



**UNIVERSITÀ  
DEGLI STUDI  
DI UDINE**

**The University of Udine - Department of Medicine  
S.M. Misericordia University Hospital - Department of Oncology  
School of Medical Oncology**

---

# **Dissecting the needle in the haystack**

**Characterizing Circulating tumor cells in Breast Cancer**

**Breast Journal Club 2017 - 10.03.2017**

**Lorenzo Gerratana**

### Is it time to rethink the entire paradigm?

- A snapshot could be not worth the effort

Cristofanilli M et al. NEJM, 351(8), 781–791  
Smerage, J. B et al. J Clin Oncol, 32(31), 1–8

### Its time to scrape under the surface

- From counting to characterizing

Mostert B, et al. Annals of Oncology, 26(3), 510–516  
Bulfoni M, Gerratana L, Del Ben F, et al. Breast Cancer Res. 2016;18

### The hidden side of the results

- Insights from unpublished data

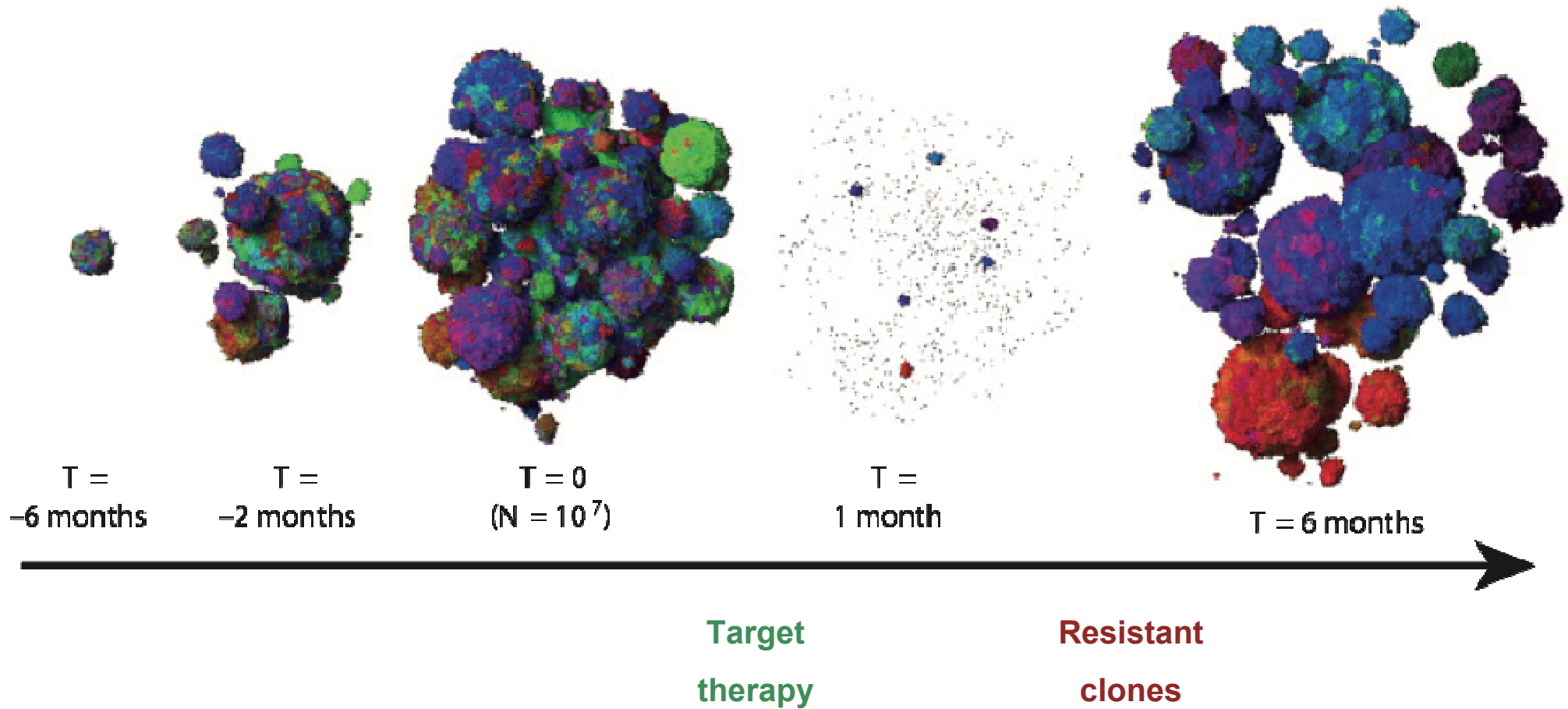
### Future perspectives

- Why are these informations useful

Guo S, Diep D, Plongthongkum N, et al. Nat Genet. 2017  
Shaw JA, Guttery DS, Hills A, et al. Clin Cancer Res 2017 Jan 1;23(1):88–96

# Is it time to rethink the entire paradigm?

A snapshot could be not worth the effort



# A long time ago in a galaxy far, far away

---

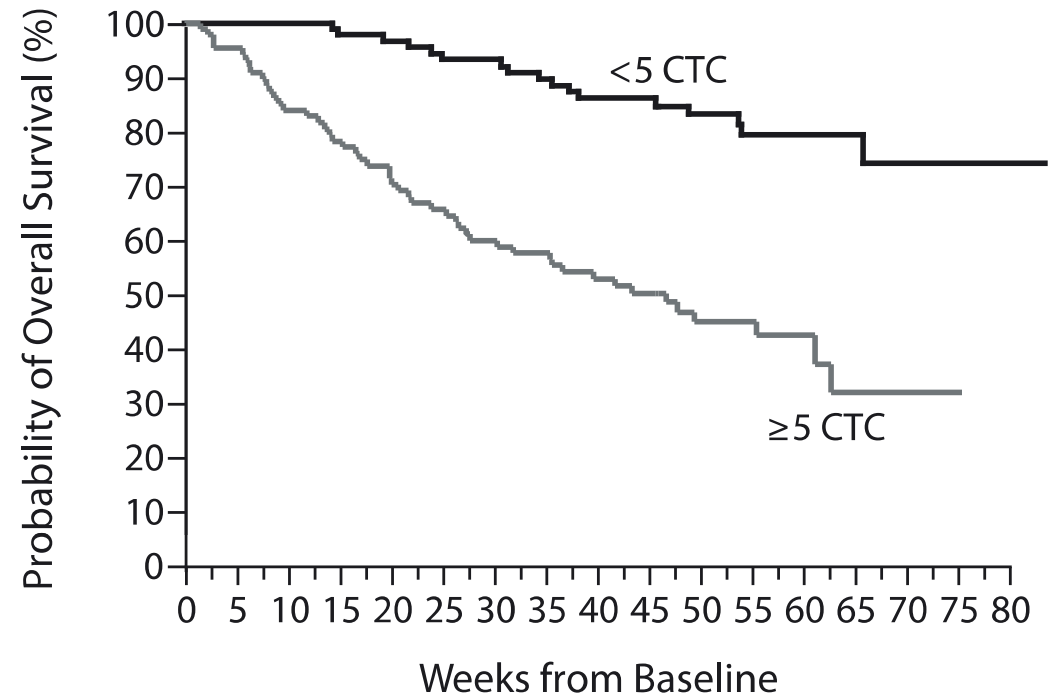
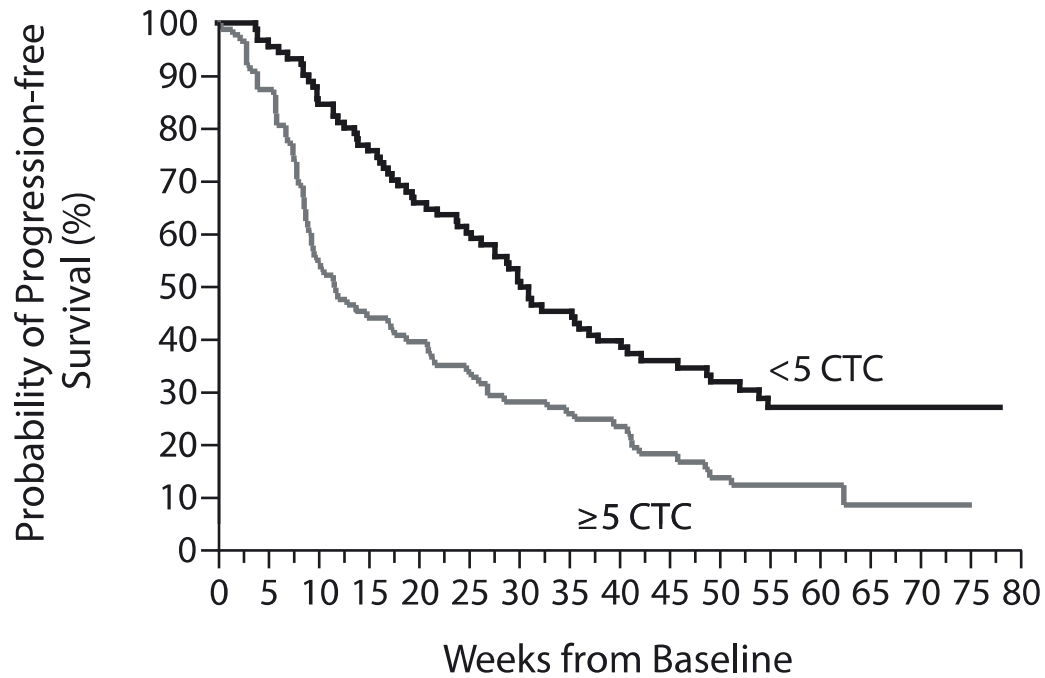
Sometimes novelties are not so new

In 1869 T.R. Ashworth described cells in the blood of a woman with metastatic breast cancer that were similar in to her primary tumor cells



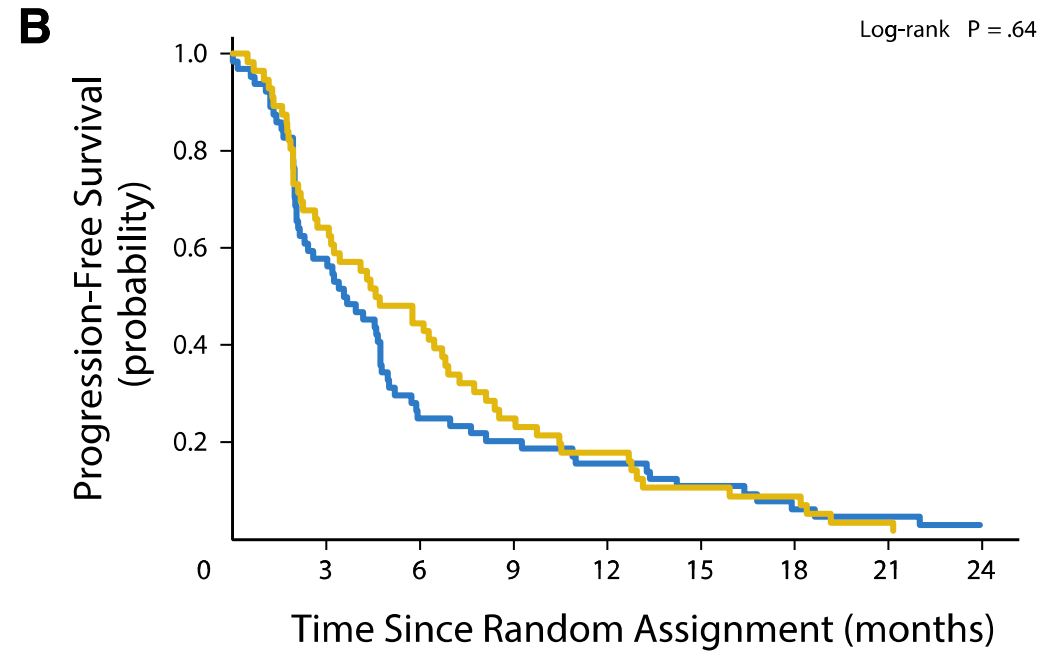
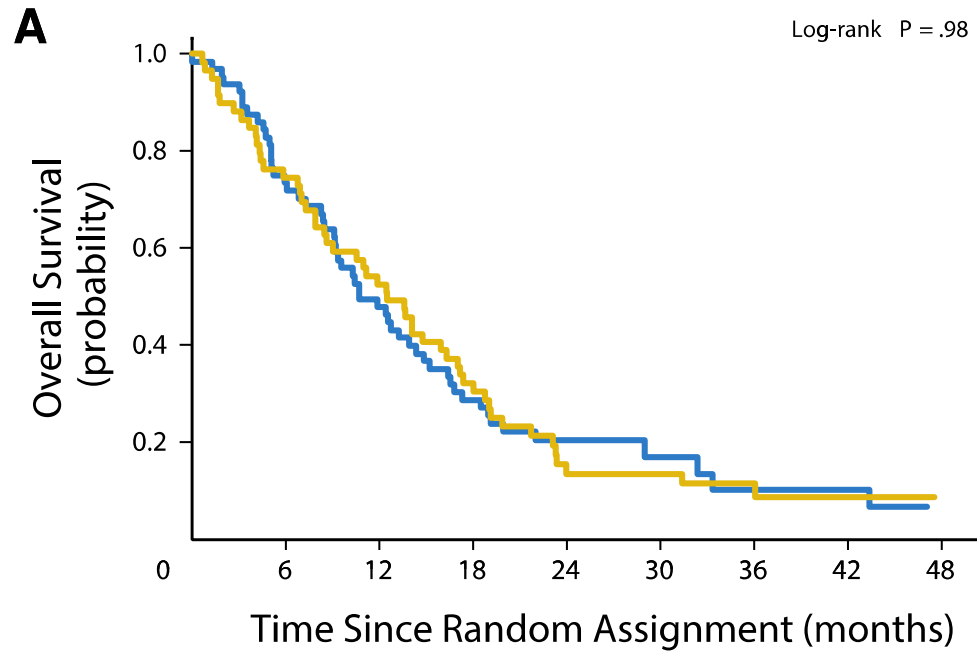
# CTC and prognosis in breast cancer

Where it all began



# The SWOG S0500 trial

## CTCs only as a prognostic factor?

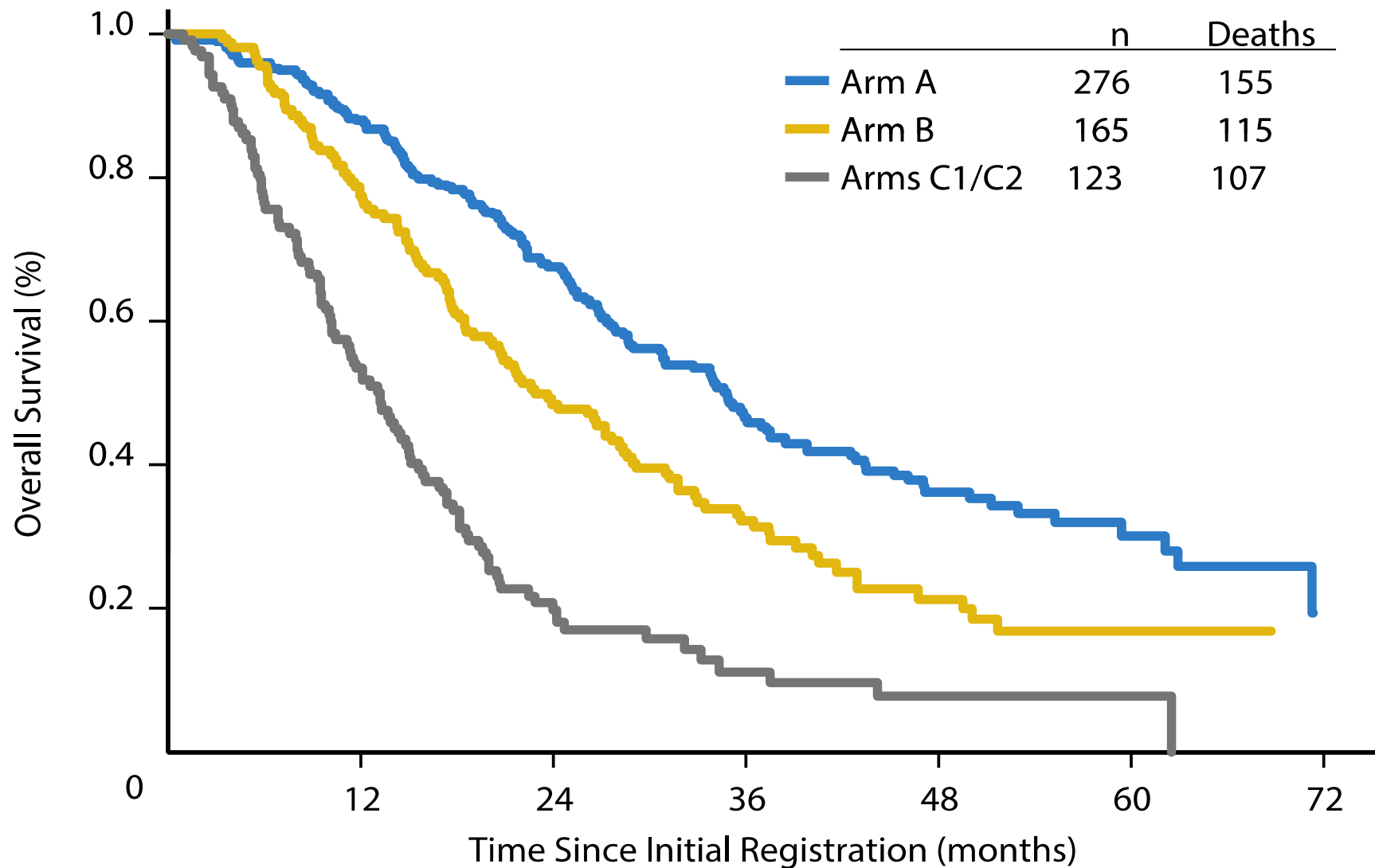


— Arm C1: Maintain therapy    — Arm C2: Change therapy

**Should we throw everything away?**

# The SWOG S0500 trial

CTCs only as a prognostic factor?

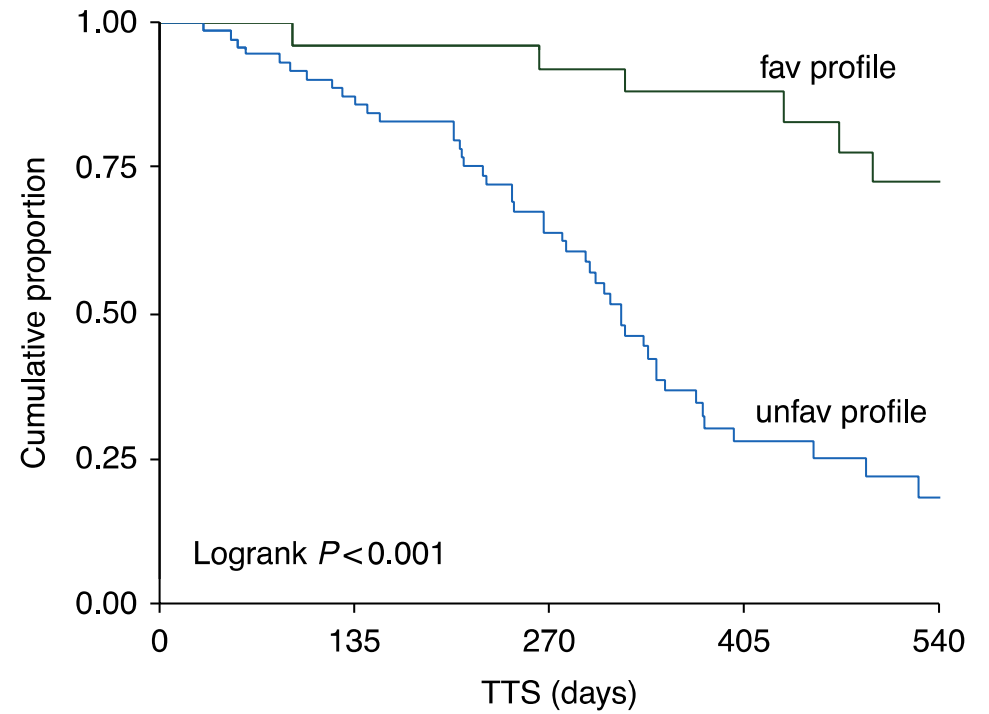
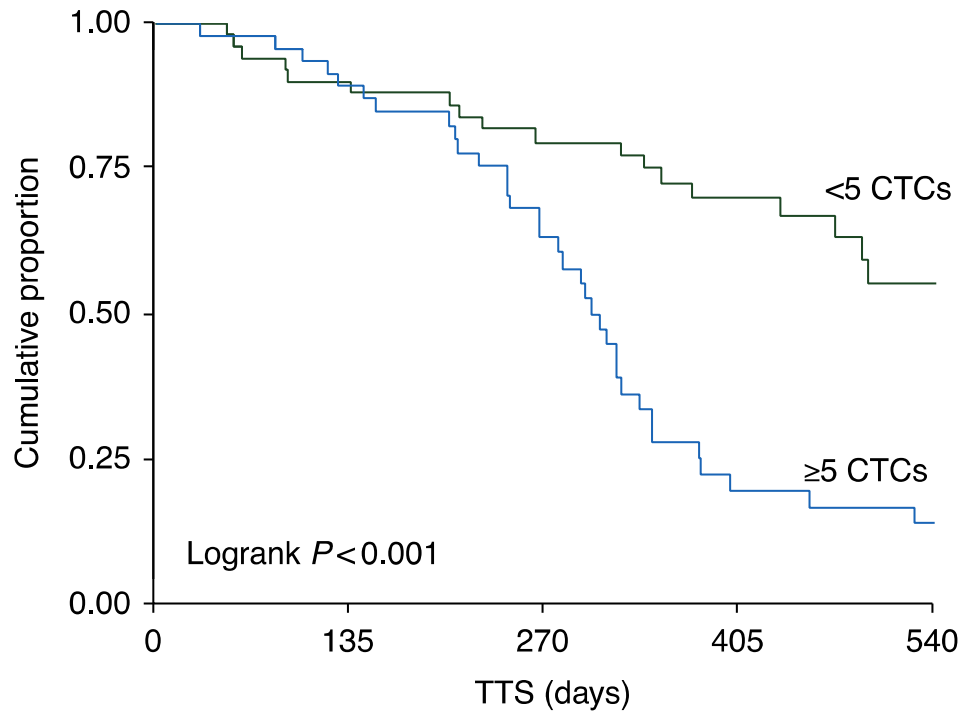




**Time to change the paradigm**

# Its time to scrape under the surface

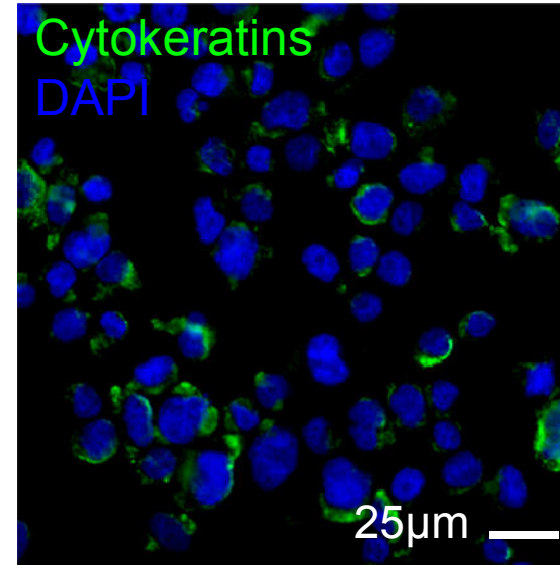
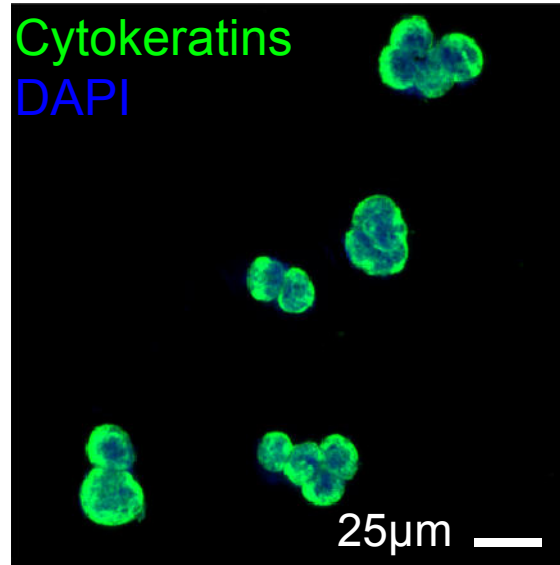
## From counting to characterizing



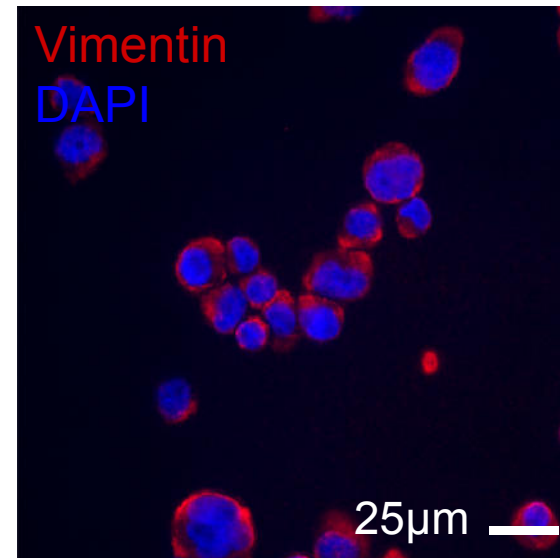
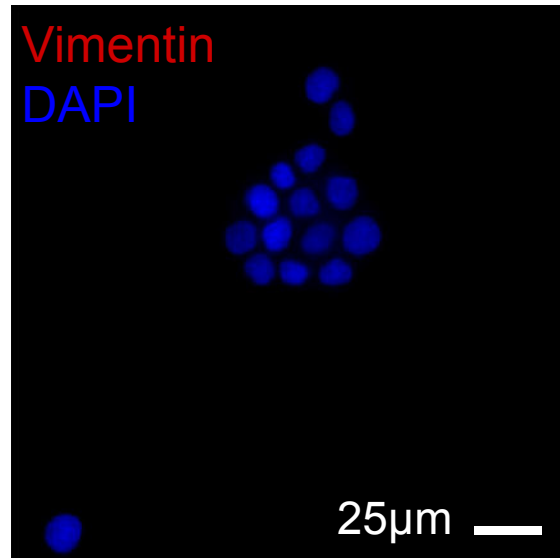
# Knowing the enemy it's the first step

## Membrane markers selection

**MCF-7**

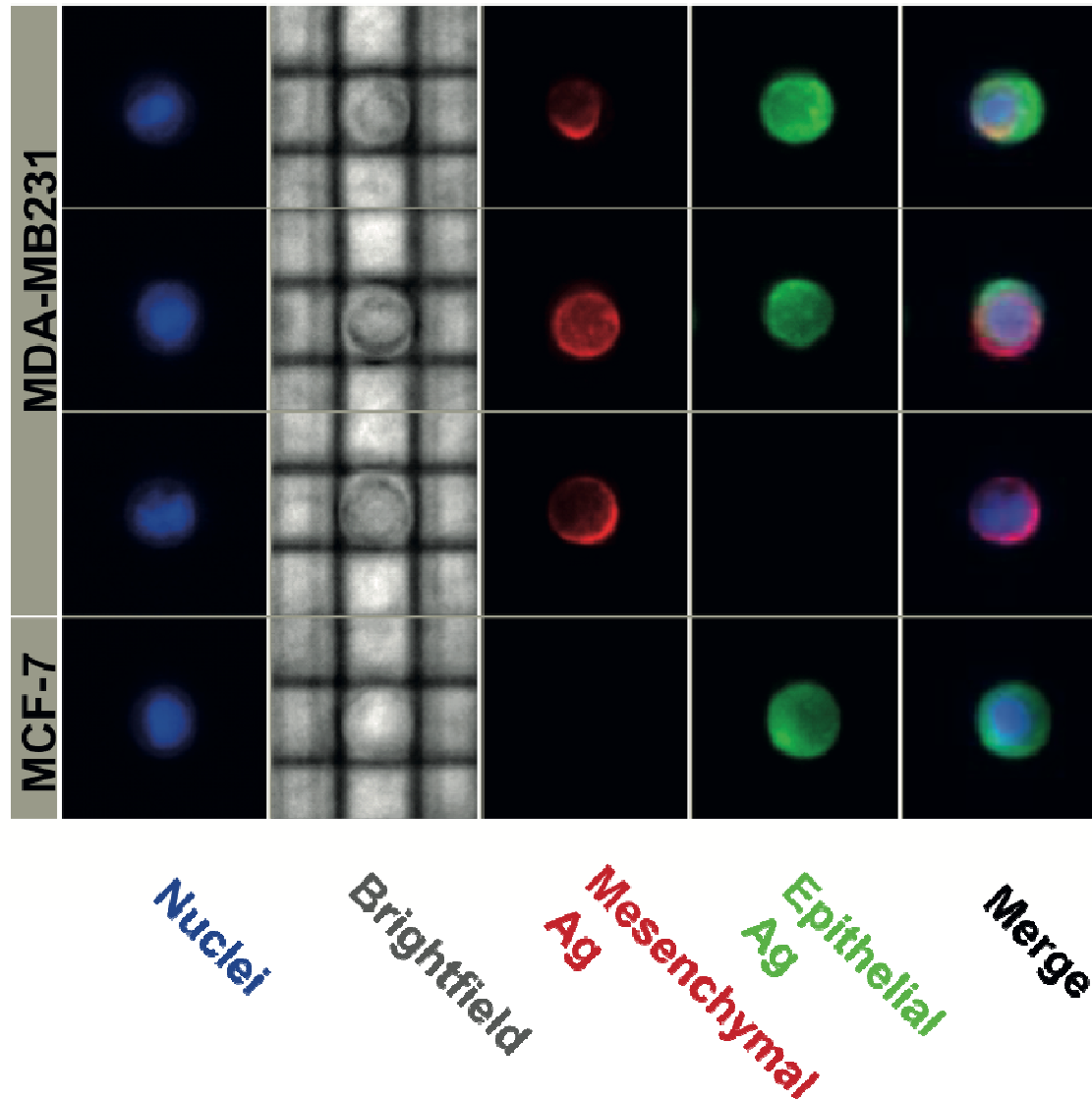


**MDA-MB231**



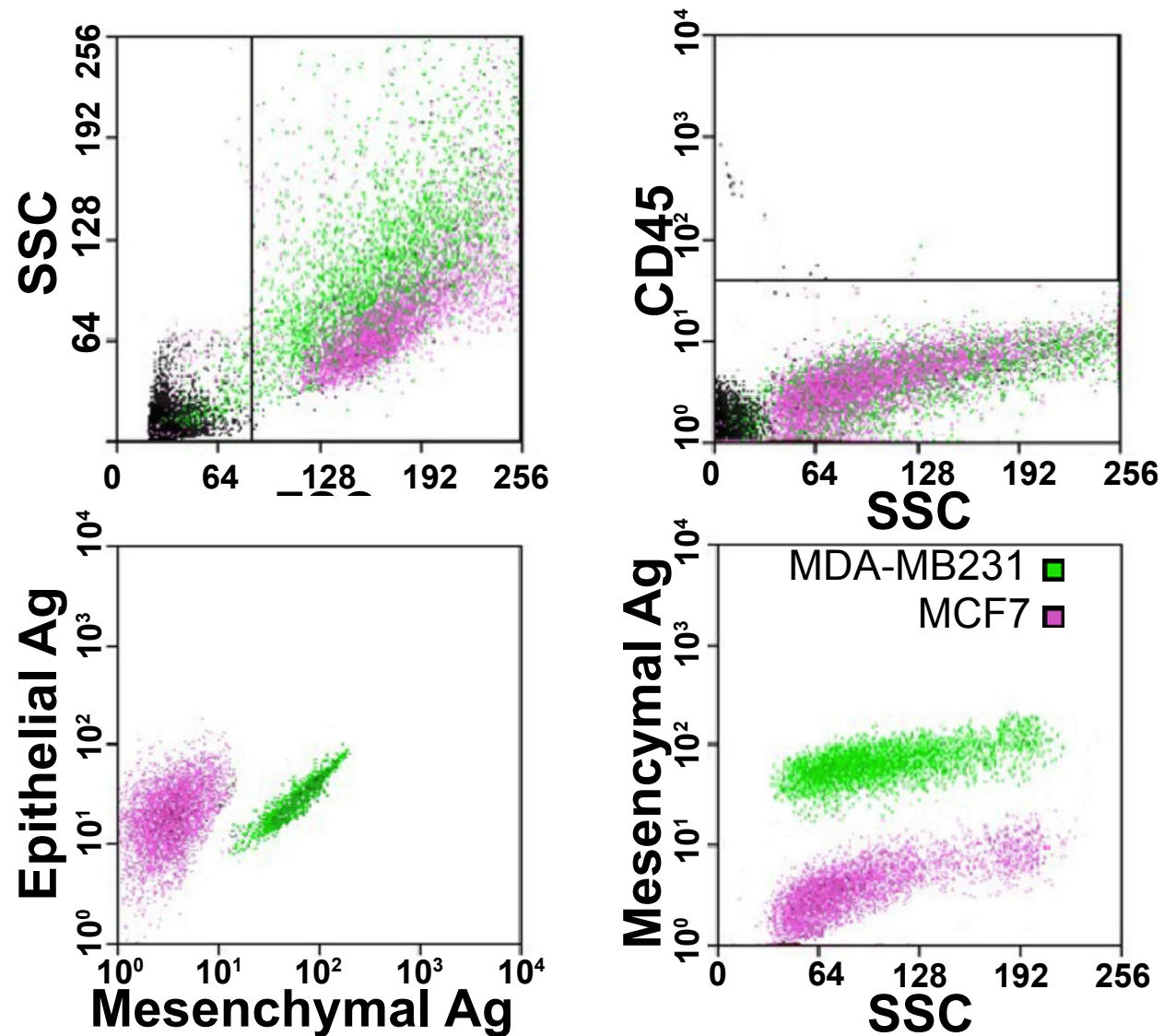
# Knowing the enemy it's the first step

## Membrane markers selection



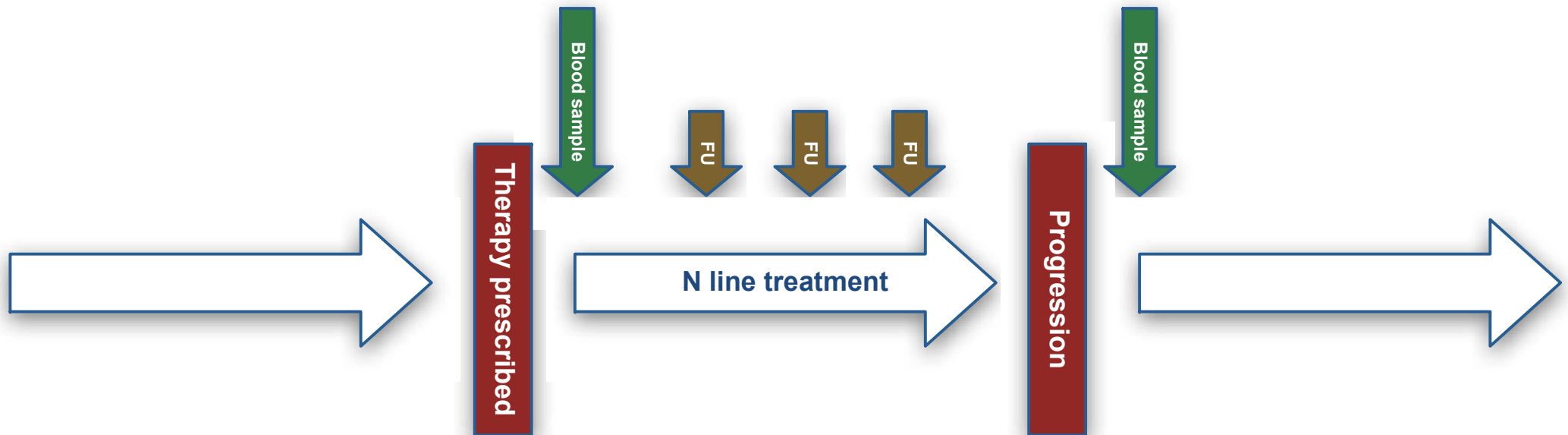
# Knowing the enemy it's the first step

## Membrane markers selection



# Its time to scrape under the surface

From counting to characterizing



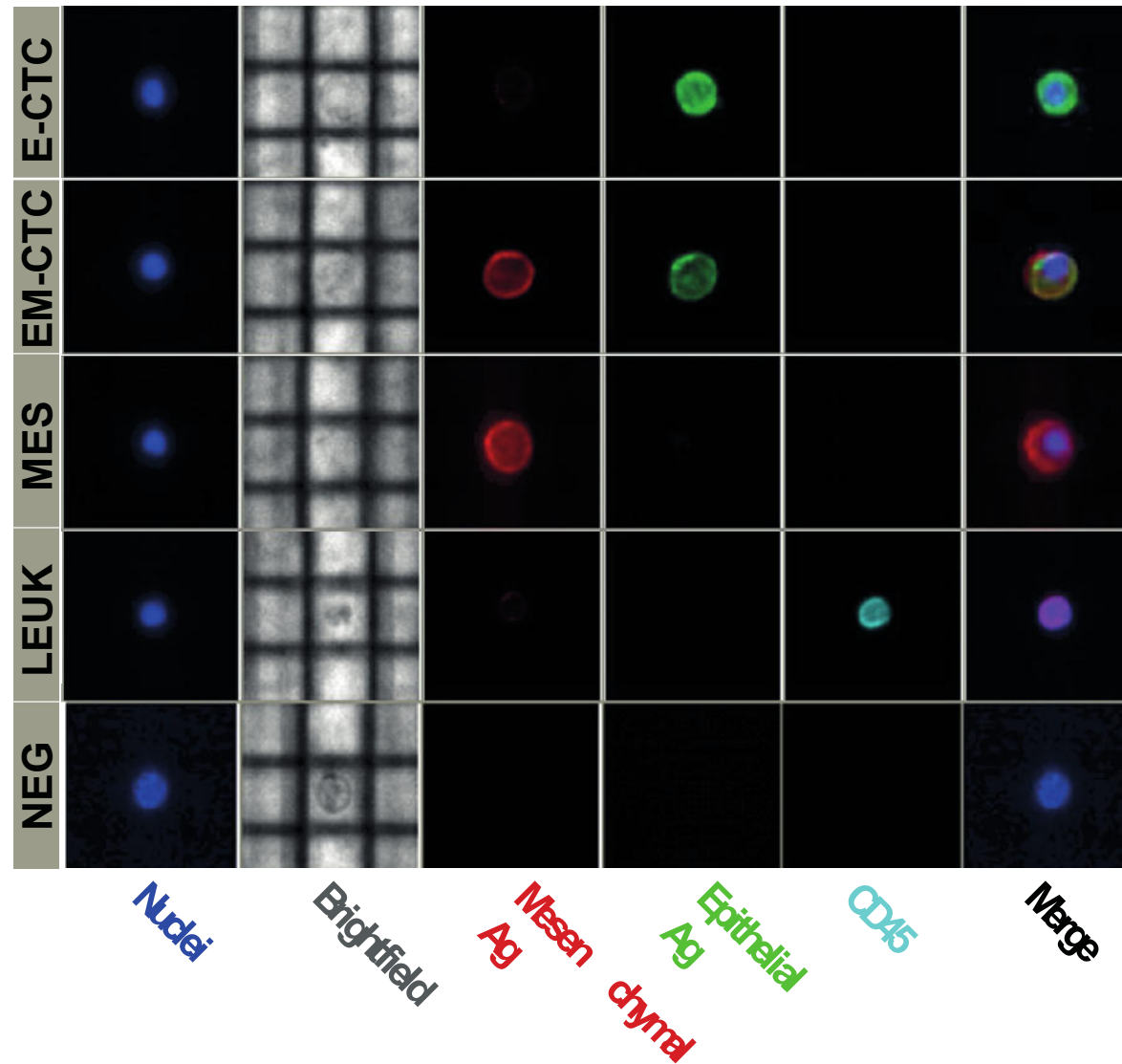
56 MBC patients regardless the line of treatment, median follow-up was 33 months, median PFS was 8.12 months.

Cell sorting experiments were performed by DEPArray

The sample was injected by the system into a microchamber consisting of 16,000 electrical cages in which individual cells are trapped.

# Knowing the enemy it's the first step

## CTC selection



# Its time to scrape under the surface

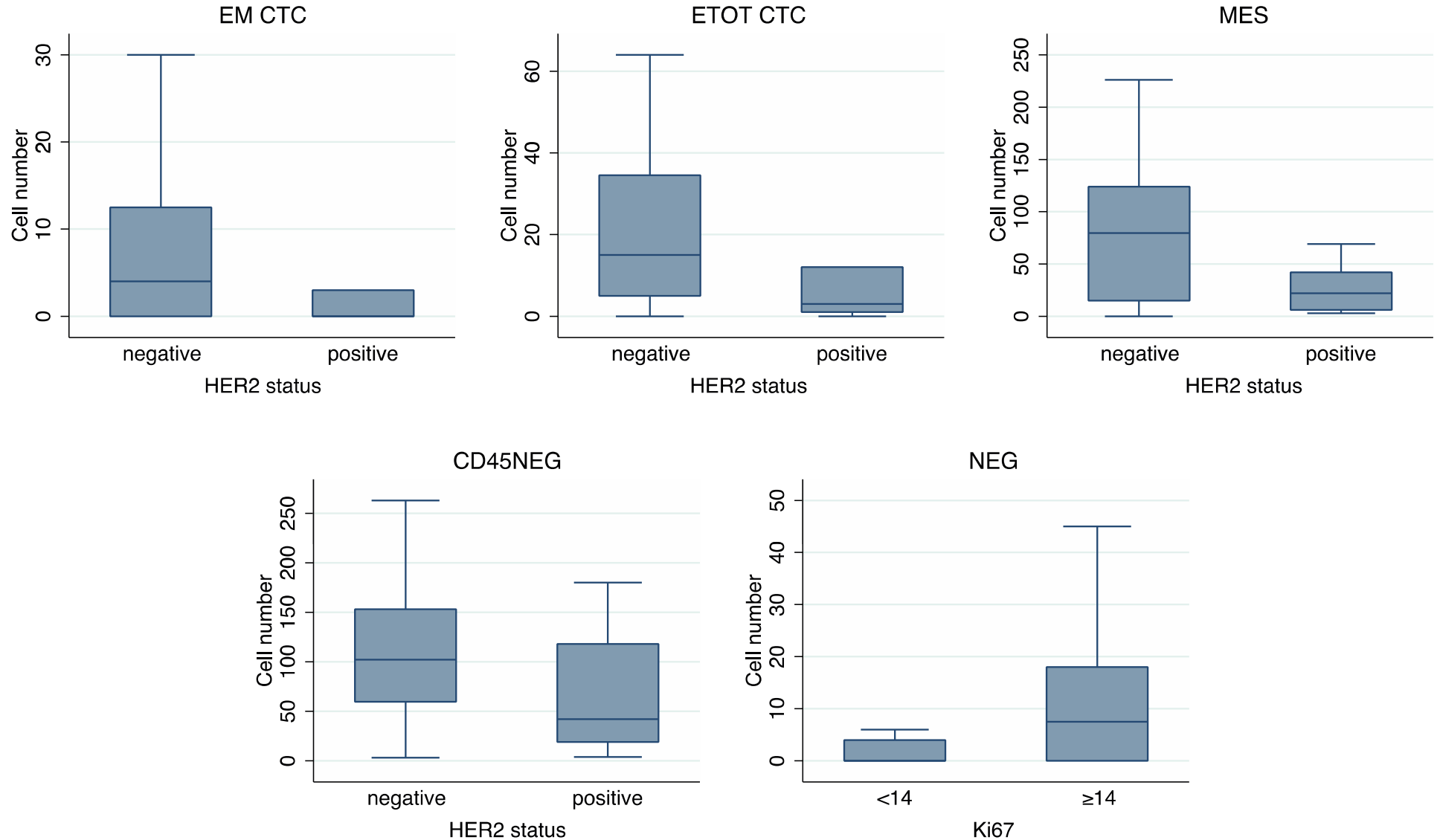
## Patients' characteristics

Characteristic	N (%)
ECOG performance status; n (%)	
0	29 (62%)
1	14 (29%)
2	4 (9%)
Immunophenotype; n (%)	
Luminal-like	25 (54%)
HER2 positive	11 (23%)
Triple Negative	11 (23%)
Histotype; n (%)	
Ductal	38 (84%)
Lobular	7 (16%)
Metastatic sites*; n (%)	
Bone	29 (62%)
Liver	22 (47%)
Lung	16 (34%)
CNS	3 (6%)
Number of metastatic sites	
1	16 (34%)
≥ 2	31 (66%)



# Realists do not fear the results of their study

## CTC subpopulations and pathological characteristics



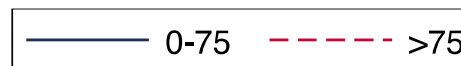
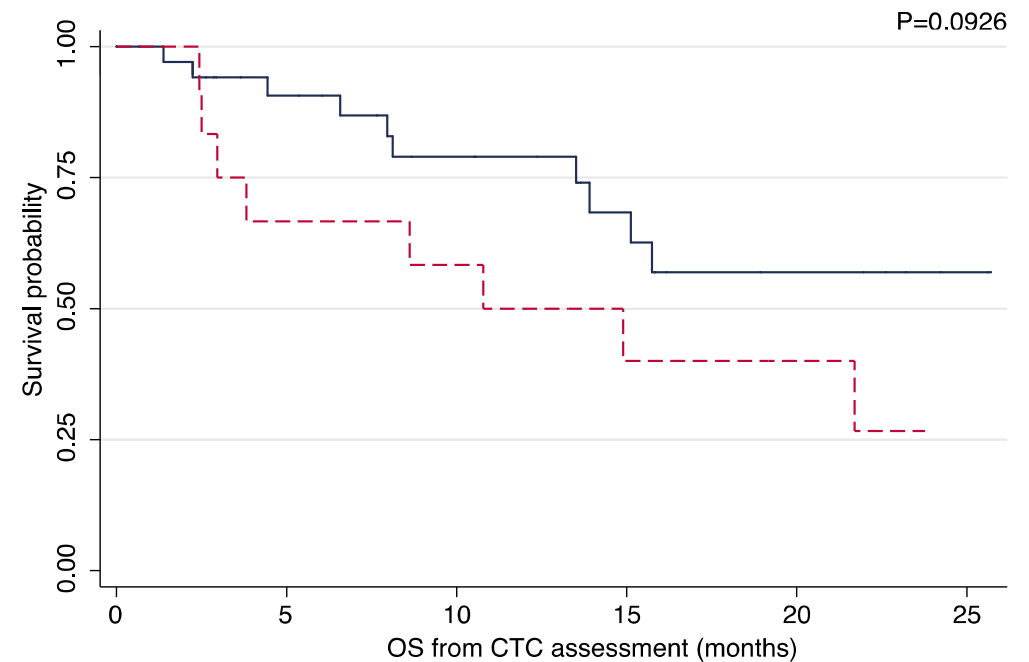
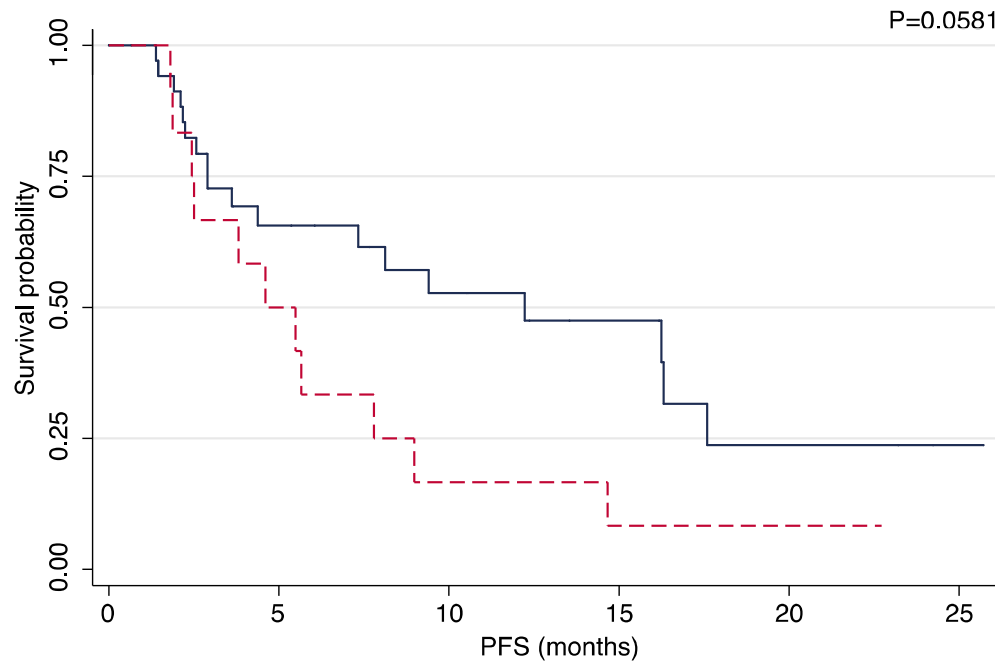
# Realists do not fear the results of their study

## CTC subpopulations and prognosis

Variable	OS (from stage IV diagnosis)			PFS		
	HR	95% CI	p	HR	95% CI	p
E CTC (No.)	1.003	0.982-1.024	0.79	1.004	0.989-1.020	0.6
EM CTC (No.)	1.018	0.994-1.043	0.13	1.020	0.998-1.043	0.81
E <sub>TOT</sub> (No.)	1.006	0.993-1.019	0.35	1.007	0.996-1.018	0.24
MES (No.)	0.998	0.991-1.005	0.56	0.998	0.992-1.003	0.41
NEG (No.)	0.998	0.981-1.015	0.79	0.997	0.986-1.008	0.58
CD45 <sup>NEG</sup> (No.)	0.999	0.994-1.004	0.79	0.999	0.995-1.003	0.56
EM CTC / CD45 <sup>NEG</sup> (%)	1.022	1.003-1.042	0.022	1.021	1.004-1.039	0.016
E <sub>TOT</sub> / CD45 <sup>NEG</sup> (%)	1.019	1.004-1.034	0.015	1.010	0.997-1.023	0.12
MES / CD45 <sup>NEG</sup> (%)	0.984	0.968-0.999	0.037	0.993	0.981-1.005	0.26
NEG / CD45 <sup>NEG</sup> (%)	0.999	0.972-1.027	0.95	0.998	0.980-1.017	0.86

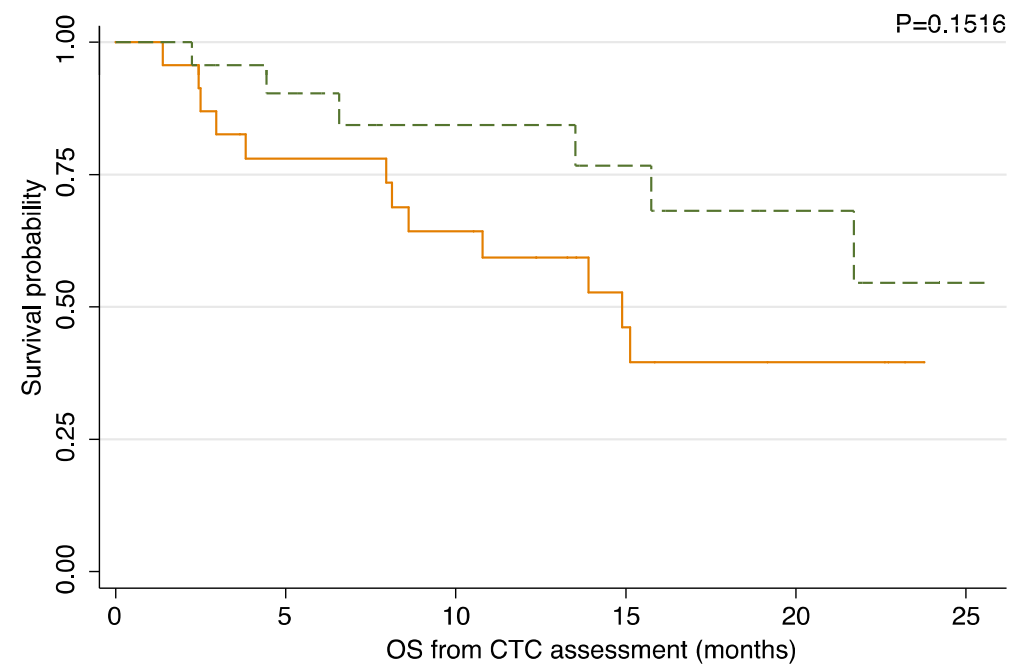
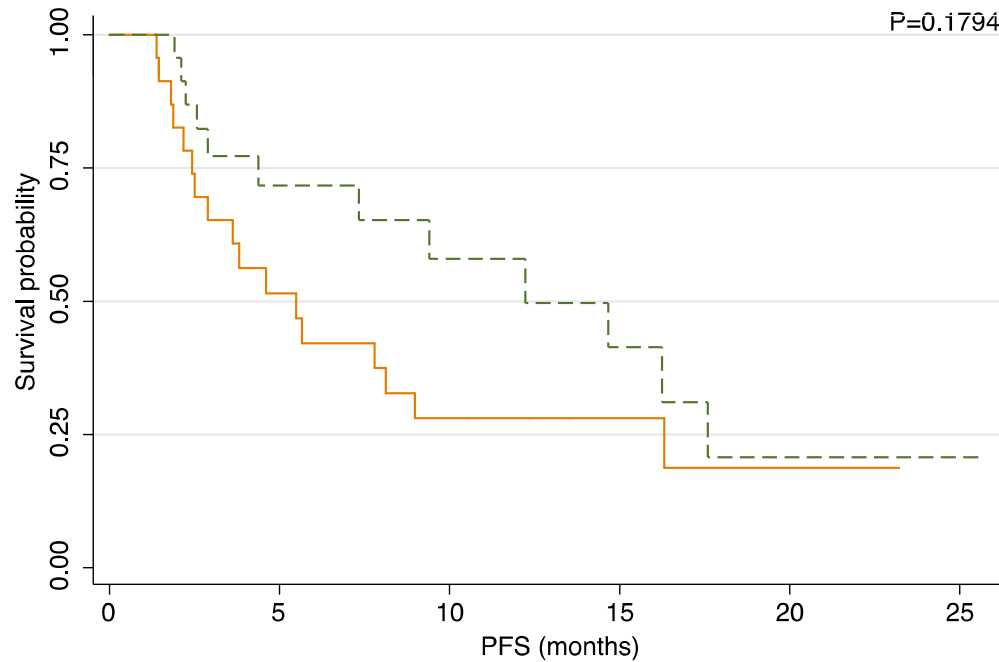
# Realists do not fear the results of their study

## CTC subpopulations and prognosis



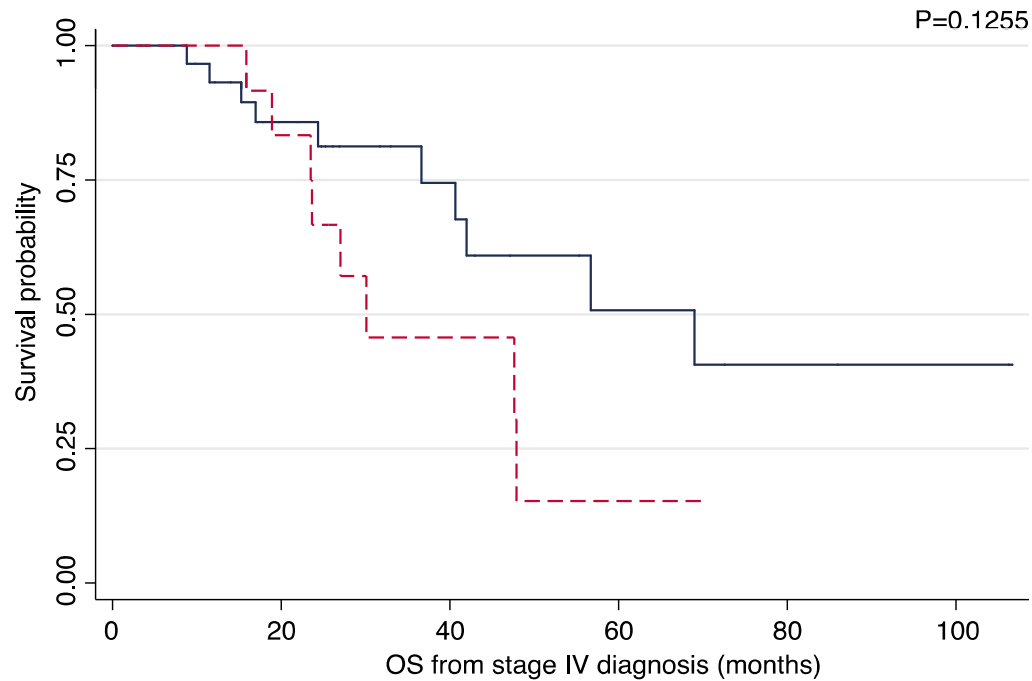
# Realists do not fear the results of their study

## CTC subpopulations and prognosis

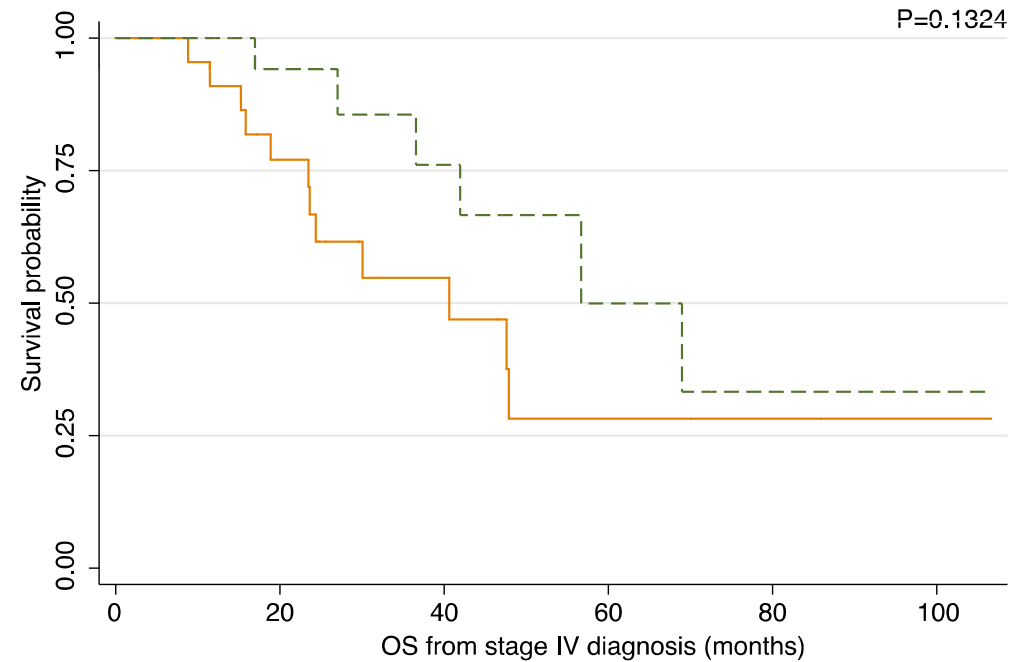


# Realists do not fear the results of their study

## CTC subpopulations and prognosis



— 0-75    - - - >75

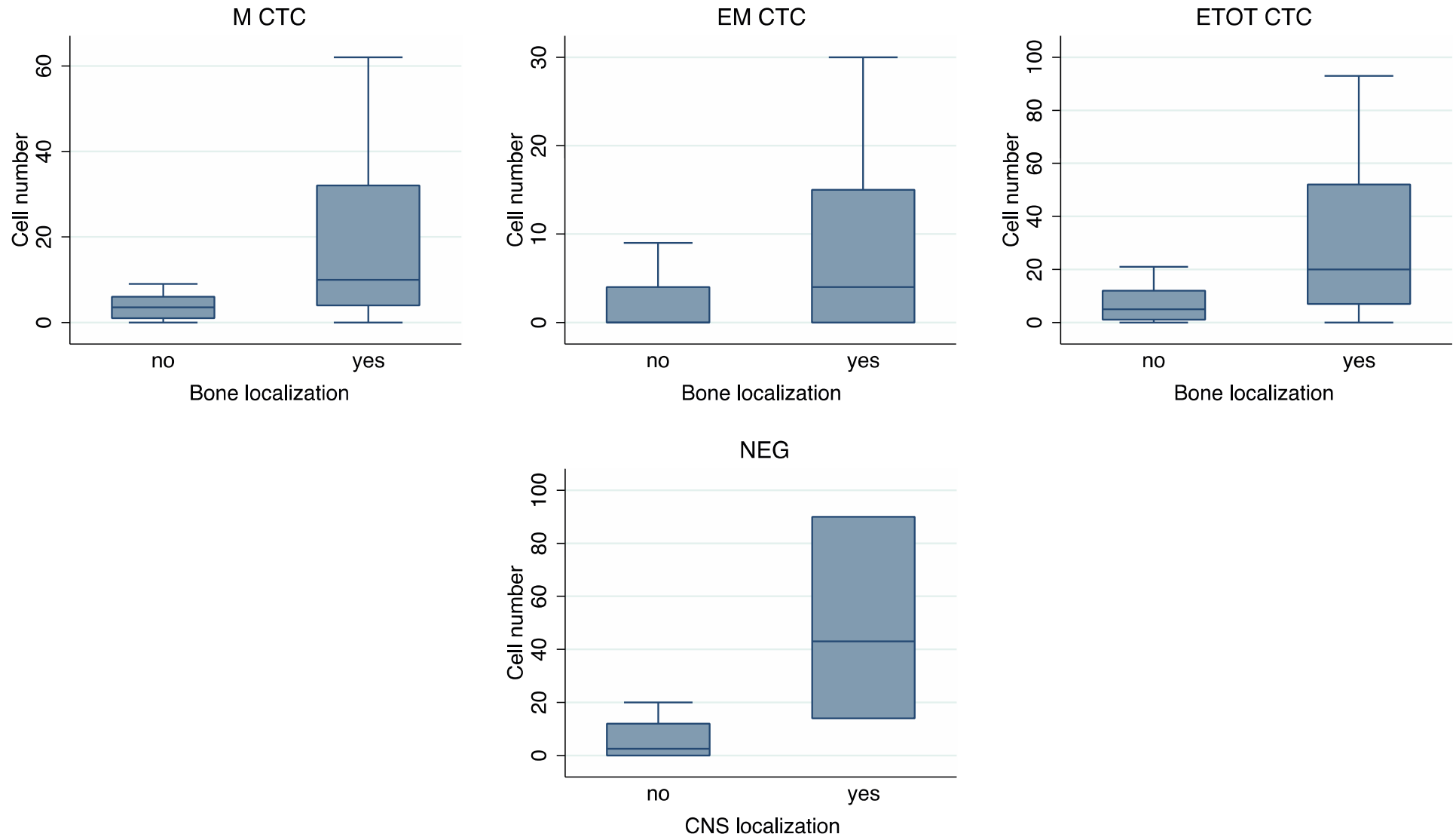


— 0-50    - - - 50-75

**But that's not all**

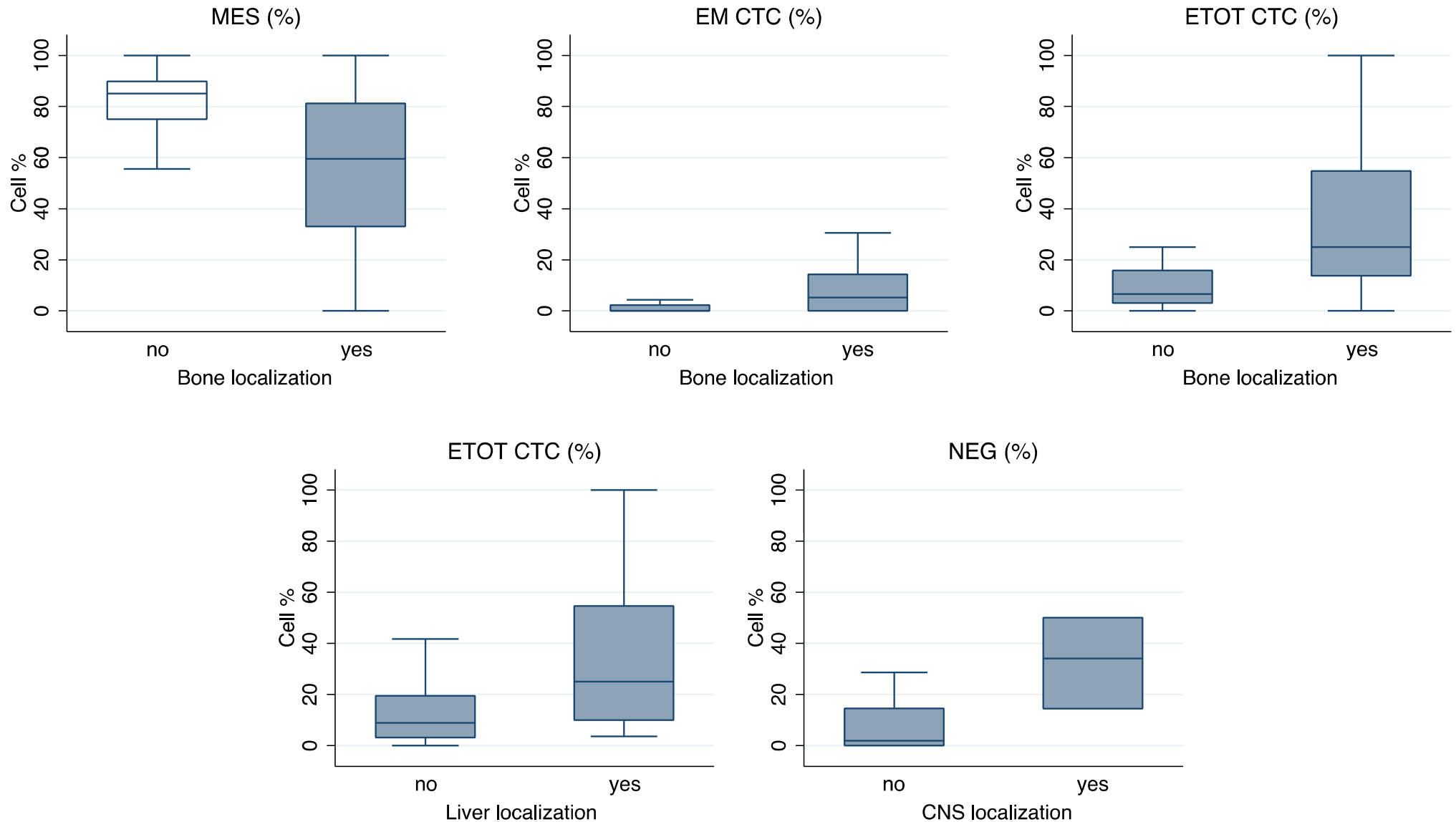
# Realists do not fear the results of their study

## CTC subpopulations and metastatization pattern



# Realists do not fear the results of their study

## CTC subpopulations and metastatization pattern

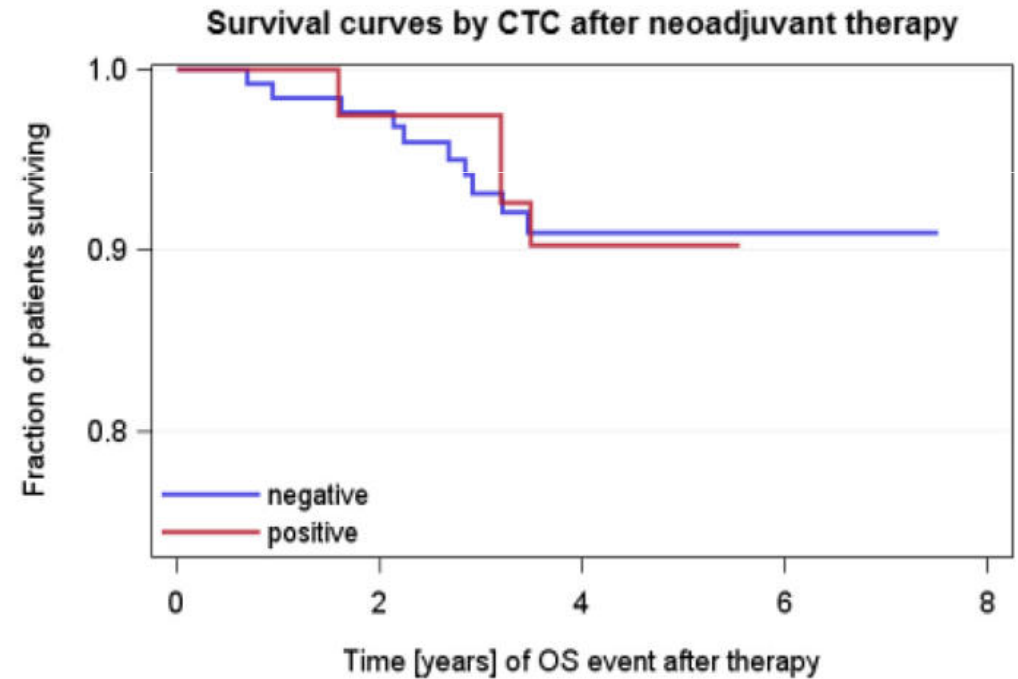
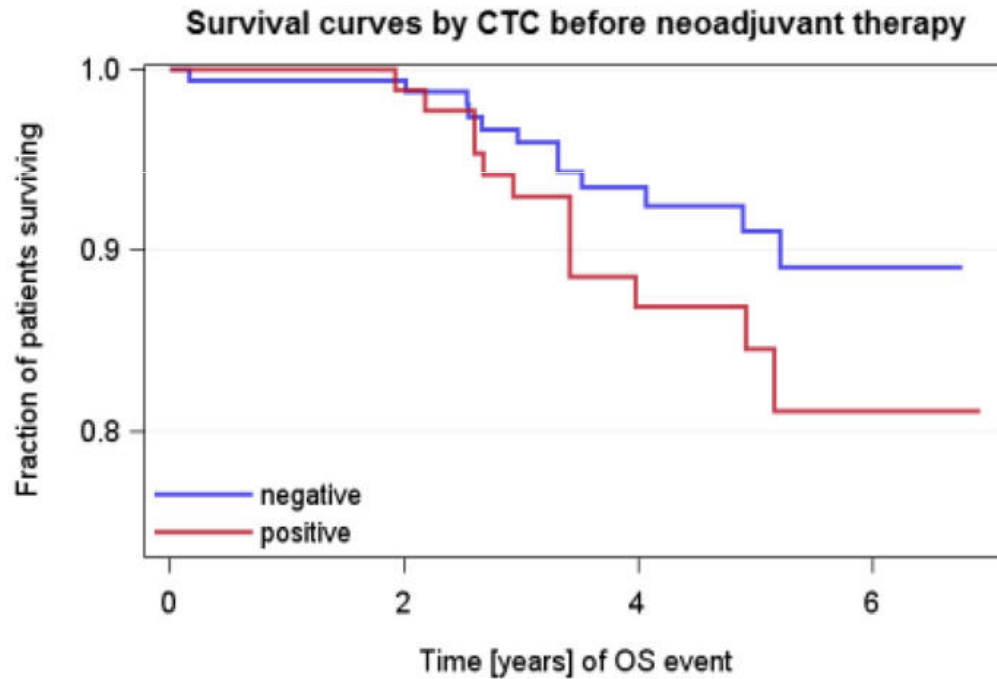




**But what's the point?**

# Why are these informations useful

## Future perspectives

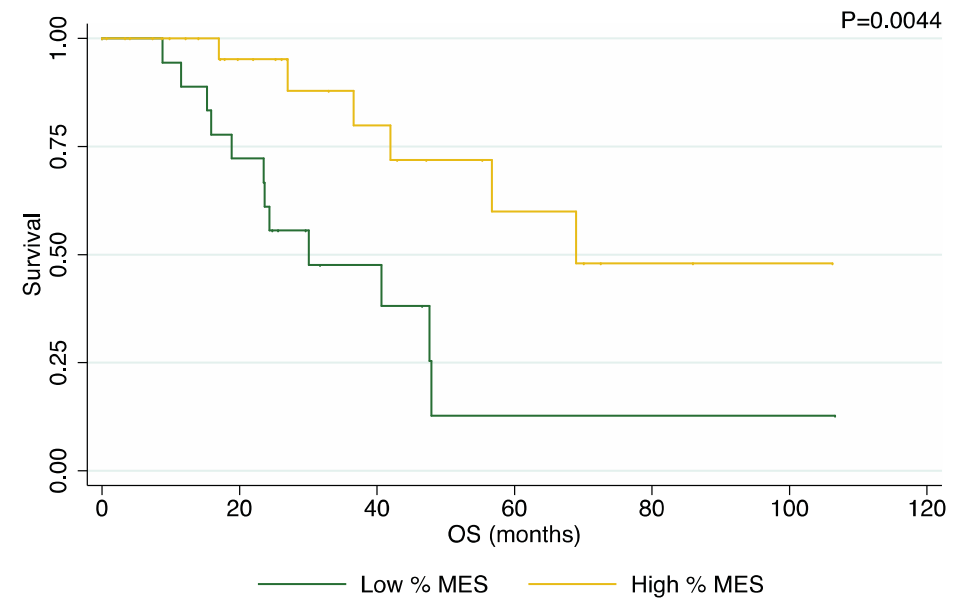
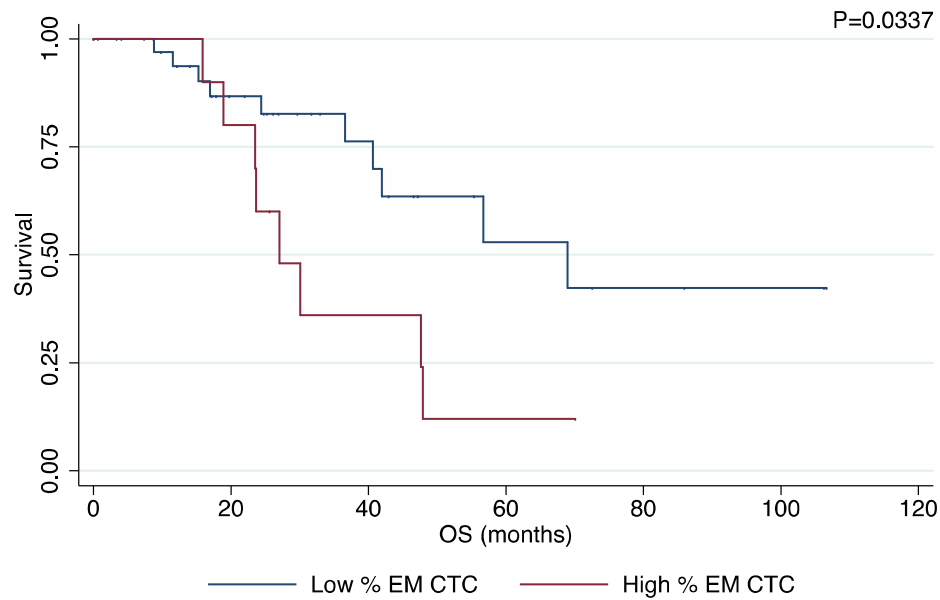


CTC before NACT	CTC after NACT	
	CTC-neg	CTC-pos
CTC-neg	58	9
CTC-pos	24	1

**One more thing...**

# The hidden side of the results

## Speculative unpublished data



Multivariate analysis, corrected for **ECOG PS** and **number of lines**, confirmed the unfavorable prognostic impact of :

**ETOT** as continuous percentage variable in terms of:

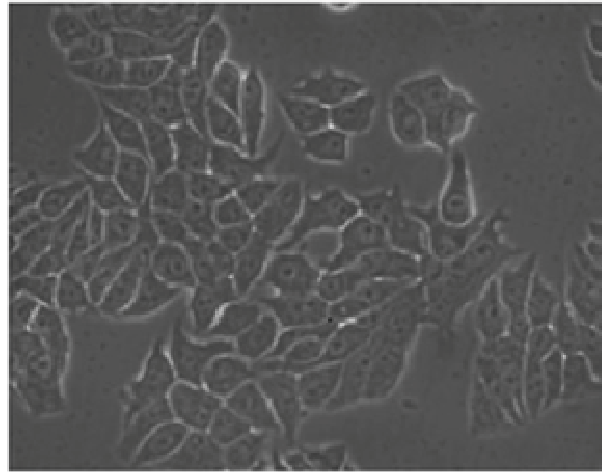
- OS at stage IV diagnosis  $P = 0.0324$
- OS at CTC assessment  $P = 0.0134$ )

**EM CTC** subpopulation in terms of:

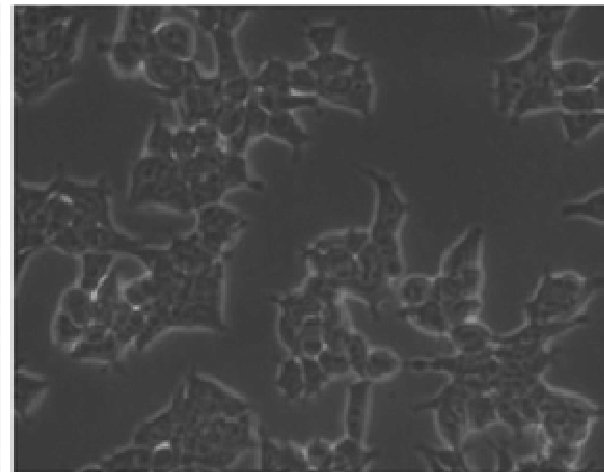
- OS at stage IV diagnosis  $P = 0.0142$
- OS at CTC assessment  $P = 0.0006$

# The hidden side of the results

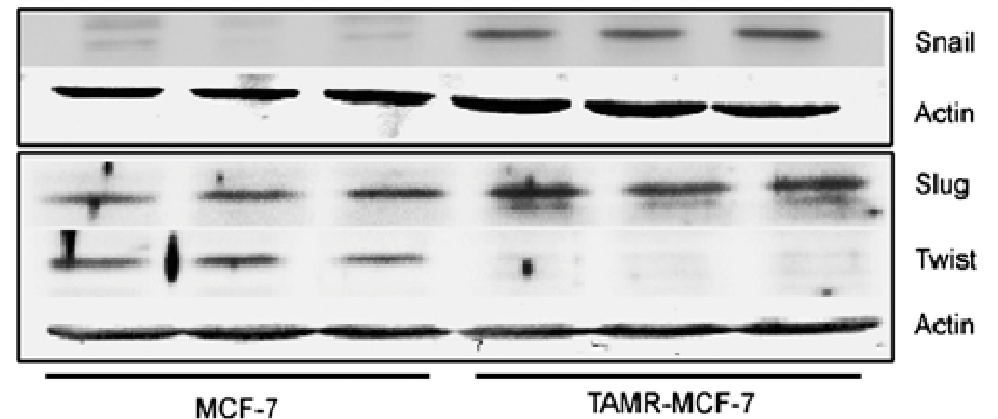
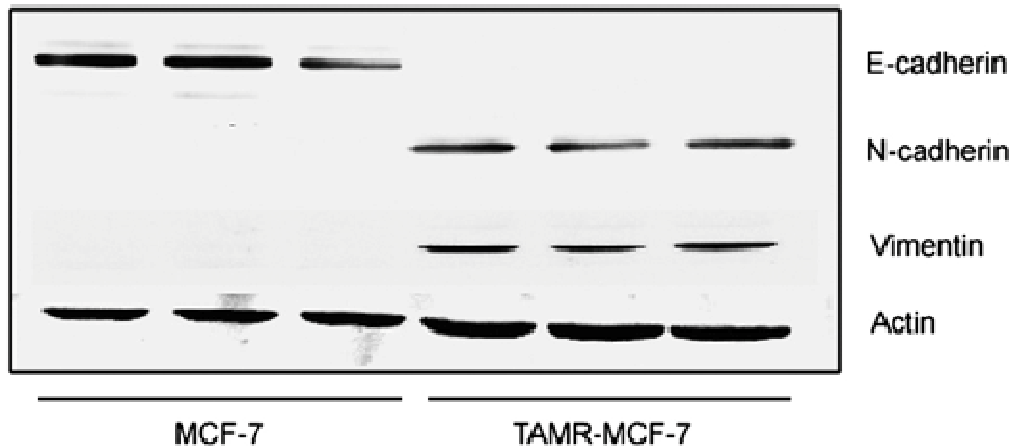
## CTC subpopulations treatments received



MCF-7

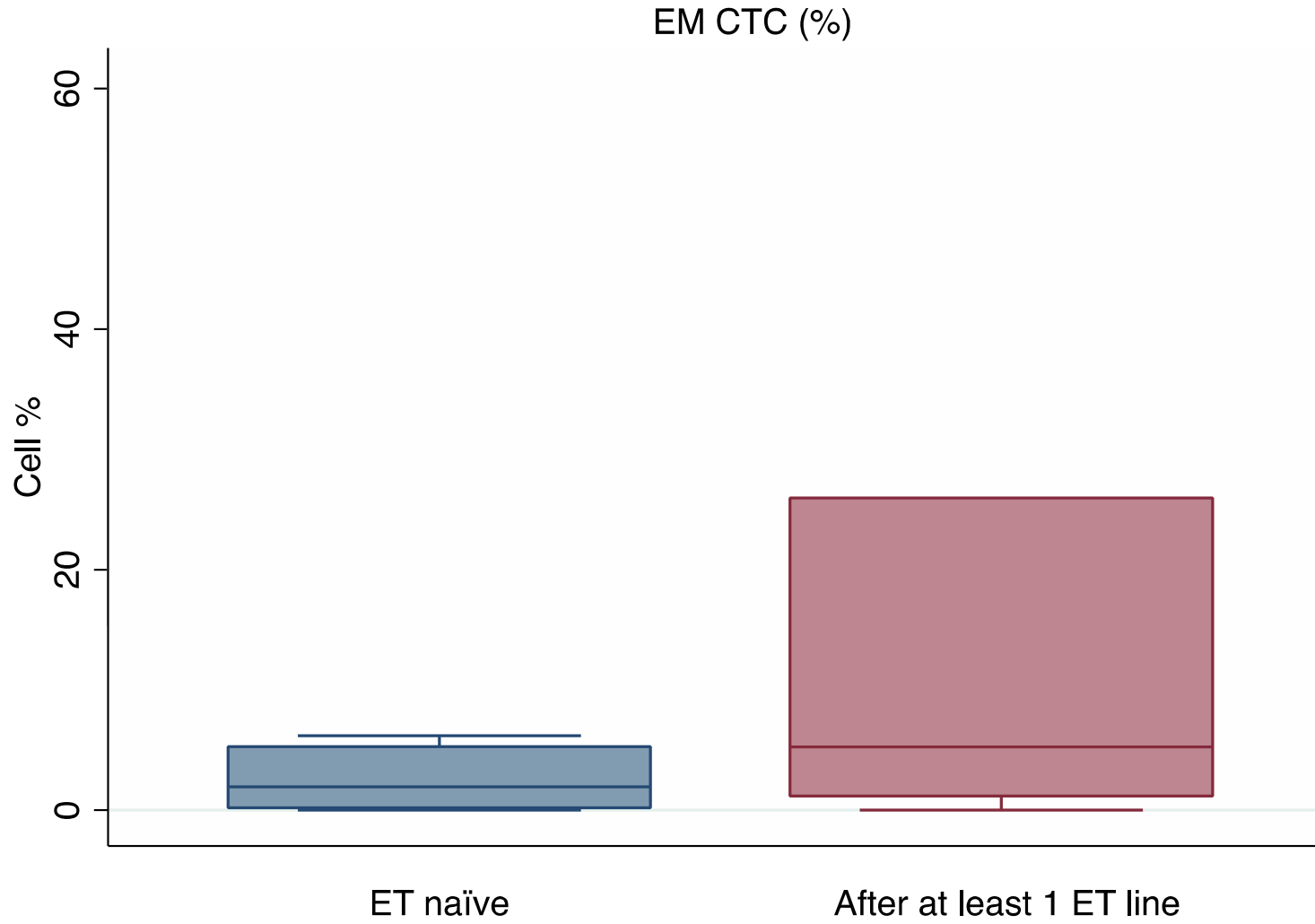


TAMR-MCF-7



# The hidden side of the results

## CTC subpopulations treatments received

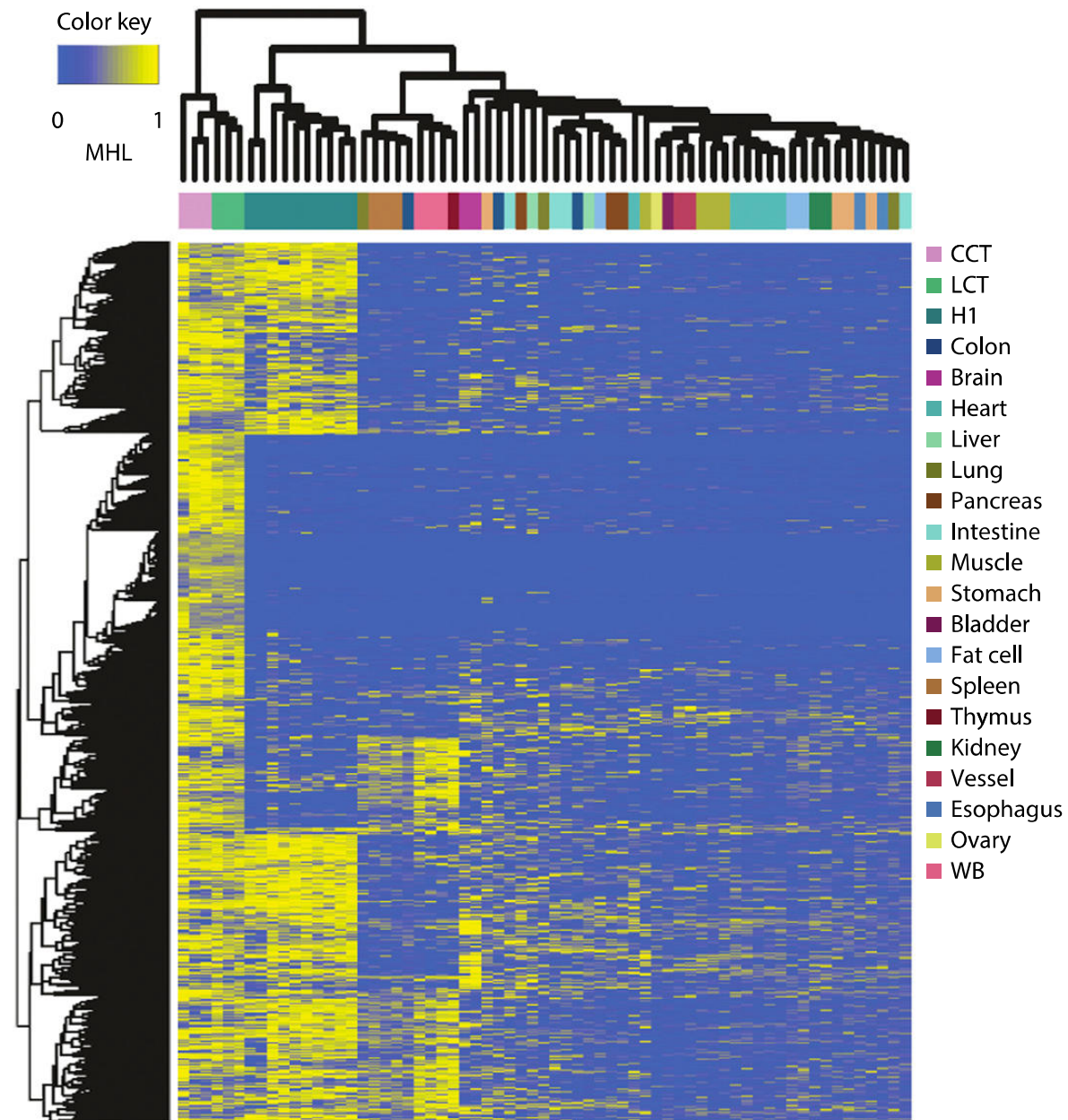


**So, what's next?**



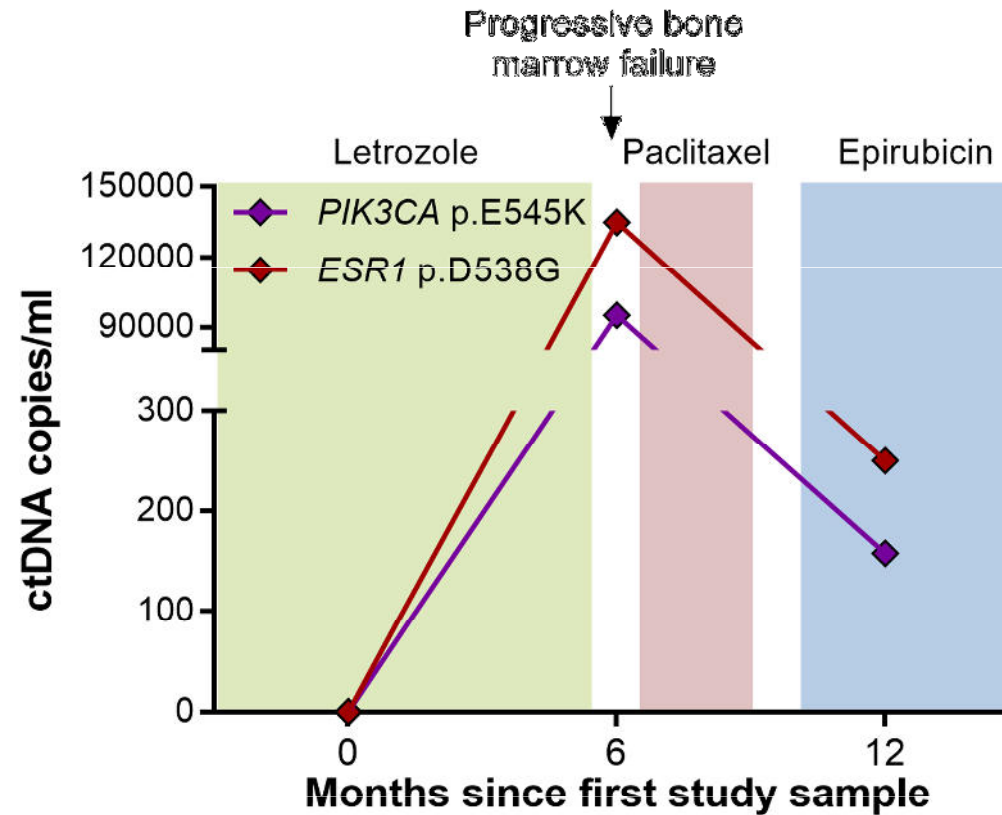
# A leap forward

And a new perspective



# A leap forward

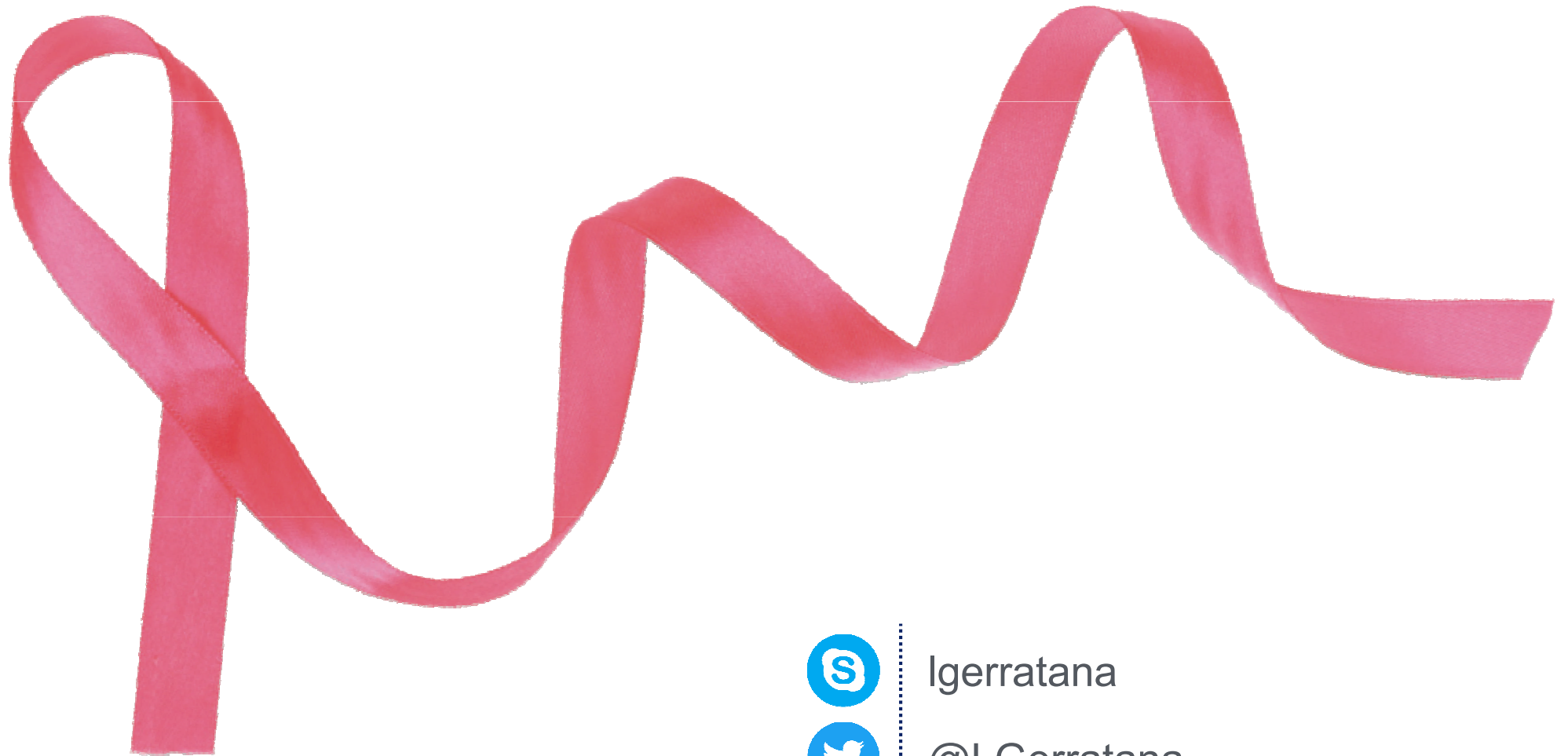
## And a new perspective



ctDNA copies/ml		2197	504545	96970
<i>PIK3CA</i> p.E545K	AF (%)	0	19.7	0.17
	mutant copies/ml	0	99395	165
<i>ESR1</i> p.D538G	AF (%)	0	28	0.27
	mutant copies/ml	0	141273	262
CTC count		1	NA	174

Thank you

---



lgerratana



@LGerratana



gerratana.lorenzo@spes.uniud.it