

---

# **Applicazione del "Targeted RNAseq" alle CTC di pazienti affette da carcinoma mammario metastatico allo scheletro**

***Targeted RNA-seq signature of circulating tumor cells (CTCs) from metastatic breast cancer (BC) patients correlates with the onset of bone-only metastases***

**Stella D'Oronzo, MD, PhD**

**Assistant Professor  
University of Bari Aldo Moro  
Bari, Italy  
Email: [stella.doronzio@uniba.it](mailto:stella.doronzio@uniba.it)**



**UNIVERSITÀ  
DEGLI STUDI DI BARI  
ALDO MORO**



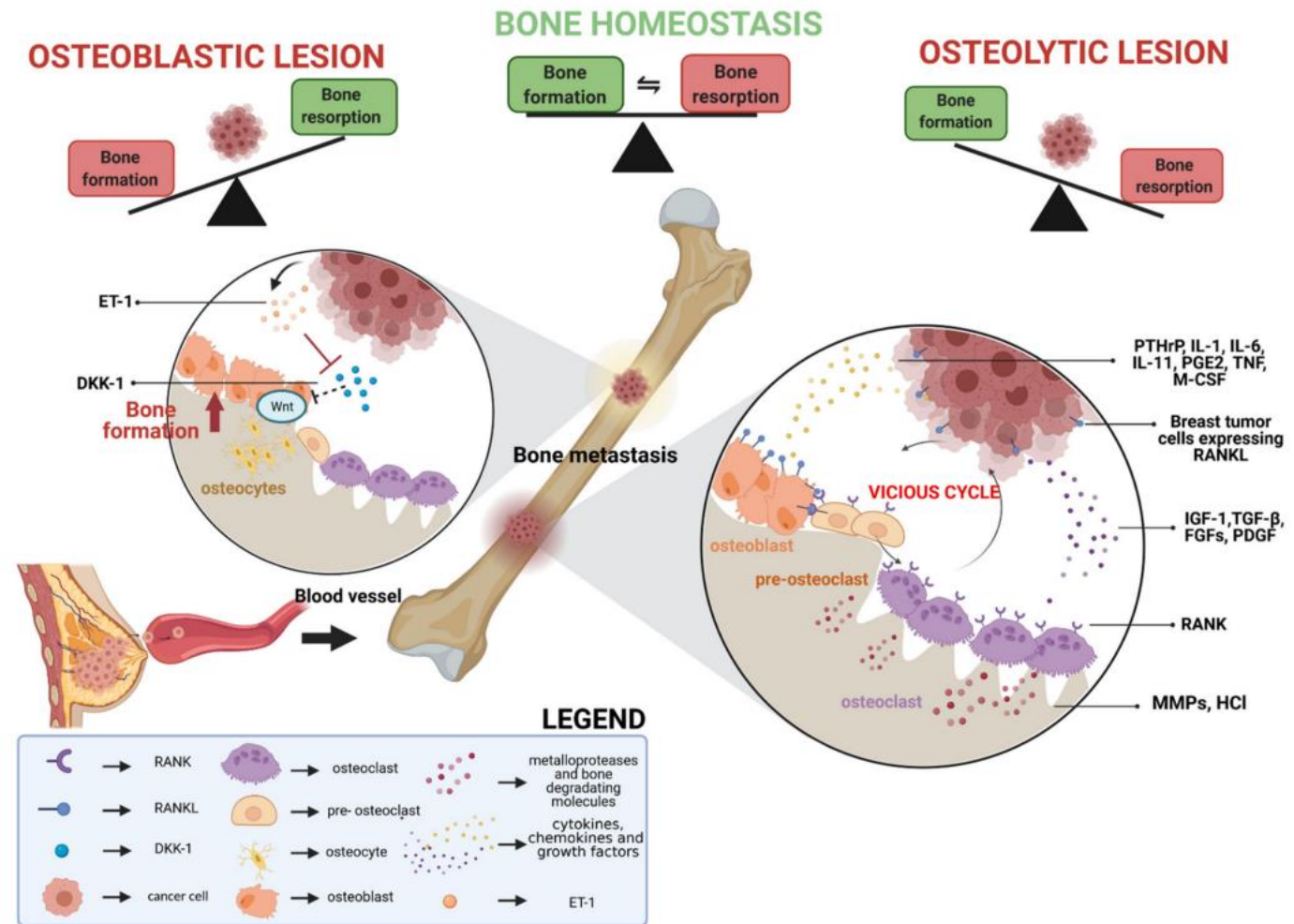
**The  
University  
Of  
Sheffield.**

---

# - BACKGROUND -

➤ Breast cancer (BC) is the most common female malignancy and the first cause of cancer-related death in women worldwide.

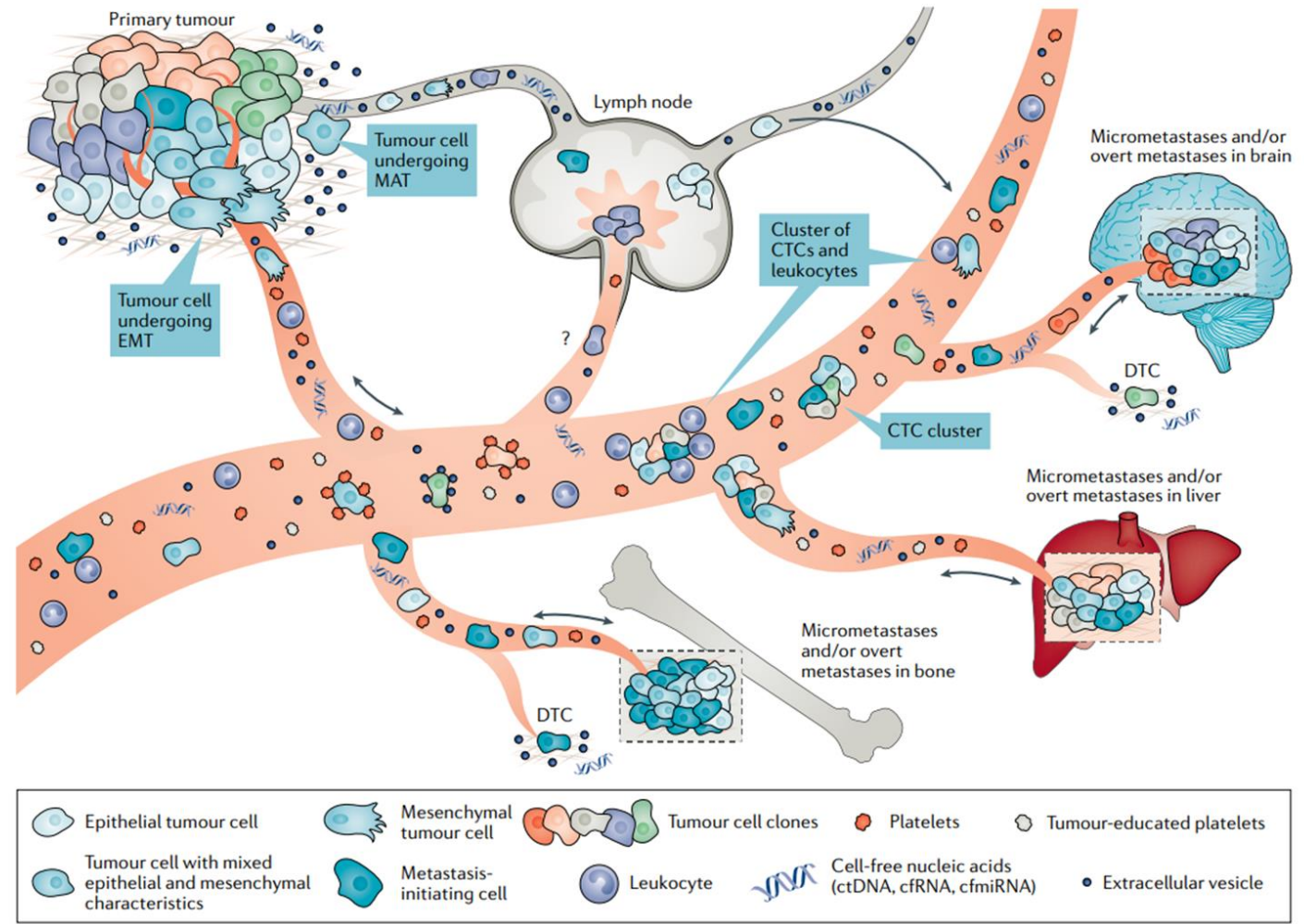
➤ Bone metastases (BM) affect up to 70% of patients with advanced BC, while approximately 13.6% of patients with stage I-III disease develop BM within 15 years.



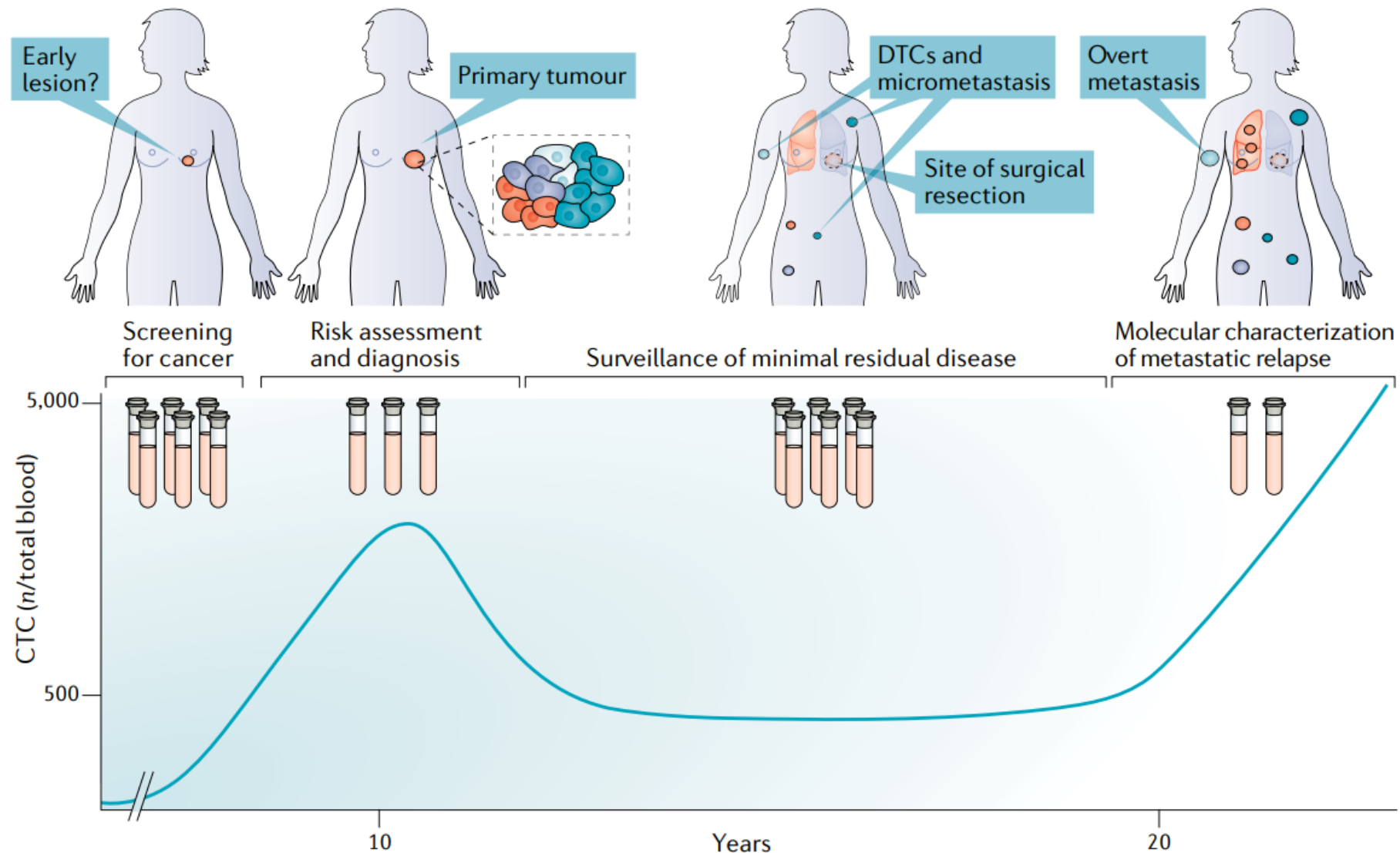
# - BACKGROUND -

➤ Distant metastasis is accomplished through the dissemination of circulating tumor cells (CTCs) from primary or metastatic lesions, their survival within the bloodstream, as well as their extravasation and colonization of target metastatic sites.

➤ As a consequence, CTCs are viewed as pro-metastasis precursors and their isolation from the blood of cancer patients is enabling a better understanding of metastasis biology, alongside with new possibilities for disease monitoring.



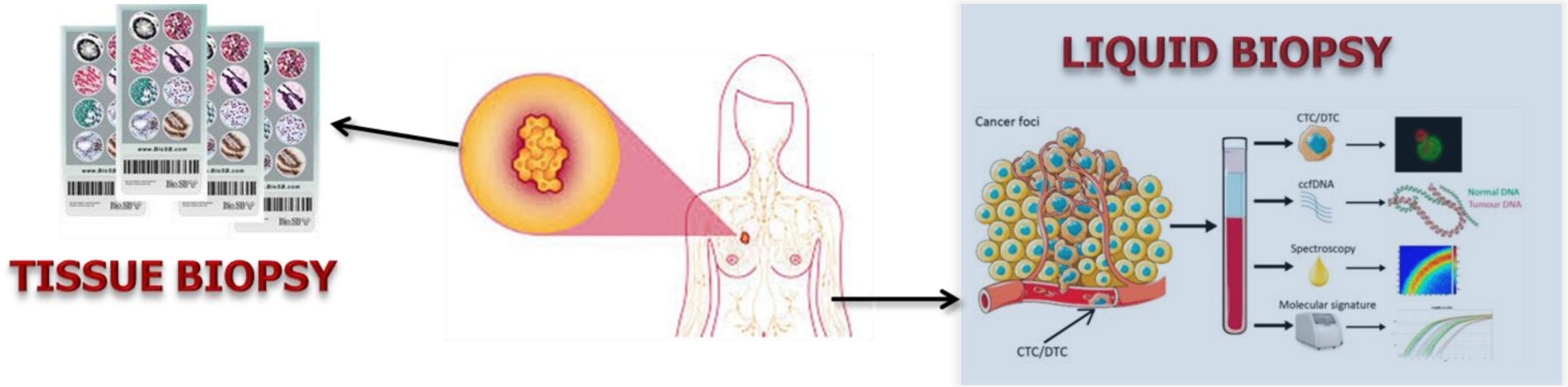
**In BC setting, several potential applications of the «liquid biopsy» have emerged, including prognostic stratification of patients and monitoring of treatment response. More recently, the potential applicability of liquid biopsy to the study of BC organotropism has also emerged.**





# - AIM OF THE STUDY -

To identify a specific «OSTEOTROPISM» gene signature  
in CTCs isolated from metastatic BC patients



# - METHODS -



**Peripheral blood (15 ml)  
from 40 stage IV BC patients**

INCLUSION criteria: ≥18 years; metastatic BC;  
treatment-naïve condition or disease progression.

EXCLUSION criteria: other synchronous or metachronous  
malignancies; systemic anti-cancer treatment in the last 21 days.



**Immune-magnetic selection**  
(anti-CD45 and anti-glycophorin microbeads)



**CTC isolation by DEPArray**

Positive selection: Epithelial markers (FITC) –  
Mesenchymal markers (PE)  
Negative selection: CD45, CD31, CD34 (APC)



**Targeted RNAseq**  
and correlation with clinical data

**Confirmation of BC-origin by  
targeted NGS-mutational analysis**

Ion AmpliSeq™ Cancer Hotspot Panel v2



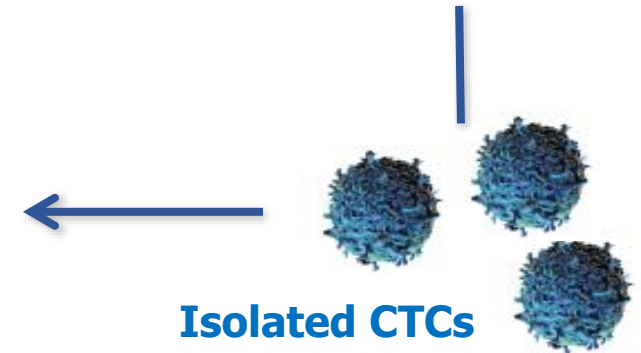
**FFPE BC samples**



**CTCs**



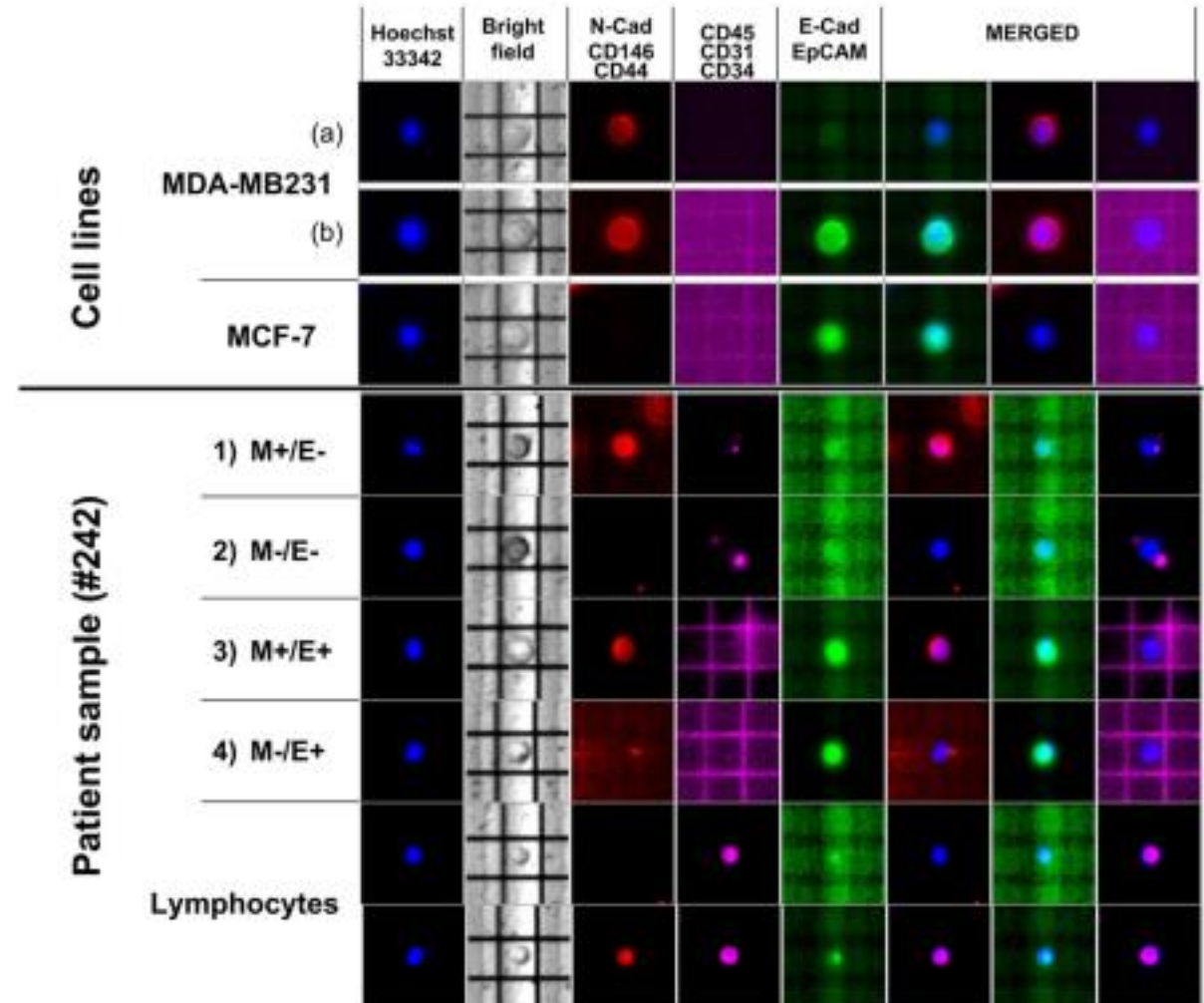
**WBCs**



**Isolated CTCs**

# - RESULTS -

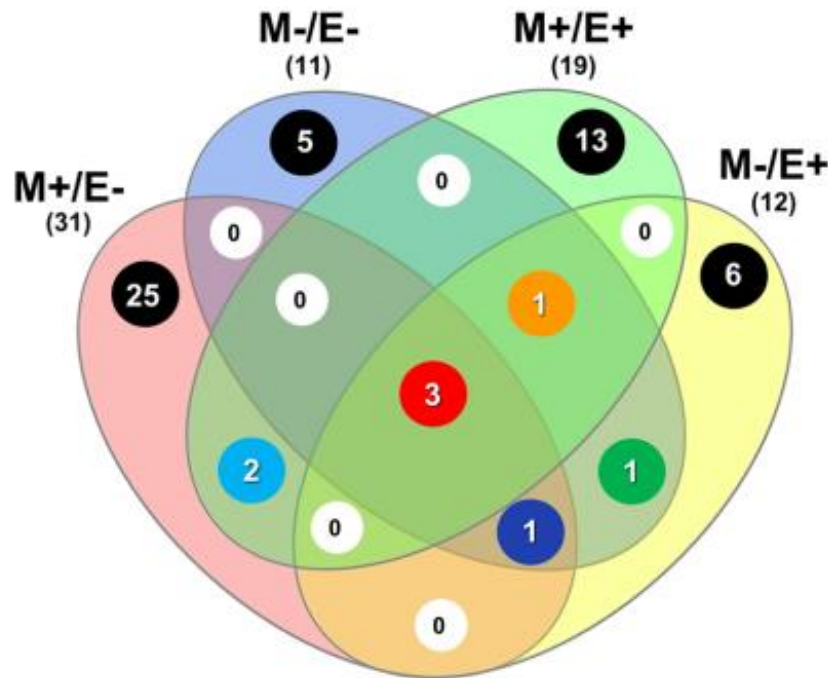
- CTCs were successfully isolated from the peripheral blood of all enrolled patients.
- CTCs exhibited phenotypical heterogeneity, in terms of epithelial and mesenchymal marker expression.
- Four phenotypes were identified, namely M+/E-, M-/E-, M+/E+, M-/E+, based on the expression of the abovementioned markers.





# - RESULTS -

- Pathogenic gene variants in major BC-related oncogenes and tumor suppressor genes were found in all the subsets of isolated CTCs, confirming their tumor origin.
- Some of them were shared with the primary tumor, while others were found only in one or more CTC subgroups, supporting the theory of cancer heterogeneity.



Shared pathogenic variants	CTC subsets			
	M+/E-	M-/E-	M+/E+	M-/E+
<b>ATM</b> c.1810 C>T	+	+	+	+
<b>FGFR3</b> c.1150 T>C	+	+	+	+
<b>TP53</b> c.388 C>A	+	+	+	+
<b>PIK3CA</b> c.3140 A>G	+	-	+	-
<b>PIK3CA</b> c.3196 G>A	+	-	+	-
<b>PIK3CA</b> c.1633 G>A	+	+	-	+
<b>PIK3CA</b> c.3140 A>T	-	+	-	+
<b>TP53</b> c.742 C>T	-	+	+	+

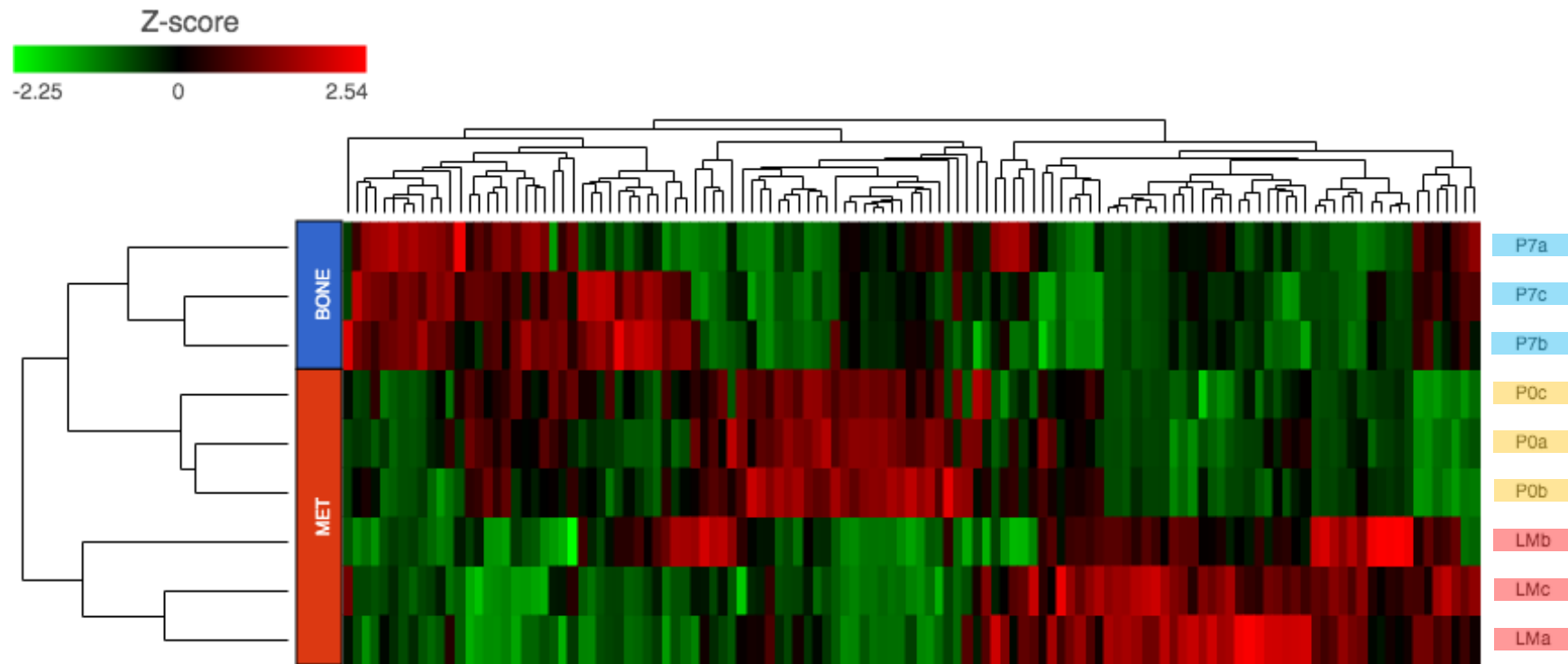
## Exclusive pathogenic variants

CTC subsets	<b>M+/E-</b>	APC c.4348 C>T; AKT1 c.528 C>A; ATM c.3878 A>G; ATM c.7328 G>A; BRAF c.1379 G>T; ERBB4 c.866 G>T; FBXW7 c.1442 C>T; GNAQ c.735+1 G>T; KDR c.2946 C>T; KIT c.1904 A>G; KRAS c.153 T>C; MET c.1124 A>G; MET c.2962 C>T; NOTCH1 c.5026 G>A; PIK3CA c.323 G>A; PTEN c.511 C>T; PTEN c.635-1G>A; PTEN c.991 G>A; PTEN c.1001 A>G; PTPN11 c.169 C>T; SMAD4 c.512 C>A; SMO c.1180 T>C; SMAD4 c.1216 G>A; STK11 c.536 C>T; TP53 c.667 C>T
	<b>M-/E-</b>	ATM c.5415 G>A; BRAF c.1800 G>A; EGFR c.2257 C>T; FGFR3 c.2115 G>A; RET c.2691 A>G
	<b>M+/E+</b>	ALK c.3836+1 G>T; CDKN2A c.241 C>T; CTNNB1 c.136 C>T; FBXW7 c.1451 G>T; FGFR3 c.2408 G>A; HNF1A c.620 G>A; KDR c.2959 G>T; NRAS c.394 G>T; PDGFRA c.1955 G>T; PIK3CA c.352+1 G>A; SMO c.646 C>T; TP53 c.713 G>A; TP53 c.1009 C>T
	<b>M-/E+</b>	EGFR c.2300 C>T; JAK3 c.2126 G>A; KRAS c.103 A>G; NRAS c.181C>A; RB1 c.1975 T>C; SMO c.1870 A>T



# - RESULTS -

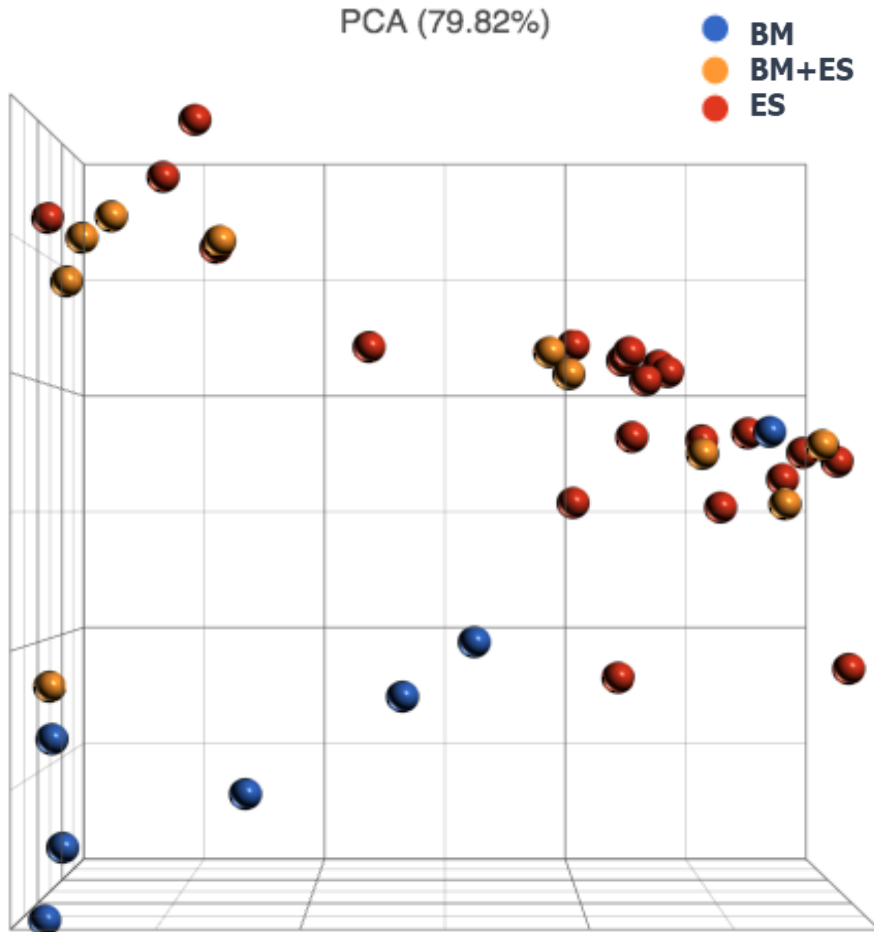
## Targeted RNA-Seq set-up on BC cell lines



PO: BC cell line without selective tissue tropism; P7: osteotropic BC cell line; LM: lung-metastatic BC cell line

# - RESULTS -

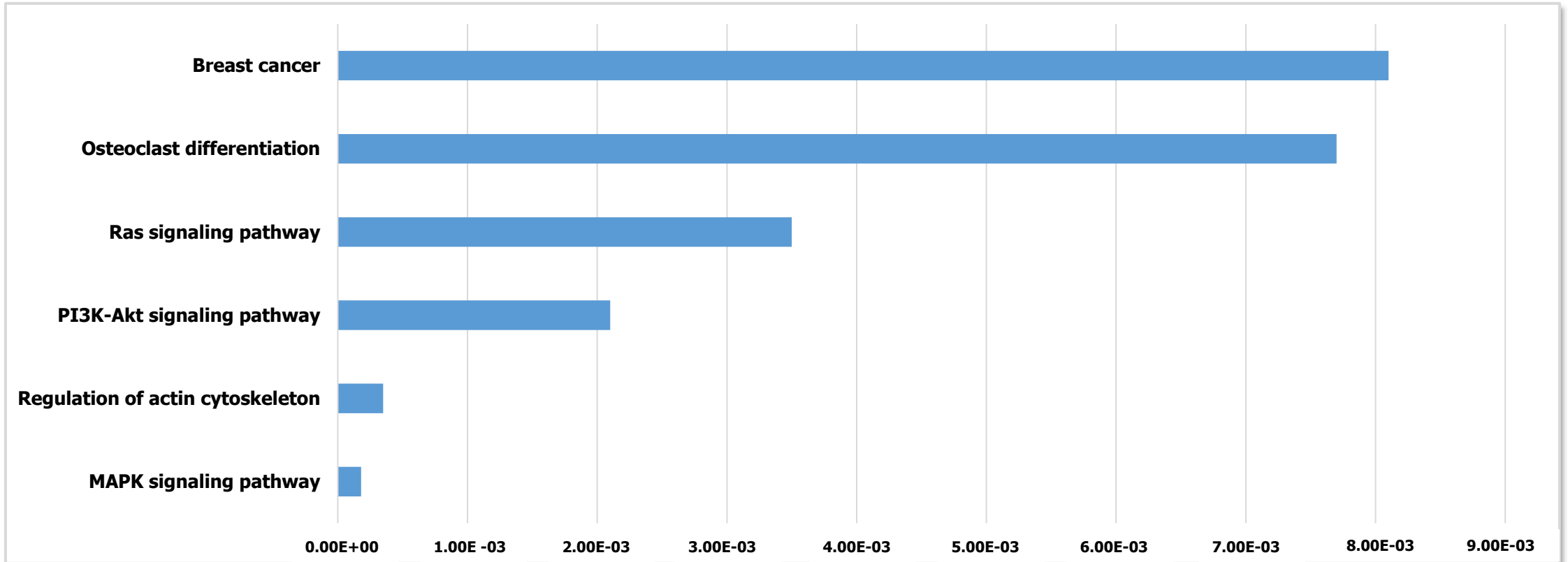
## Targeted RNA-Seq of BC CTCs



Gene symbol	Total counts	P-value	FDR step up	Fold change
<i>CAPG</i>	25167.12	1.73E-02	2.02E-01	30.79
<i>HRAS</i>	2512.24	1.34E-02	2.02E-01	11.67
<i>IL1B</i>	8545.97	2.46E-02	2.02E-01	5.37
<i>FGFR4</i>	6704.33	1.99E-02	2.02E-01	5.28
<i>MAF</i>	6006.48	2.38E-02	2.02E-01	4.39
<i>SERPINB2</i>	2163.33	2.79E-02	2.02E-01	4.38
<i>CTSK</i>	4328.66	1.91E-02	2.02E-01	4.06
<i>MAFA</i>	9498.79	2.77E-02	2.02E-01	3.92
<i>COL3A1</i>	8489.81	8.53E-03	2.02E-01	3.75
<i>TTYH1</i>	872105.32	1.37E-03	2.02E-01	3.62
<i>AURKB</i>	3179.91	3.38E-02	2.08E-01	3.48
<i>HMMR</i>	9998.47	1.66E-02	2.02E-01	2.97
<i>NAP1L3</i>	510327.99	2.59E-02	2.02E-01	2.88
<i>EPHB3</i>	2214.79	3.04E-02	2.02E-01	2.82
<i>SYNM</i>	32105.67	5.47E-02	2.64E-01	2.74
<i>GIPC1</i>	5979.75	3.06E-02	2.02E-01	2.43
<i>RERGL</i>	1011.85	3.03E-02	2.02E-01	2.38
<i>ITGB4</i>	1230.12	3.38E-02	2.08E-01	2.34
<i>PRDX1</i>	19180.03	2.23E-02	2.02E-01	2.25
<i>ST3GAL1</i>	954.23	8.12E-03	2.02E-01	2.20
<i>MEF2C</i>	2094.35	3.79E-02	2.24E-01	2.19
<i>DKK1</i>	2432.94	5.46E-02	2.64E-01	2.18
<i>MAPK1</i>	11070.76	4.30E-02	2.32E-01	2.17
<i>FGF5</i>	15117.97	3.01E-02	2.02E-01	2.15
<i>SOX9</i>	96130.9	4.92E-02	2.57E-01	2.04
<i>FGFR3</i>	7484.52	1.30E-02	2.02E-01	-2.75
<i>HPRT1</i>	2456.14	1.24E-02	2.02E-01	-2.79
<i>SMAD2</i>	46948.61	2.62E-02	2.02E-01	-2.86
<i>HMGA2</i>	13845.8	2.33E-03	2.02E-01	-4.11
<i>MCM2</i>	52952.37	3.91E-02	2.24E-01	-4.56
<i>ANLN</i>	383828.91	9.02E-03	2.02E-01	-12.02

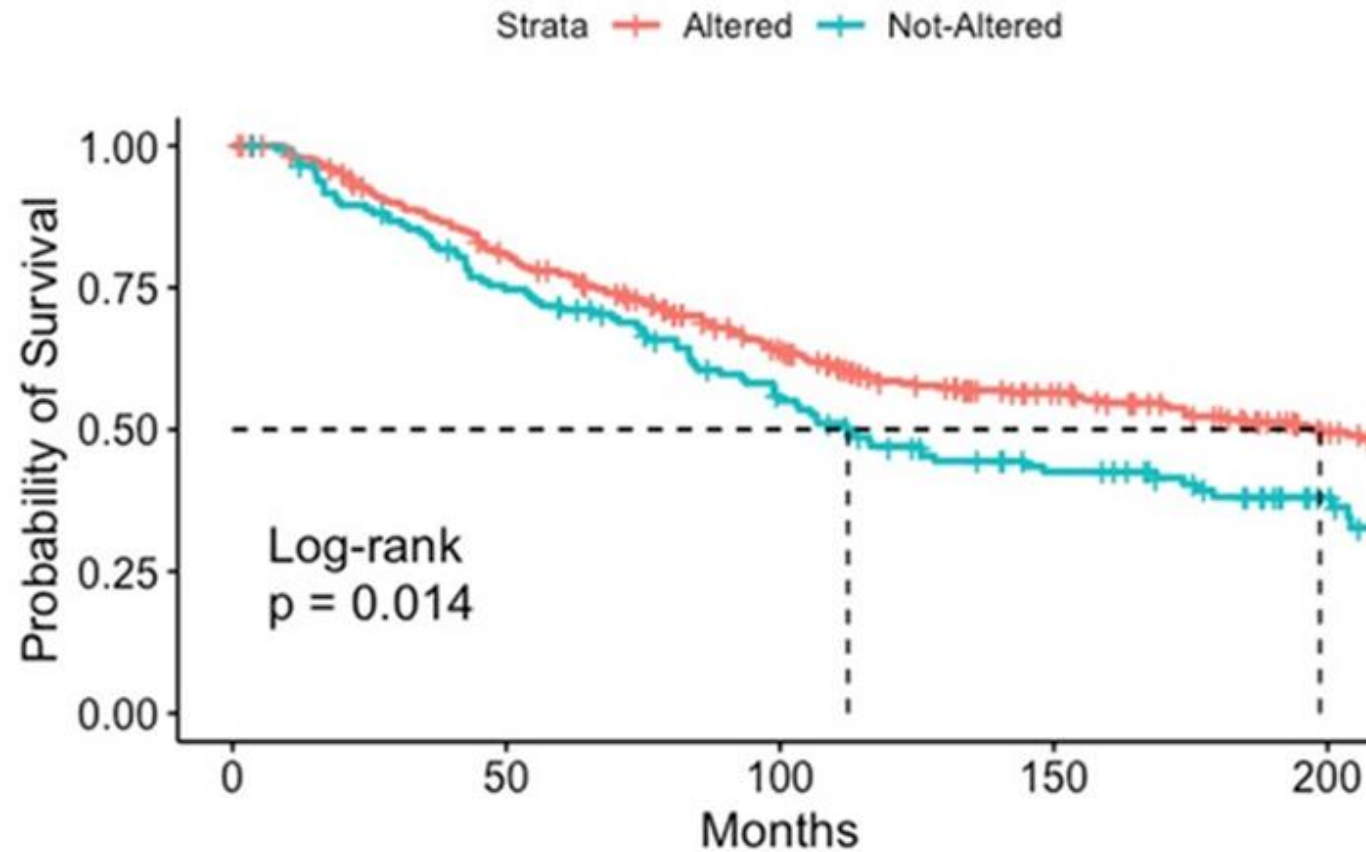
# - RESULTS -

## KEGG enrichment analysis of the significantly up-regulated genes in "BM" CTCs



# - RESULTS -

## Prognostic meaning of «top10» DEGs (METABRIC dataset)









*Thank You!*

# **- ACKNOWLEDGEMENTS -**



**UNIVERSITÀ  
DEGLI STUDI DI BARI  
ALDO MORO**

**C. Porta  
F. Silvestris**

**P. Cafforio  
E. Lauricella  
D. Lovero  
R. Palmirotta**



**The  
University  
Of  
Sheffield.**

**J.E. Brown  
R.E. Coleman**

**M. Oliva  
S. Woods**