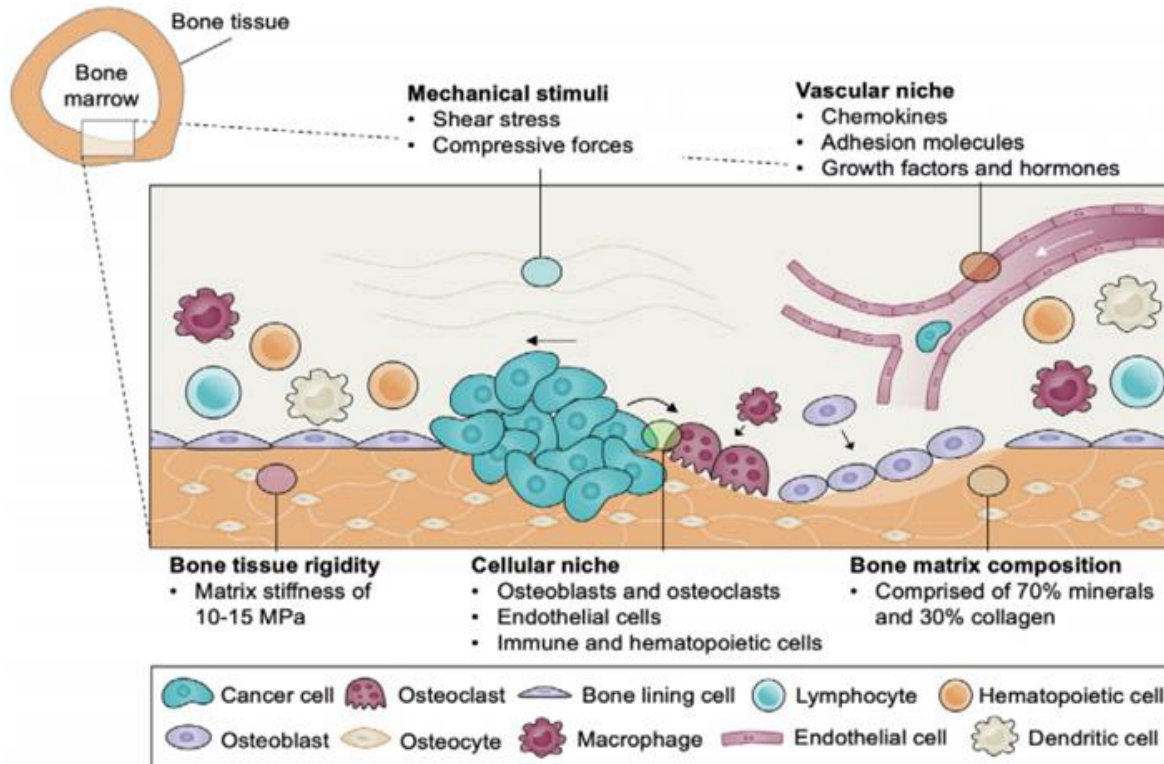
CHIARA S.  
Ricercatrice

# Metastasi ossea: un processo multi-step



Kang Y., Pantel K., *Cancer Cell*, 2013

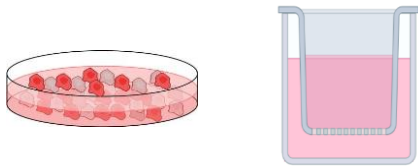
# Ruolo del microambiente e della matrice extracellulare (ECM) nella metastasi ossea



# Modelli preclinici in Osteoncologia

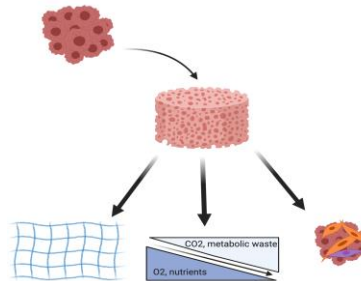
- Approfondire le nostre conoscenze sui meccanismi molecolari coinvolti nel processo di metastasi può aiutare ad identificare nuove strategie terapeutiche più efficaci
- Necessità di implementare i nostri modelli *in vitro* ed *in vivo* di metastasi ossea

## 2D *in vitro* models



- Cost-effective
- Easy to use
- Easy downstream processes
- Less biologically relevant models
- Reduced cell-to-cell interactions
- Poor clinical relevance
- Unnatural adhesion forces

## 3D *in vitro* models



- Mimics *in vivo* environment more accurately
- More relevant 3D cell-to cell and cell-ECM interactions
- More suitable as drug resistance models
- More complex than 2D models
- Complex downstream processed
- Lack the vasculature

## *In vivo* models



- Whole organism interactions
- Physiologically and clinically relevant
- Ethical concerns
- High Cost
- Time consuming
- Not human, different immune system



# Dai modelli bidimensionali 2D...

Bone 66 (2014) 214–222

Contents lists available at ScienceDirect

Bone

journal homepage: [www.elsevier.com/locate/bone](http://www.elsevier.com/locate/bone)



Original Full Length Article

CSF-1 blockade impairs breast cancer osteoclastogenic potential in co-culture systems



Chiara Liverani <sup>a,\*</sup>, Laura Mercatali <sup>a</sup>, Chiara Spadazzi <sup>a</sup>, Federico La Manna <sup>a</sup>, Alessandro De Vita <sup>a</sup>, Nada Riva <sup>a</sup>,



International Journal of  
Molecular Sciences



Article

The Effect of Everolimus in an In Vitro Model of Triple Negative Breast Cancer and Osteoclasts

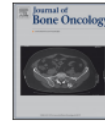
Laura Mercatali  
Alberto Bongioanni

Journal of Bone Oncology 16 (2019) 100227

Contents lists available at ScienceDirect

Journal of Bone Oncology

journal homepage: [www.elsevier.com/locate/jbo](http://www.elsevier.com/locate/jbo)



Research Paper

mTOR inhibitor and bone-targeted drugs break the vicious cycle between clear-cell renal carcinoma and osteoclasts in an in vitro co-culture model



Chiara Spadazzi, Federica Recine, Laura Mercatali <sup>\*</sup>, Giacomo Miserocchi, Chiara Liverani, Alessandro De Vita, Alberto Bongioanni, Valentina Fausti, Toni Ibrahim

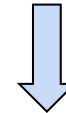
Osteoncology and Rare Tumors Center, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Via P. Maroncelli 40, 47014 Meldola, FC, Italy

Modello adatto per studiare strategie terapeutiche per bloccare il «circolo vizioso» della metastasi ossea

Tumor Cells



Osteoclasts  
Differentiation

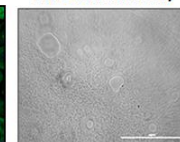
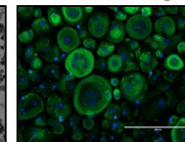
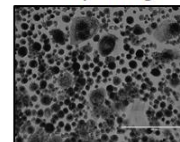


Trap staining

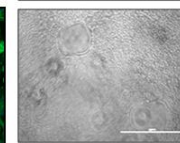
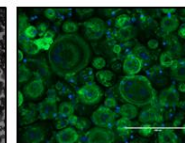
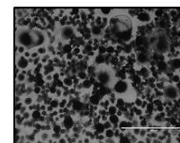
Phalloidin staining

Pit Formation Assay

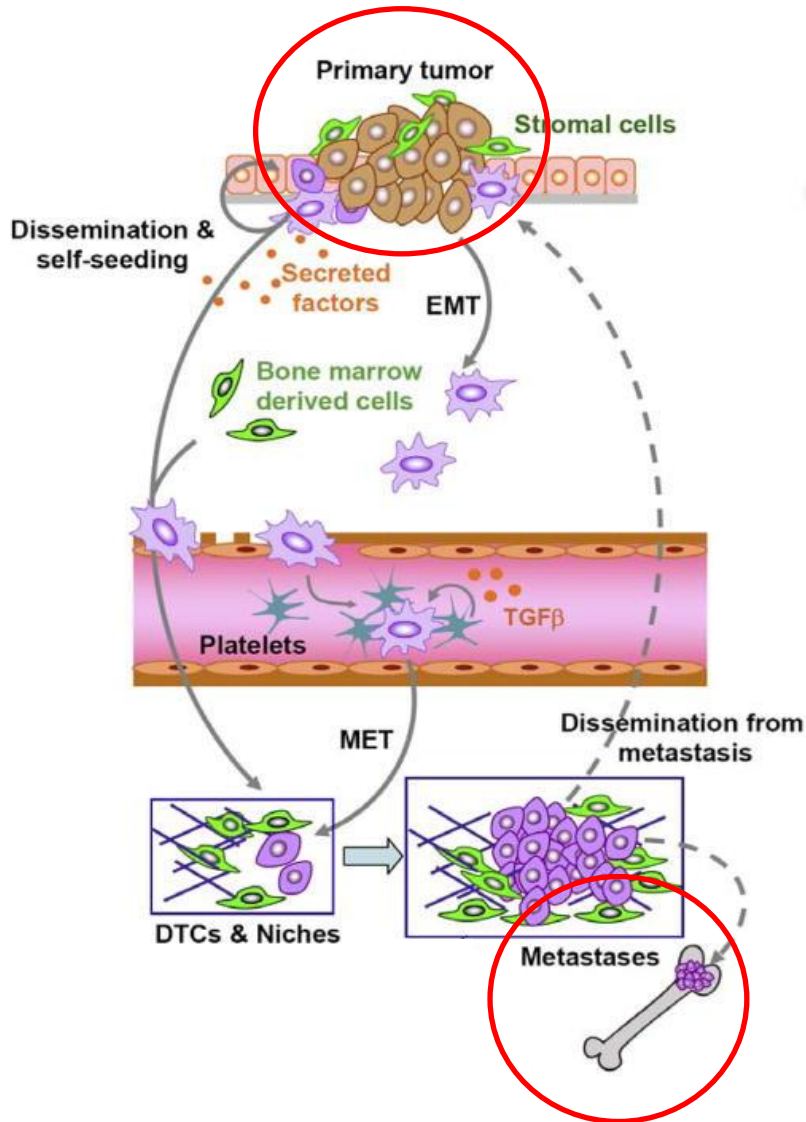
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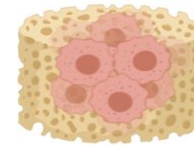
Co-Culture



# ...Ai modelli tridimensionali 3D



## In Vitro Modeling

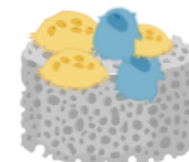


*In vitro* 3D  
collagen scaffold



Secreted  
Factors

Pre-Metastatic  
Niche



*In vitro* 3D  
collagen/HA  
scaffold

# Obiettivo del Progetto

Interazioni cellule tumorali di carcinoma di mammella ed ECM

Collagene-Idrossiapatite  
Scaffold 3D  
(30: 70 ratio)



Fattori solubili secreti dalle  
cellule tumorali



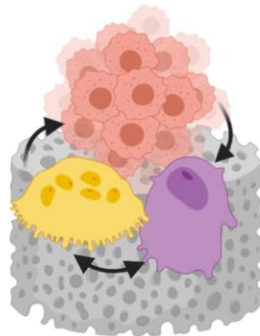
Differenziamento di  
osteoclasti e  
osteoblasti  
indotto dalle cellule  
tumorali



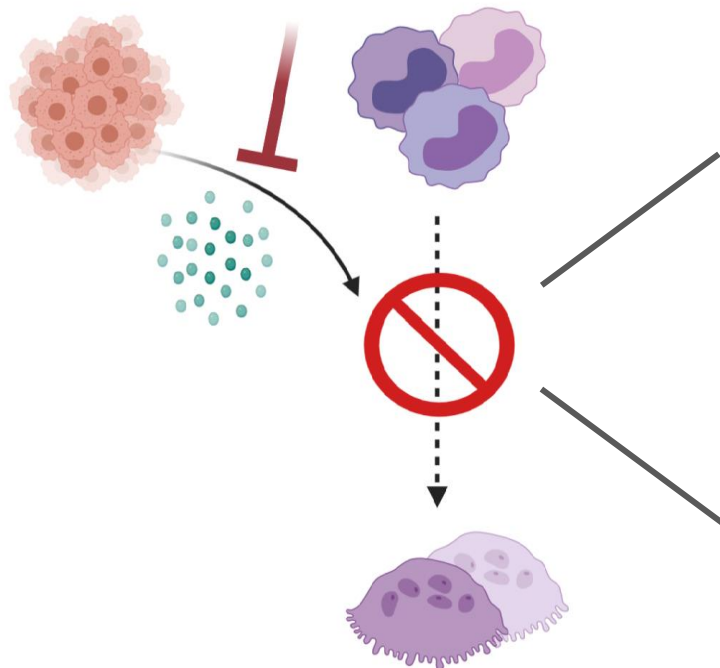
Strategie Terapeutiche?



**Modello 3D di metastasi  
ossea *in vitro*:**  
*Circolo vizioso in vitro*

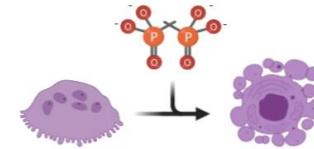


# Strategie Terapeutiche: Focus su Bone Targeted Therapies



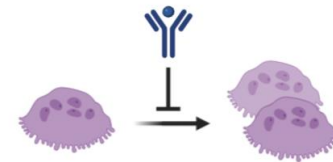
①

**Bisphosphonates**  
High osteoclast affinity  
Promotes apoptosis



②

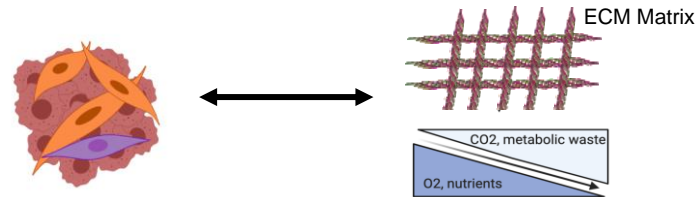
**Denosumab**  
Monoclonal antibody vs. RANKL  
Inhibits osteoclast proliferation





# Sviluppo dello scaffold 3D mineralizzato

I. Indagare le interazioni cellula tumorale-matrice



II. Ottimizzare differenziamento di osteoclasti e osteoblasti nel modello 3D

Mesenchimal Cells  
– Osteoblast  
precursor cells



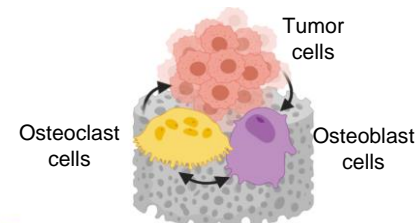
Osteoblast  
cells

Monocytes -  
Osteoclast  
precursor cells



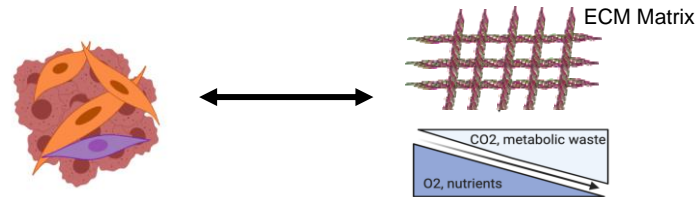
Osteoclast  
cells

III. Sviluppo di un modello 3D *in vitro* di metastasi ossea :  
interazione diretta tra cellule tumorali-osteoblasti-osteoclasti



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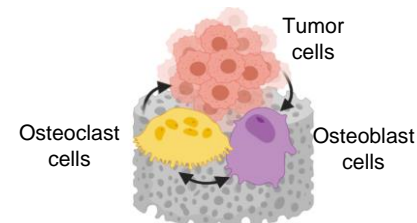
Osteoblast  
cells

Monocytes -  
Osteoclast  
precursor cells



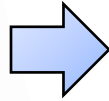
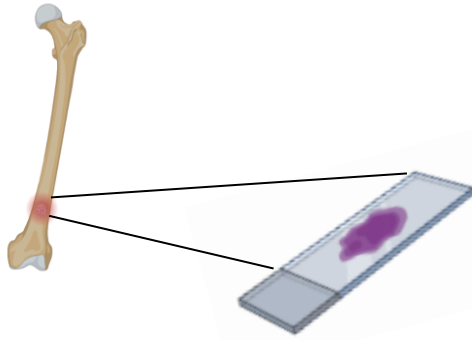
Osteoclast  
cells

## III. Sviluppo di un modello 3D *in vitro* di metastasi ossea : interazione diretta tra cellule tumorali-osteoblasti-osteoclasti



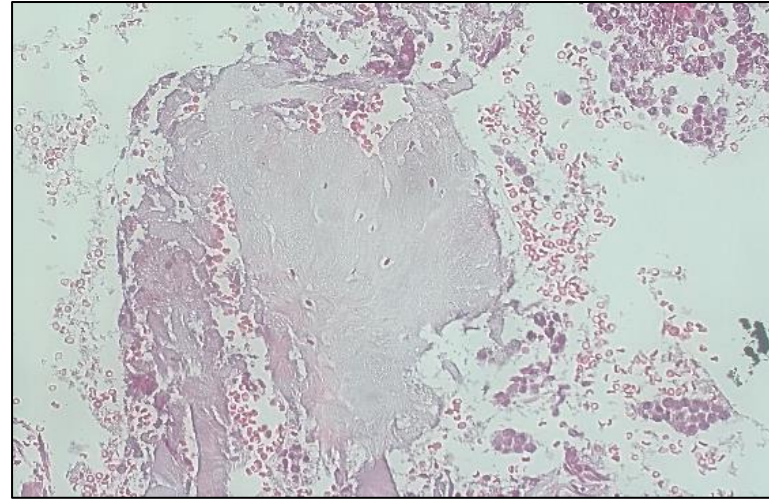
# Il modello 3D mineralizzato mimma la matrice extracellulare ossea

Bone Metastasis

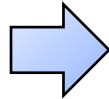


H&E

Bone Metastasis from ER+ BC

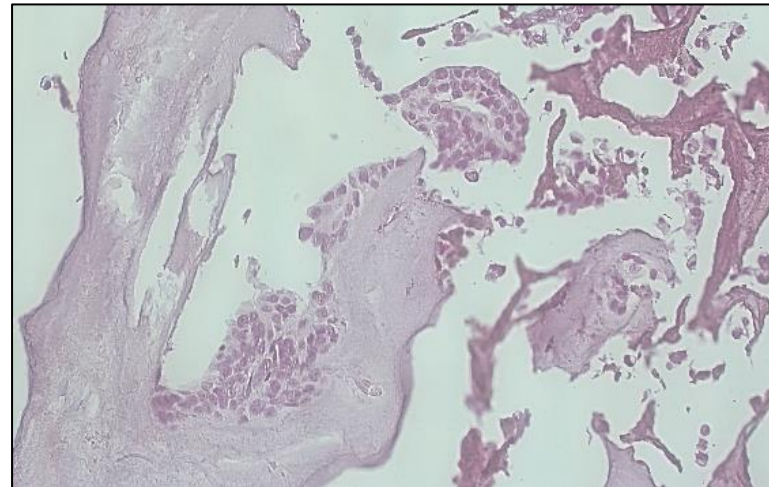


3D Scaffold Collagen I -  
Hydroxyapatite (30:70 ratio)



H&E

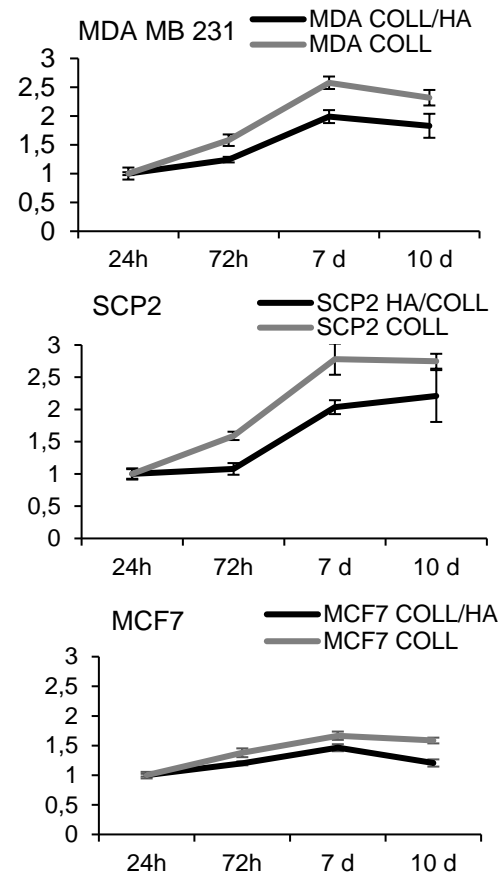
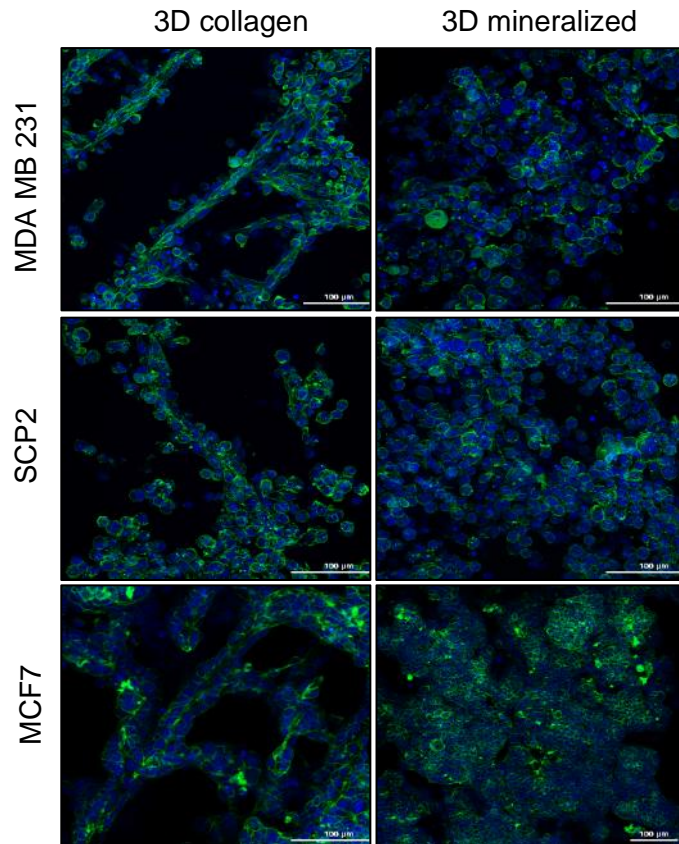
MCF7 ER+ BC in 3D mineralized scaffold



# Scaffold 3D collagen VS Scaffold 3D mineralizzato

Linee Cellulari

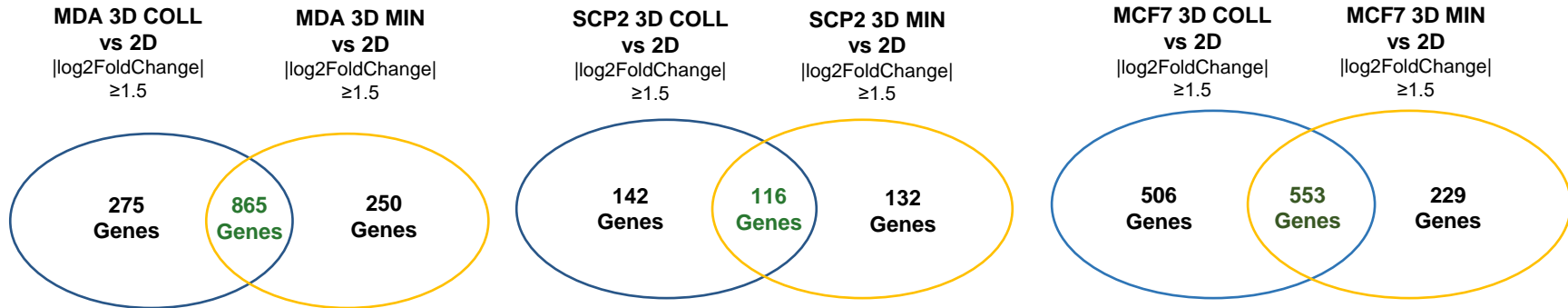
<b>A) MDA-MB-231:</b> ER- cell line	Basal- like molecular subtype	Fenotipo aggressivo, invasivo e scarsamente differenziato, metastatico in differenti siti secondari (principalmente viscerali)
<b>B) SCP2:</b> ER- cell line	osteotropic subclone of MDA-MB-231	Fenotipo aggressivo, linea cellulare metastatica all'osso
<b>C) MCF7:</b> ER+ cell line	Luminal A molecular subtype	Fenotipo non invasivo, dormiente



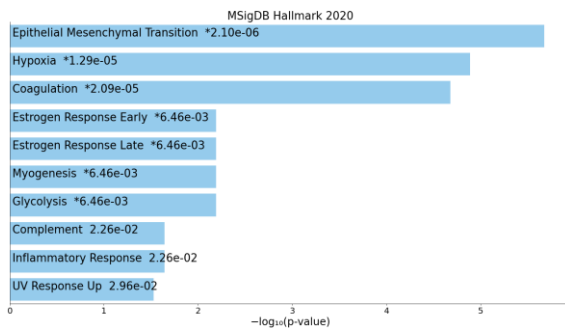
➤ La matrice extracellulare influenza il profilo di crescita delle cellule tumorali

# Un differente microambiente influenza il comportamento di cellule tumorali di carcinoma della mammella

- Analisi di RNA Seq mostra una disregolazione significativa di alcuni pathways relati al processo di metastasi ossea, indotta dalla differente matrice extracellulare

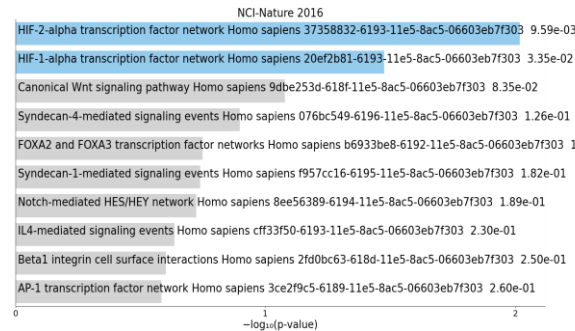


**Top Significant Dysregulated Pathways**  
MDA 3D MIN vs MDA 3D COLL



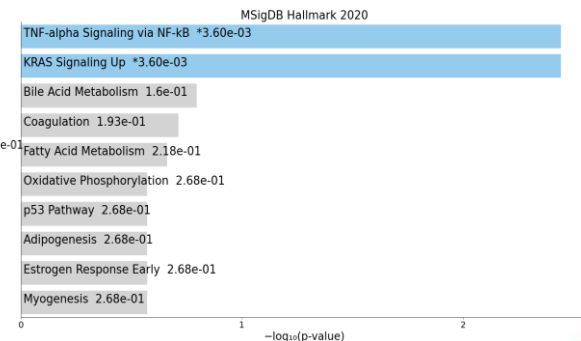
MSigDB Hallmark Database

**Top Significant Dysregulated Pathways**  
SCP2 3D MIN vs SCP2 3D COLL



NCI-NATURE 2016 Database

**Top Significant Dysregulated Pathways**  
MCF7 3D MIN vs MCF7 3D COLL

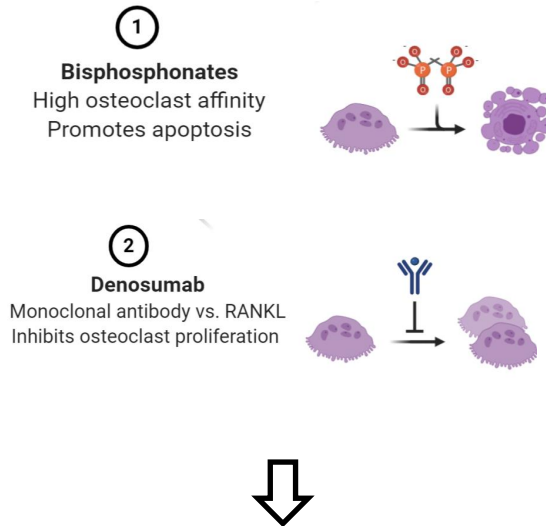


MSigDB Hallmark Database

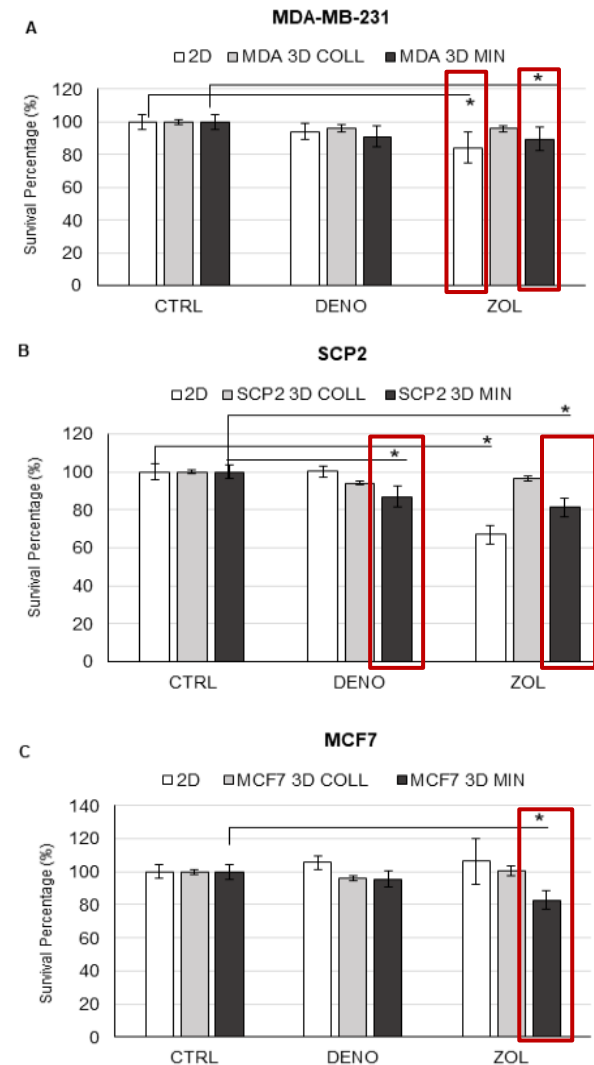


# La matrice extracellulare influenza la sensibilità alla bone targeted therapy in linee cellulari di carcinoma della mammella

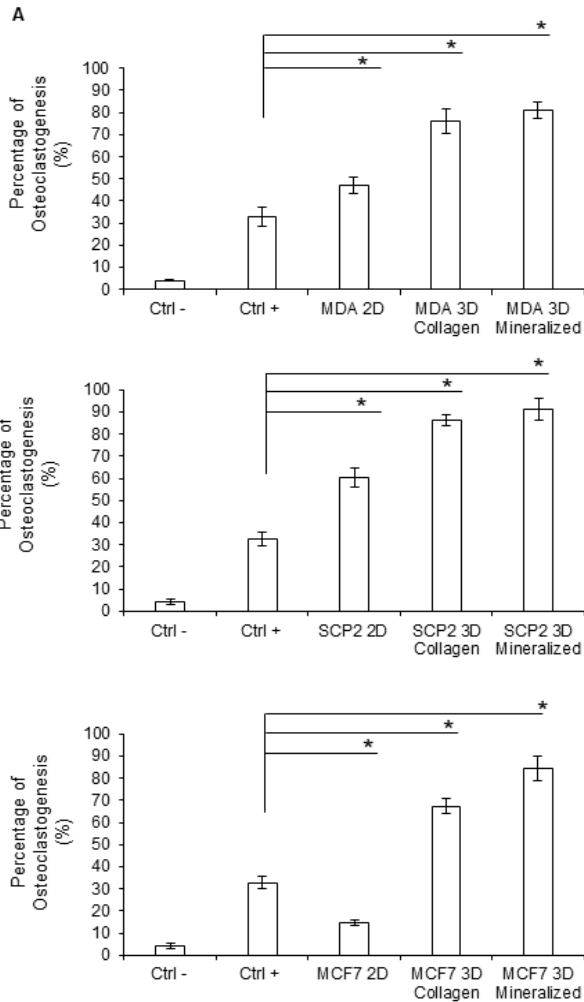
## Bone targeted therapy



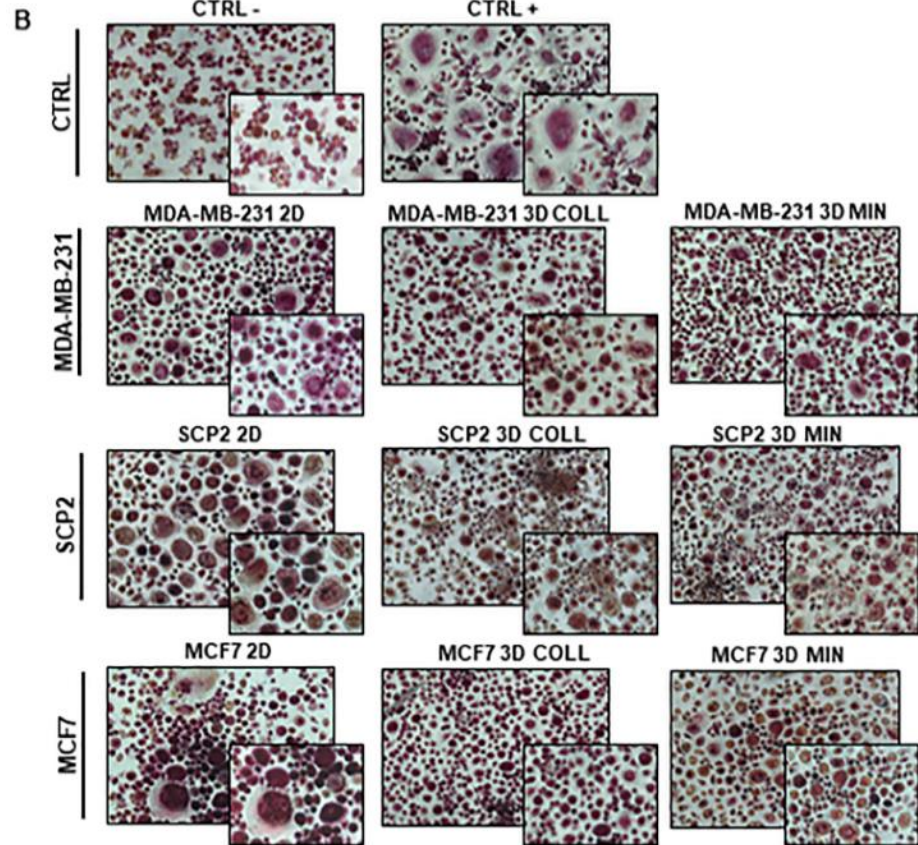
- La Bone Targeted Therapy influenza non solo le cellule dell'osso ma anche le cellule tumorali
- La sopravvivenza delle cellule tumorali è influenzata dalla differente matrice extracellulare



# Cellule di carcinoma della mammella cresciute in modello 3D influenzano il differenziamento osteoclastico



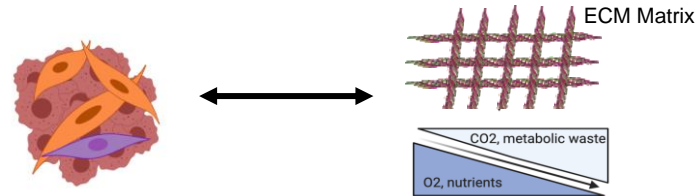
## Colorazione TRAP



Definizione di osteoclasta : cellule positive alla colorazione TRAP con più di 4 nuclei

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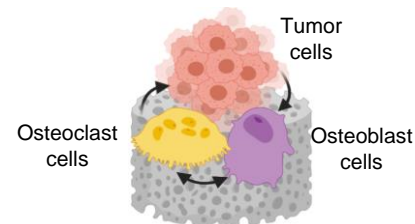
Osteoblast  
cells

Monocytes -  
Osteoclast  
precursor cells



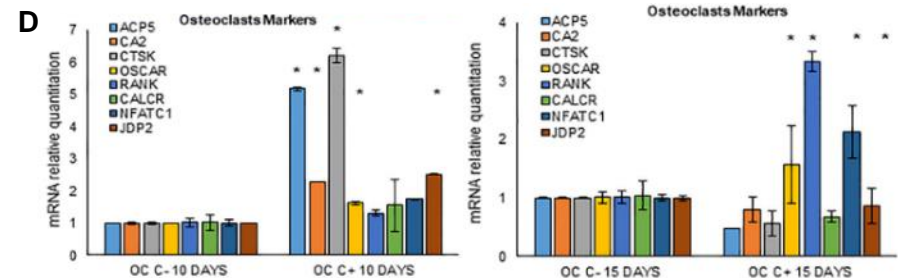
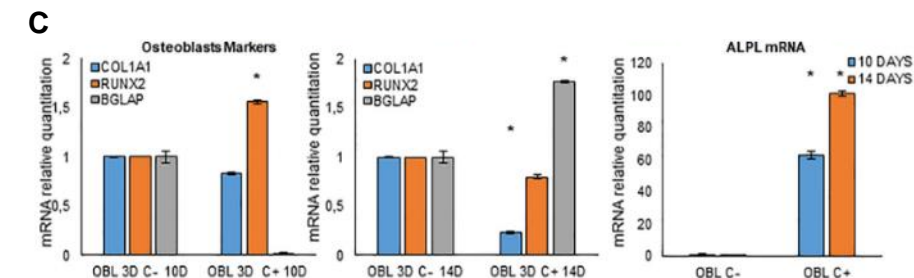
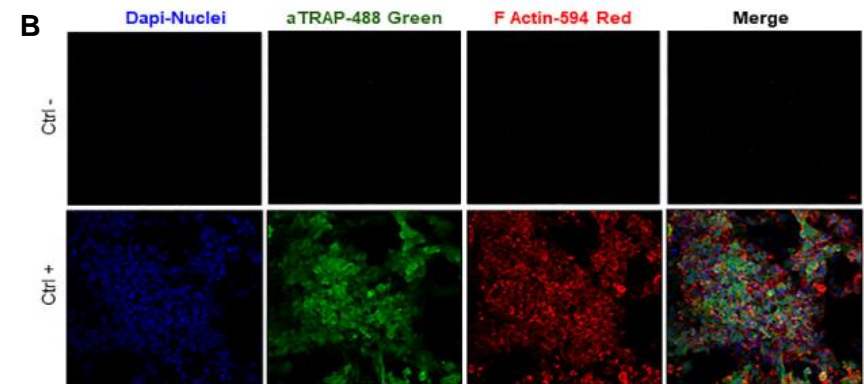
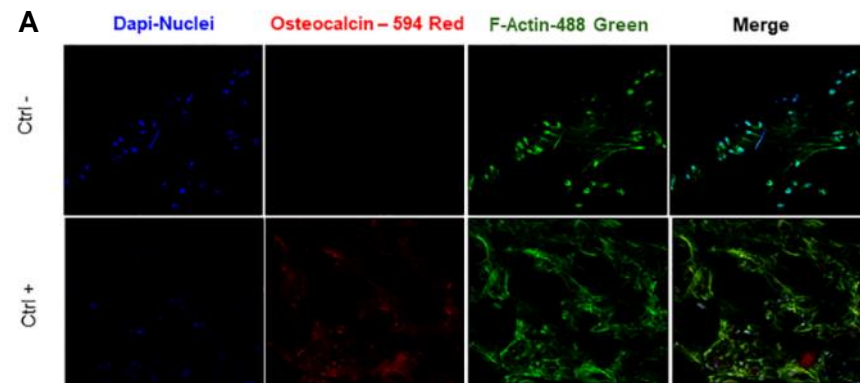
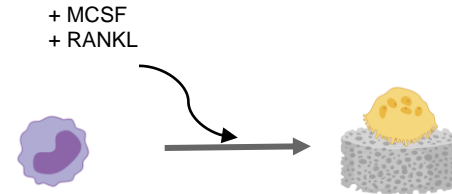
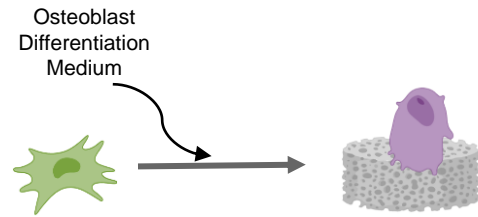
Osteoclast  
cells

III. Sviluppo di un modello 3D *in vitro* di metastasi ossea :  
interazione diretta tra cellule tumorali-osteoblasti-osteoclasti



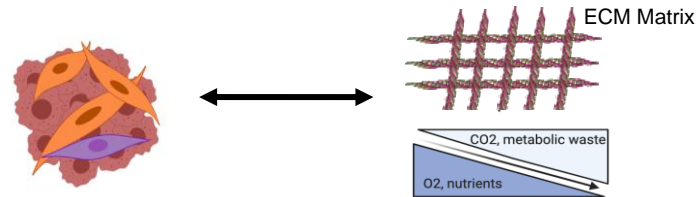
# Osteoblastogenesi ed Osteoclastogenesi nel modello 3D mineralizzato

- ✓ Ottimizzazione del numero di cellule mesenchimali o monociti da seminare nel modello 3D, necessario per il differenziamento osteoblastico ed osteoclastico



# Sviluppo dello scaffold 3D mineralizzato

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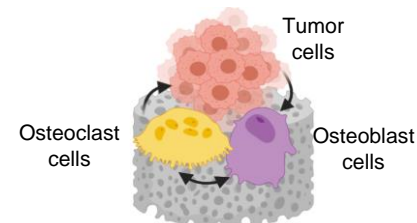
Osteoblast  
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Monocytes -  
Osteoclast  
precursor cells



Osteoclast  
cells

III. Sviluppo di un modello 3D *in vitro* di metastasi ossea :  
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✓ IN PROGRESS



# Conclusioni e prospettive future

- I. E' importante sviluppare modelli *in vitro* adatti a mimare la cascata sequenziale di eventi coinvolti nella patogenesi della metastasi ossea per:
  - caratterizzare i meccanismi molecolari coinvolti
  - Identificare nuove ed efficaci strategie terapeutiche per inibire la metastasi ossea
- II. Lo sviluppo del modello 3D mineralizzato è una piattaforma adatta per mimare la metastasi ossea: la struttura meccanica e minerale della matrice ossea può influenzare il comportamento delle cellule tumorali e il differenziamento delle cellule ossee
- III. Il modello sarà implementato da una tri-coltura di osteoblasti, osteoclasti e cellule tumorali per meglio indagare le interazioni coinvolte nella formazione della metastasi e per identificare nuove strategie terapeutiche.
- IV. Il modello sarà confermato con campioni clinici di paziente per aumentare il suo potere traslazionale.

# Ringraziamenti

## Ricercatori di Lab:

Dr.ssa Laura Mercatali (Coordinatrice e Co-Supervisore PhD)

Dott.ssa Chiara Liverani

Dott. Alessandro De Vita

Dott. Giacomo Misserocchi

Dott.ssa Claudia Cocchi

Dott.ssa Silvia Vanni

Dott.ssa Chiara Calabrese

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Dott. Sebastiano Calpona

Dott.ssa Nicoletta Ranallo

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## Università di Bologna:

### Supervisore PhD:

Prof. Nicola Baldini



SERVIZIO SANITARIO REGIONALE  
EMILIA-ROMAGNA  
Istituto Romagnolo per lo Studio dei Tumori "Dino Amadori"  
Istituto di Ricovero e Cura a Carattere Scientifico



ALMA MATER STUDIORUM  
UNIVERSITÀ DI BOLOGNA





# Grazie

per l'attenzione

ISTITUT  
SCIENTIFIC  
ROMAGNOLI  
PER LO STUDIO E LA CURA  
DEI TUMORI

 SERVIZIO SANITARIO REGIONALE  
EMILIA-ROMAGNA  
Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori  
Istituto di Ricovero e Cura a Carattere Scientifico