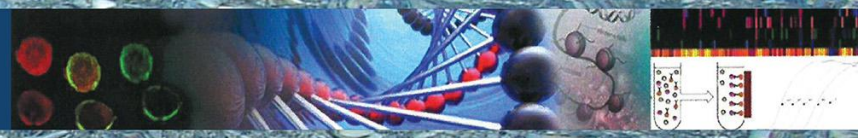
7th ACTC Advances in Circulating Tumor Cells
"Liquid Biopsy: From Bench to Bedside"
September 24-27, 2025 Makedonia Palace Hotel / Thessaloniki · Greece



Circulating tumor cells (CTCs) and CTC-clusters in patients with early-stage breast cancer (eBC) using enrichment-free technology: increased sensitivity for an improved cellular residual disease (CRD) detection

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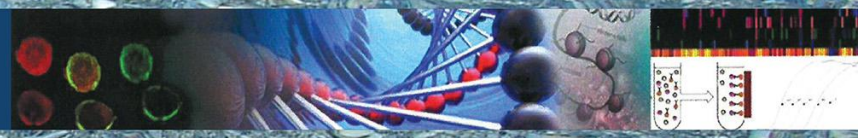
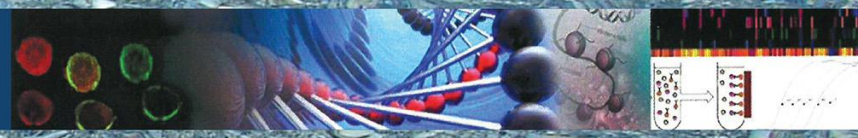
TETHIS



Conflicts of Interest Disclosure

- No scientific COI to disclose



Background

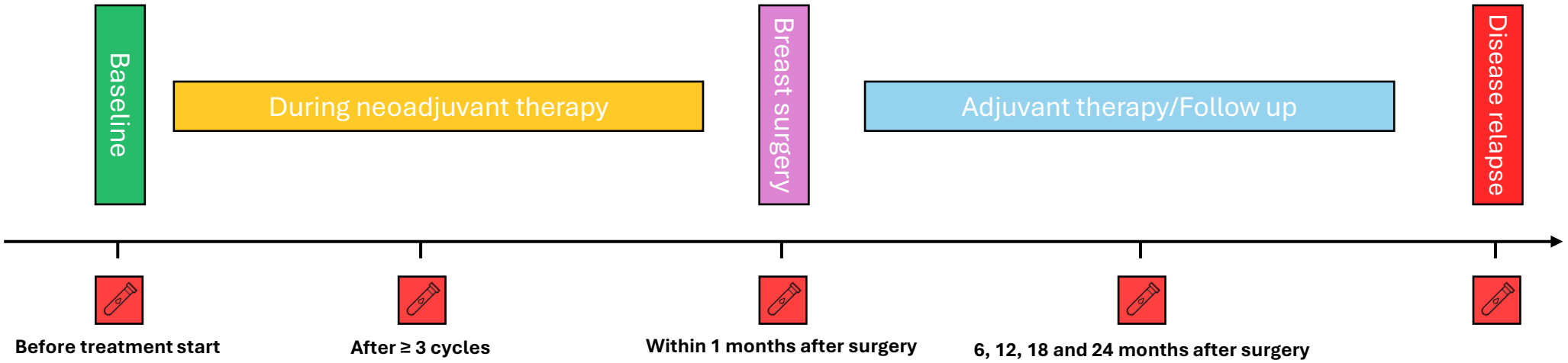
- Approximately **2/3 of all breast cancer cases** are diagnosed at an **early stage**[1]
- Treatment has a **potential curative intent**, but relapse could still develop in up to 15-30% of cases[2]
- **Minimal residual disease** is gaining recognition as a **molecular predictor of relapse**[3,4]
  - Molecular residual disease (**MRD**) → cell-free DNA (**cfDNA**) evaluation
  - Cellular residual disease (**CRD**) → circulating tumor cells (**CTCs**) enumeration
- CRD has **prognostic value** in early breast cancer[5,6], but **low CTCs detection rates** have limited its use so far
- **Aim of this study**: investigate a **novel, semi-automated, enrichment-free** CTCs detection assay as a contributor to CRD definition

[1] Siegel RL, Kratzer TB, Giaquinto AN et al., CA Cancer J Clin. 2025 Jan-Feb;75(1):10-45. doi: 10.3322/caac.21871; [2] Geurts YM, Witteveen A, Bretveld R et al., Breast Cancer Res Treat. 2017 Oct;165(3):709-720. doi: 10.1007/s10549-017-4340-3; [3] Medford AJ, Moy B, Spring LM et al., Clin Cancer Res. 2023 Nov 14;29(22):4540-4548. doi: 10.1158/1078-0432.CCR-23-0757; [4] Serafini MS, Molteni E, Nicolò E et al., J Liq Biopsy. 2023 Dec 12;3:100132. doi: 10.1016/j.jlb.2023.100132; [5] Janni WJ, Rack B, Terstappen LW et al., Clin Cancer Res. 2016 May 15;22(10):2583-93. doi: 10.1158/1078-0432.CCR-15-1603; [6] Rack B, Schindlbeck C, Jückstock J et al.,J Natl Cancer Inst. 2014 May 15;106(5):dju066. doi: 10.1093/jnci/dju066

Methods

- Clinical Stage II-III eBC
- Any biological subtype
- Eligible to receive neoadjuvant therapy

N ≈ 30 (planned)



- Prospective, monocentric, observational study
- Treatment and disease assessments as per clinical practice

- 18 mL blood samples collected at each timepoint
- Processing with See.d® platform (Tethis S.p.A.) for:
  - plasma collection → stored for downstream cfDNA analyses
  - CTCs identification and phenotyping → staining on SBS slides

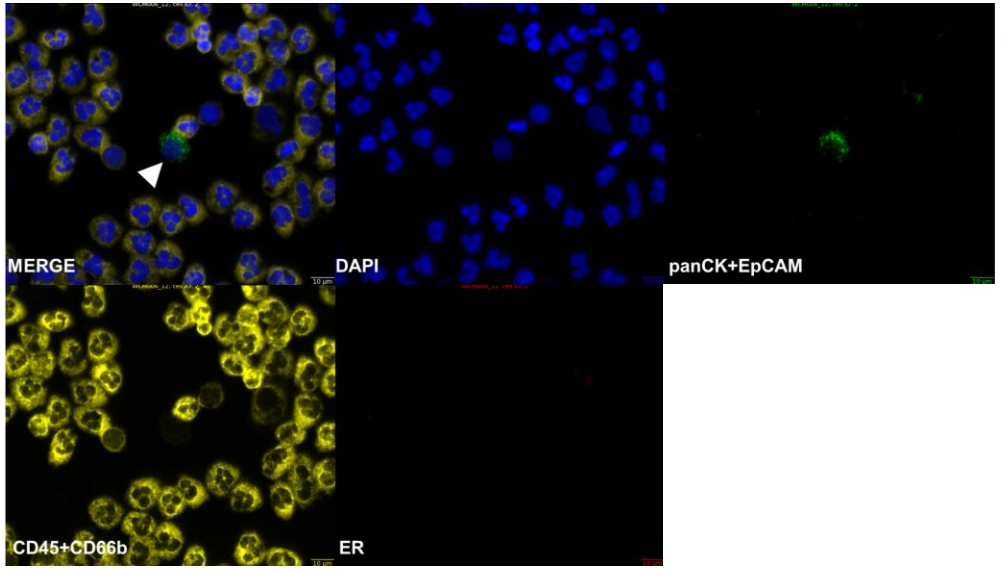
Methods

- CTCs evaluation through 3 staining steps:
  1) Immunofluorescence using…
     - epithelial (**EpCAM/panCK**), nuclear (**DAPI**), and leukocyte (**CD45/CD66**) markers for CTCs identification → **Epithelial^{pos} CTCs**
     - additional tumor markers (HER2/ER/PD-L1), according to tissue subtype, for CTCs phenotyping → **Tumor^{pos} CTCs**

  2) Cytological staining to confirm CTCs malignancy

  3) Fluorescence in situ hybridization (FISH) for HER2

1



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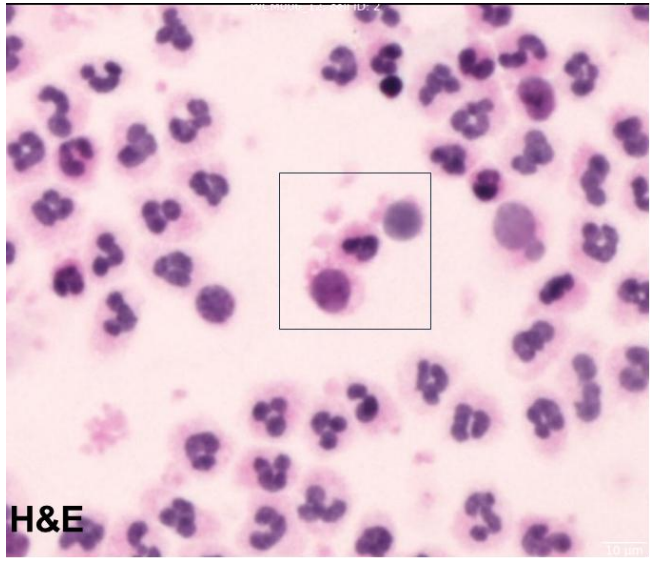

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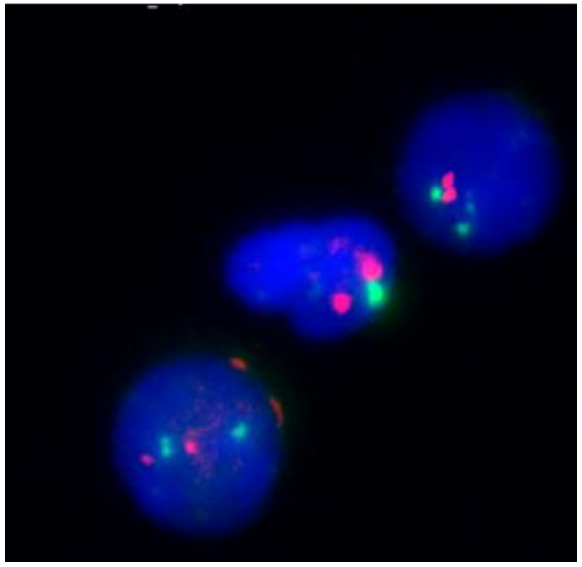
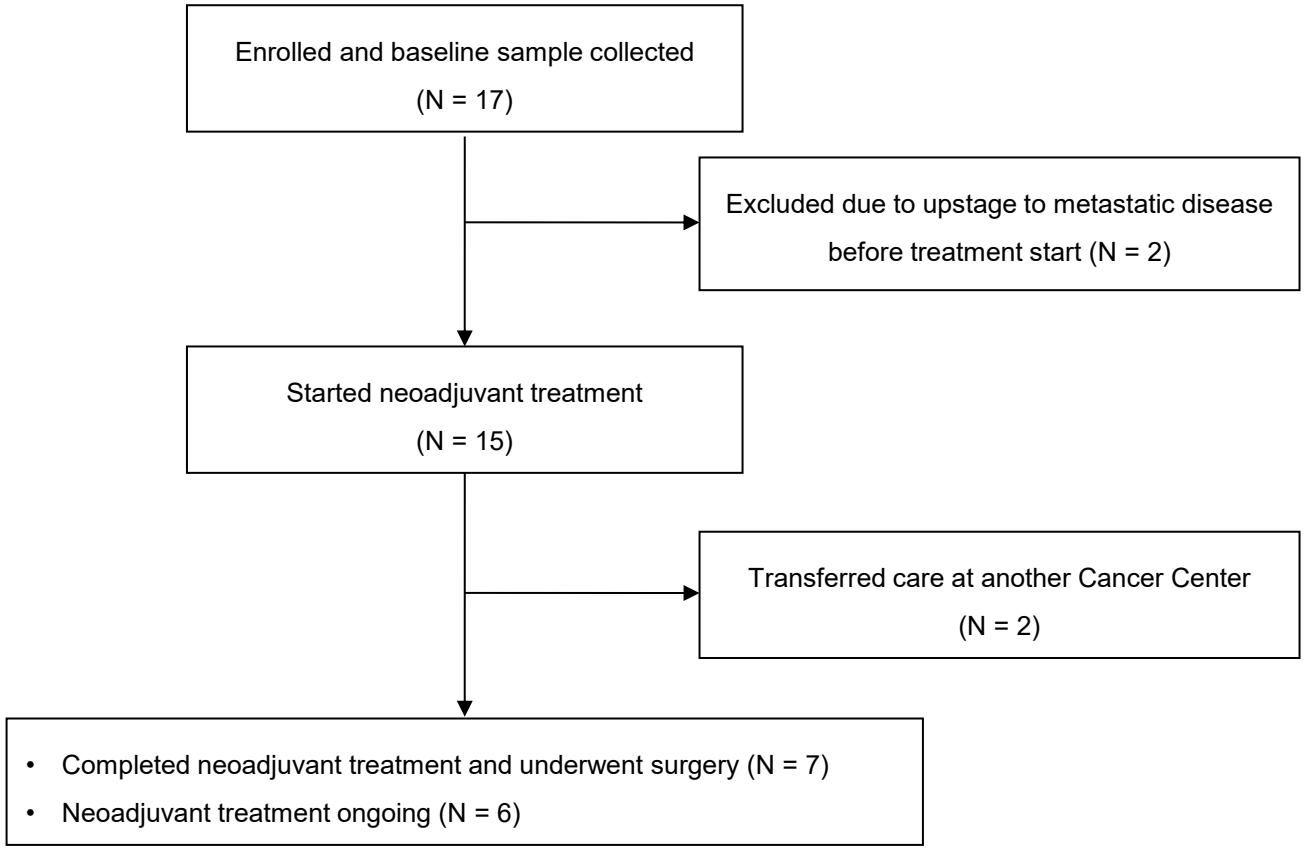


Results

Patient disposition and sample collection



Database lock: September 18, 2025
Enrollment timeframe: September 2024 – September 2025

Timepoint	N° of samples collected
Baseline	17*
During therapy	10°
Post surgery	5^
TOTAL	**32**

Notes:
*3 baseline run failures due to technical issues
°1 patient with missing «During therapy» timepoint, 2 patients awaiting collection
^1 patient refused «Post surgery» timepoint, 1 patient awaiting collection

Results

Baseline characteristics – neoadjuvant treatment cohort (N = 15)

	N	%
Age at diagnosis		
< 50	7	46.7
≥ 50	8	53.3
Sex		
Female	14	93.3
Male	1	6.7
Race		
White	10	66.7
Asian	2	13.3
Unknown/Not declared	3	20.0
Ethnicity		
Hispanic or Latino	2	13.3
Not Hispanic or Latino	12	80.0
Unknown/Not declared	1	6.7
Menopausal status		
Pre-menopausal	8	53.3
Post-menopausal	6	40.0
Men	1	6.7

	N	%
Stage at diagnosis		
II	10	66.7
III	5	33.3
Inflammatory breast cancer		
Yes	2	13.3
No	13	86.7
Histotype		
NST – Ductal	13	86.7
Lobular	1	6.7
Mixed	1	6.6
Biological subtype		
HR-positive/HER2-negative	6	40.0
HER2-positive	4	26.7
Triple negative	5	33.3
Pathological complete response (pCR) achieved		
Yes	1	40.0
No	6	6.7
Not available	8	53.3

Results

Baseline samples – 14 processed, 8 analyzed

Sample	Timepoint	Biological subtype	Tumor marker	Blood volume (mL)	Epithelial^{pos} CTCs (n°/mL)	Epithelial^{pos}/Tumor^{pos} CTCs (n°/mL)	Tumor^{pos} CTCs (n°/mL)	CTC clusters (≥ 2 Epithelial^{pos} CTCs)
LB134	Baseline	TNBC	PD-L1	5.85	2.6	0.0	0.2	no
LB135	Baseline	TNBC	PD-L1	3.80	3.7	0.0	0.0	**yes**
LB153	Baseline	HR$^+$/HER2$^-$	ER	3.10	2.9	0.3	1.6	no
LB157	Baseline	HR$^+$/HER2$^+$	ER	2.30	0.4	0.0	0.0	no
			HER2	2.30	0.9	0.0	0.0	no
LB164	Baseline	HR$^-$/HER2$^+$	HER2	2.07	1.0	0.0	0.0	no
LB167	Baseline	HR$^+$/HER2$^+$	ER	5.80	0.5	0.0	0.0	no
LB168	Baseline	TNBC	PD-L1	3.80	0.5	0.0	0.0	no
LB172	Baseline	HR$^+$/HER2$^+$	ER	6.30	0.0	0.0	0.0	no

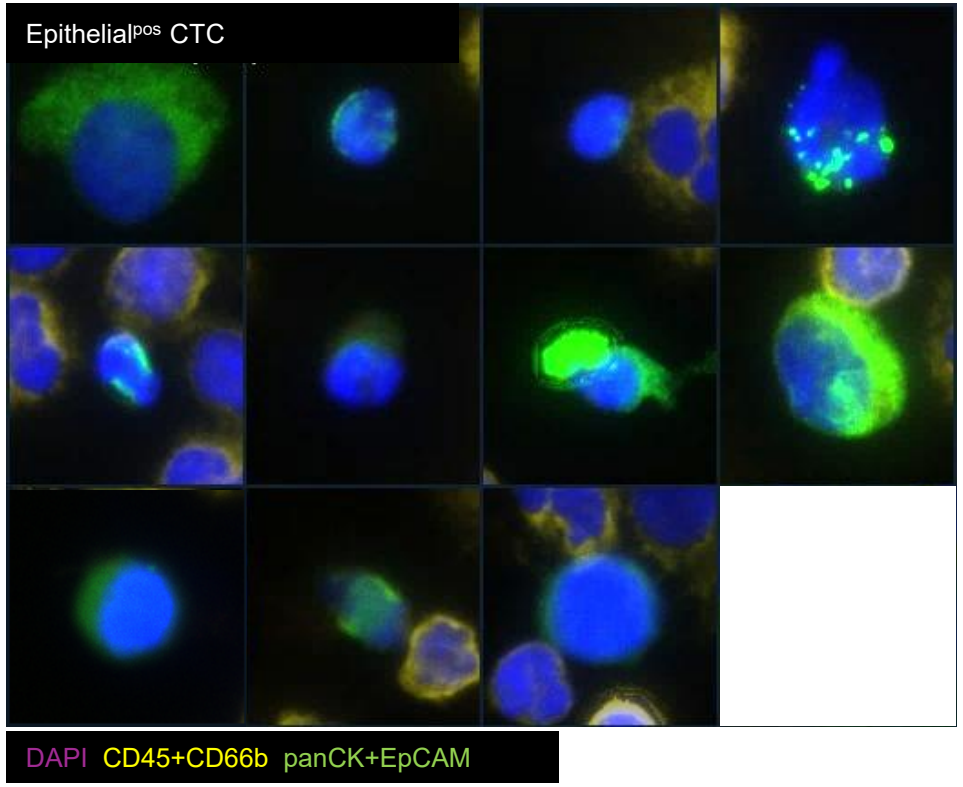
- Epithelial^{pos} CTCs detected in **7/8 baseline samples** (median **0.9/mL**, range 0.0 – 3.7)
- **CTC-clusters** detected in 1 patient with triple-negative disease
- **Tumor^{pos} CTCs with ER staining in aggregates** detected in 1 baseline sample, no detection of HER2 and PD-L1

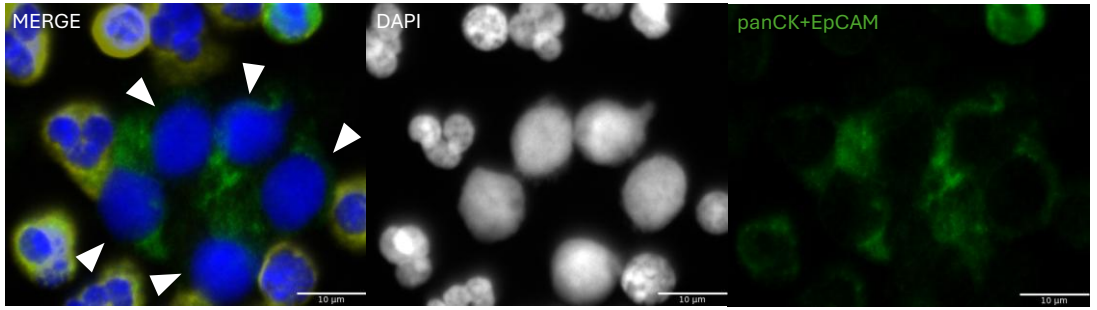
Results

Epithelial^{pos} CTCs and CTC-clusters

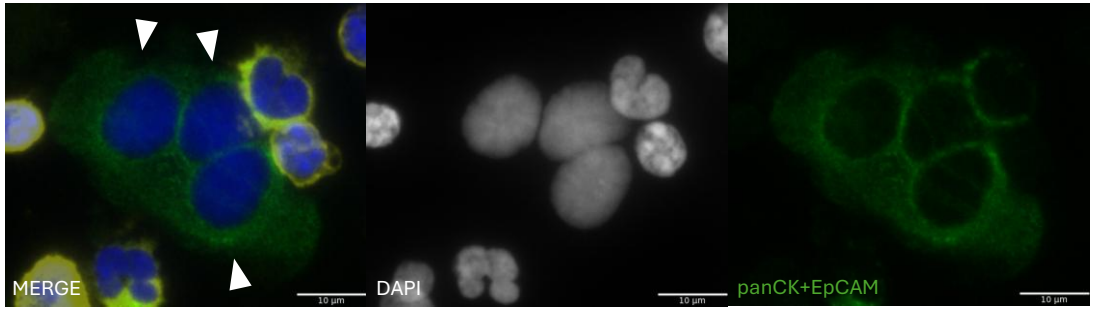
LB135

- Female, 35 y.o. at diagnosis
- **Stage at diagnosis:** II (cT2 cN0)
- **Biological subtype:** TNBC





A cluster of 5 CTCs, with low epithelial markers' expression



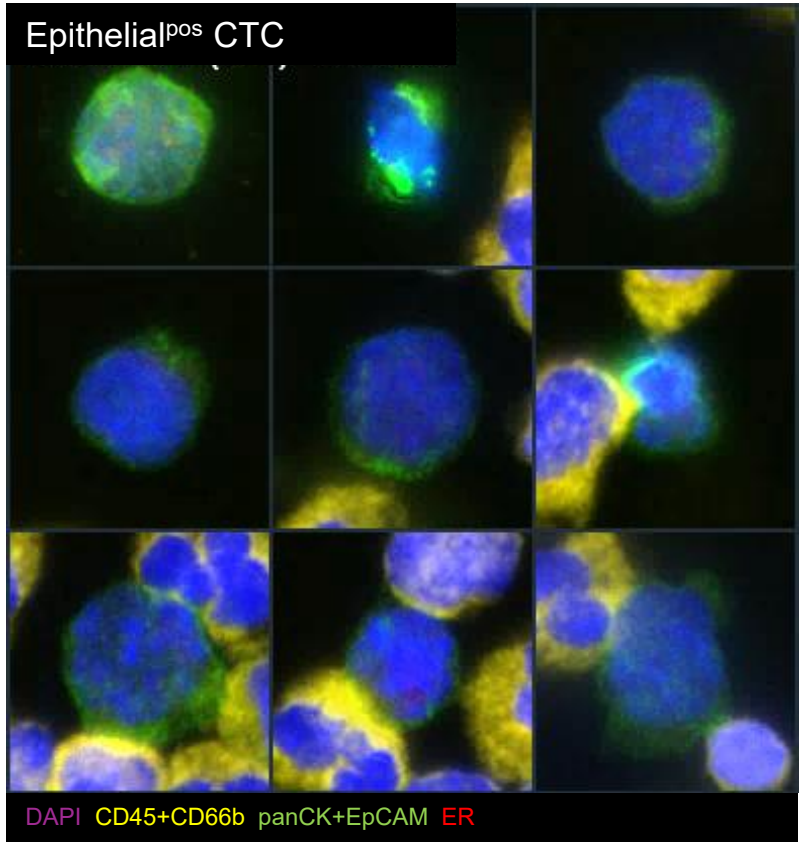
A cluster of 3 CTCs

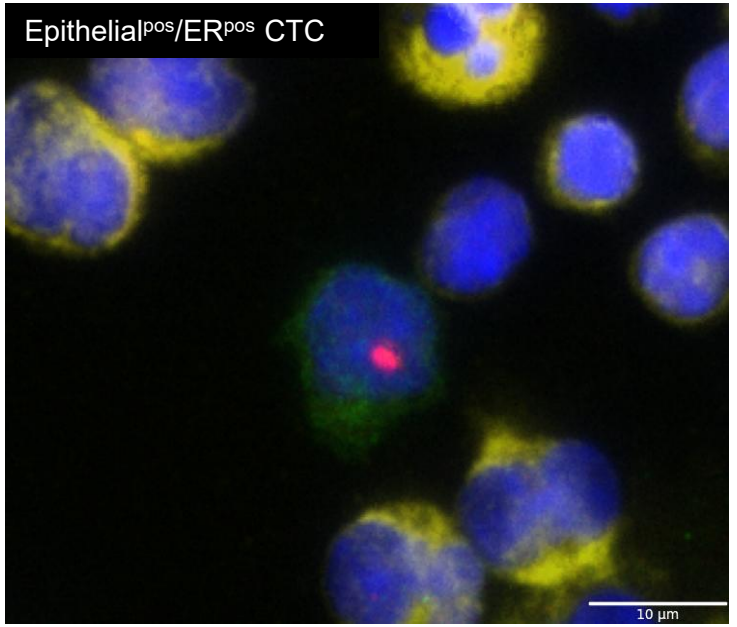
Results

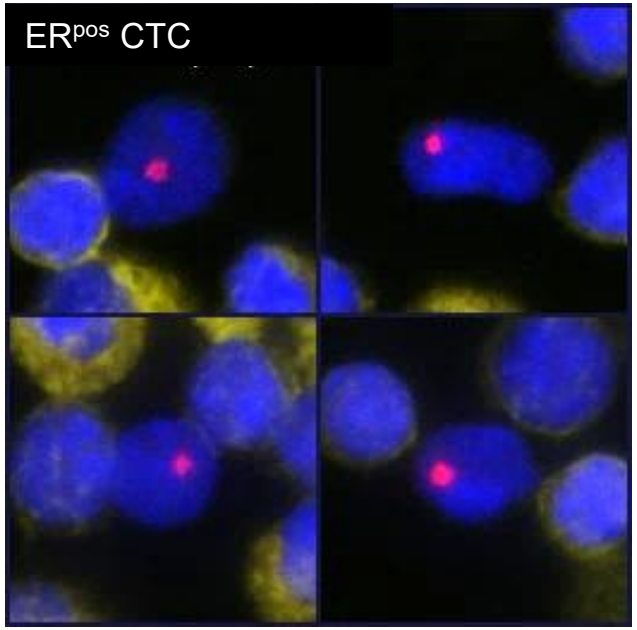
ER phenotyping

LB153

- Female, 65 y.o. at diagnosis
- **Stage at diagnosis:** II (cT2 cN0)
- **Biological subtype:** $HR^{+}/HER2^{-}$

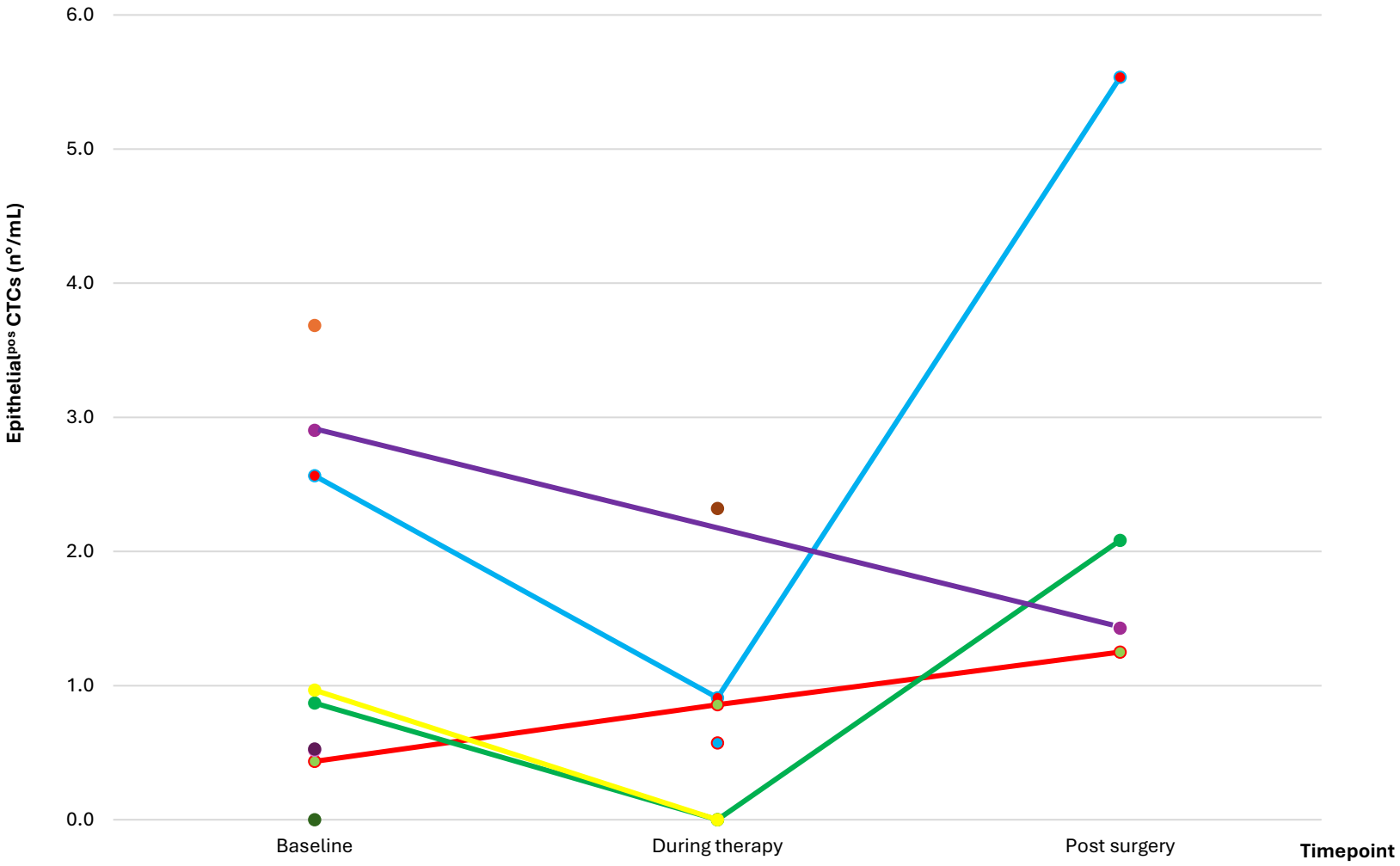






Results

All samples – 29 collected, 16 analyzed



- Early trends on Epithelial^{pos} CTCs count:
  - **decrease** at «During therapy» timepoint (3/4 samples)
  - **increase** at «Post surgery» timepoint (3/4 samples)
- **To be further investigated and assessed for association with response and survival**, with additional follow-up

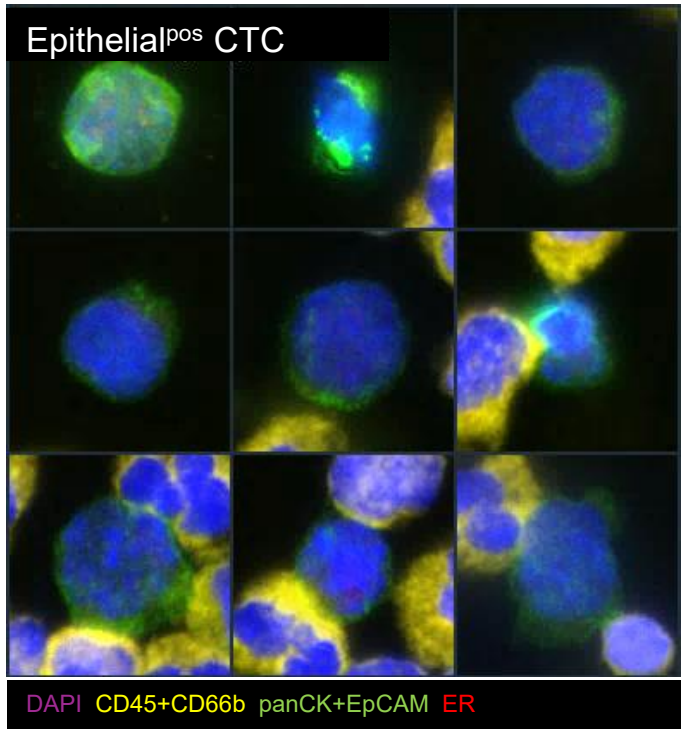
Results

LB153

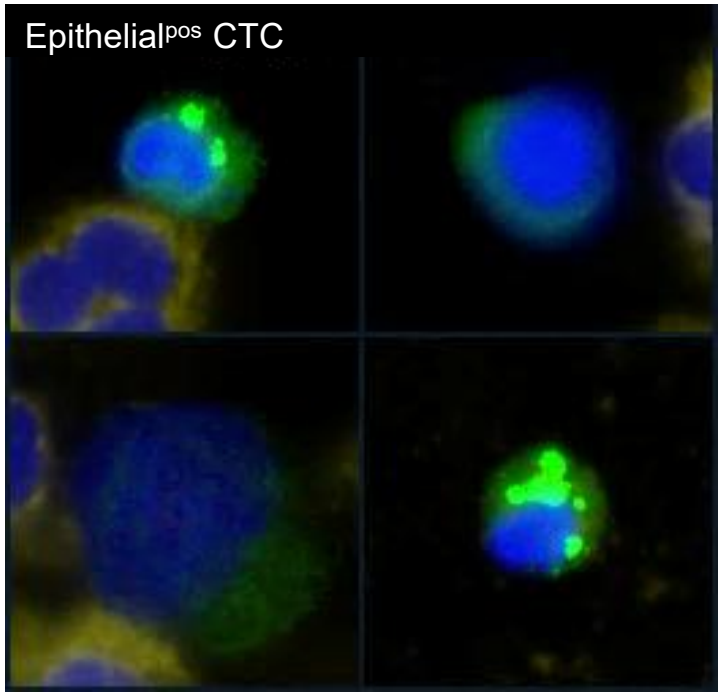
Morphological changes under treatment pressure

- Female, 65 y.o. at diagnosis
- **Stage at diagnosis:** II (cT2 cN0)
- **Biological subtype:** $HR^{+}/HER2^{-}$
- **Neoadjuvant treatment:** endocrine therapy + radiation therapy within clinical trial
- **Stage after surgery:** I (ypT1c ypN0), RCB-II

Baseline



Post surgery



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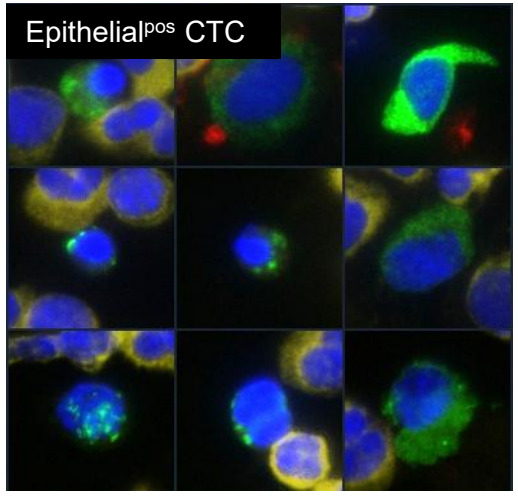
Results

LB134

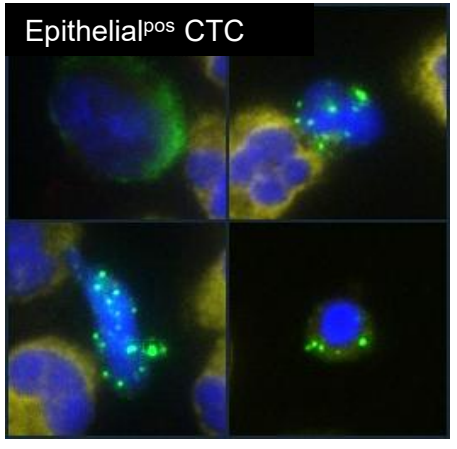
Longitudinal monitoring, CTC-clusters detection and heterogeneity capture

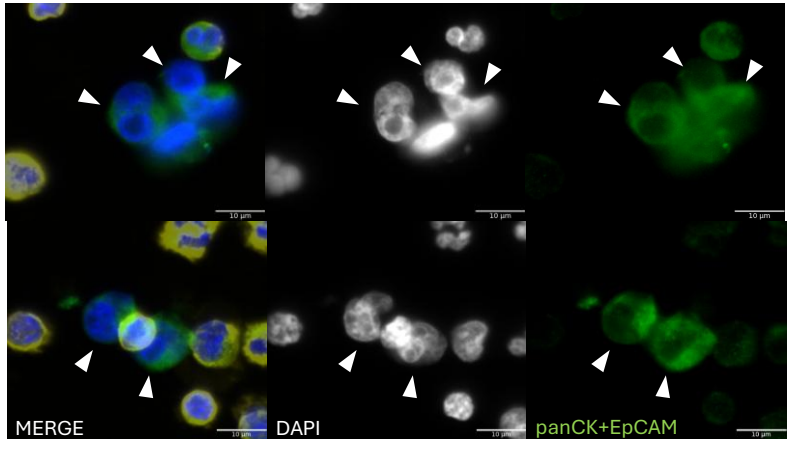
- Female, 70 y.o. at diagnosis
- **Stage at diagnosis:** III (cT4 cN3) – IBC
- **Biological subtype:** TNBC
- **Neoadjuvant treatment:** chemotherapy + immunotherapy (KEYNOTE-522 scheme)
- **Stage after surgery:** 0 (ypT0 ypN0), pCR

Baseline

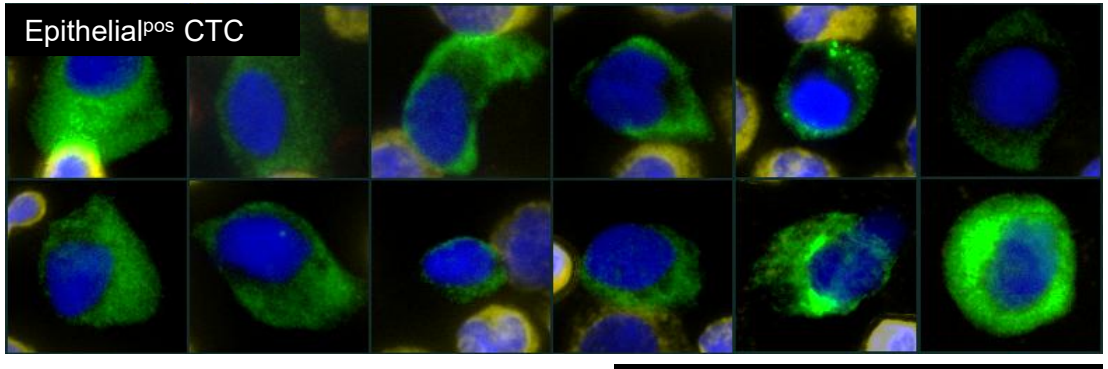


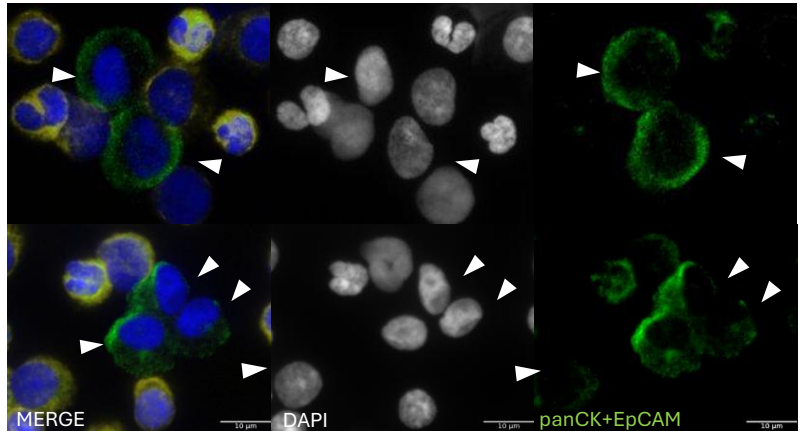
During therapy





Post surgery





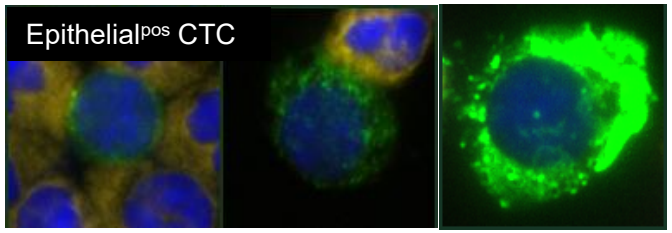
Results

Multimodal analysis

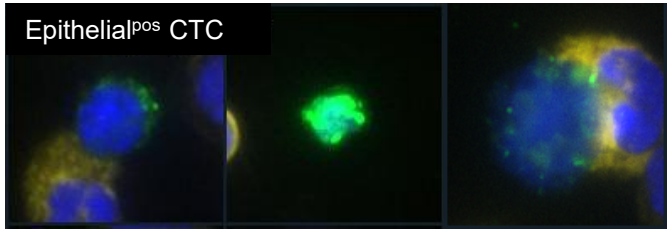
LB157

- Female, 45 y.o. at diagnosis
- **Stage at diagnosis:** II (cT2 cN0)
- **Biological subtype:** $HR^+/HER2^+$
- **Neoadjuvant treatment:** chemotherapy + antiHER2 therapy (TRAIN-2 scheme)
- **Stage after surgery:** I (ypT1c ypN0), RCB-II

Baseline

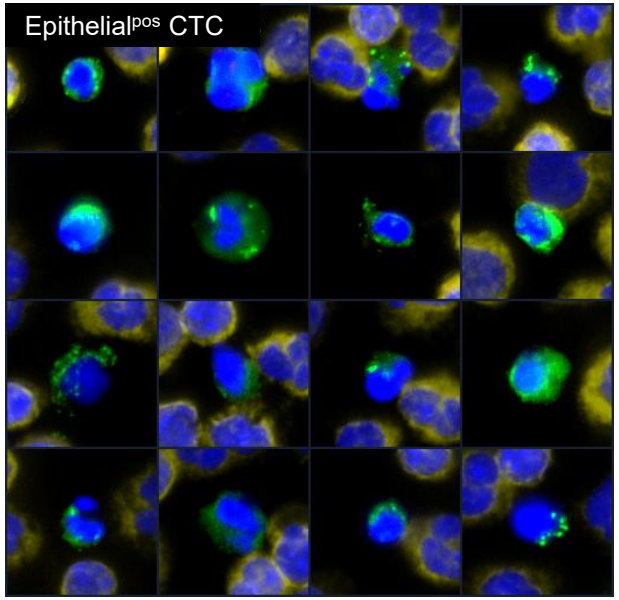


During therapy



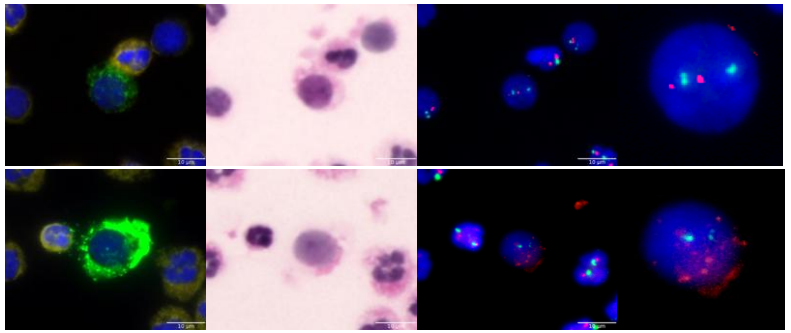
DAPI CD45+CD66b panCK+EpCAM

Post surgery

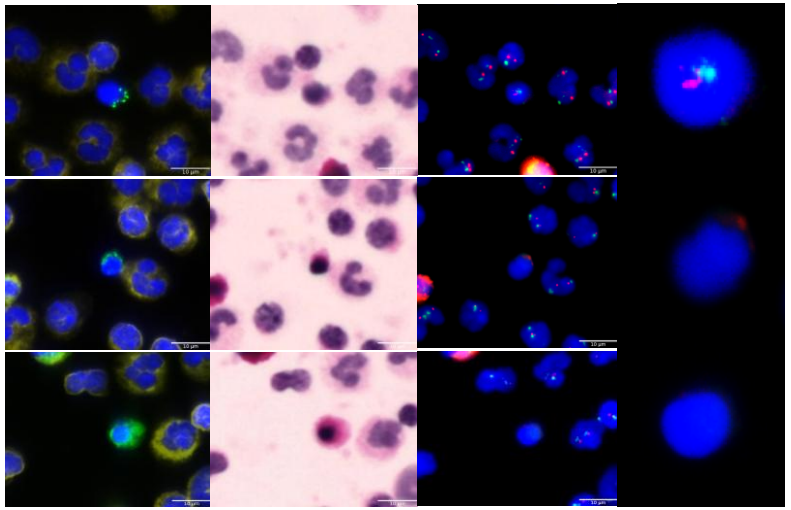


MERGE | Hematoxylin Eosin | ERBB2 SE 17 FISH

Baseline



Post surgery



Conclusions

- These results suggest that this **semi-automated**, **enrichment-free assay** is **informative** in the setting of early breast cancer

- Staining on SBS slides allows for:
  - **Enhanced CTCs detection**
  - **CTC-clusters identification**
  - **CTCs phenotyping with some biomarkers**
  - **In-depth evaluation of morphological heterogeneity**
  - **Multimodal downstream analyses**

- CTCs **evolve under treatment pressure**, not only in terms of enumeration, but also on their morphology and phenotypes

- **Expanding opportunities for CRD monitoring in early breast cancer**
- **Combining MRD and CRD information will provide further insights into disease biology**
- **Further step towards precision in treatment planning**

Acknowledgements

- **Patients and their families involved**

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  - Weill Cornell Medicine
  - University of Udine
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• Hellenic Society of Liquid Biopsy

Thank you for your attention!

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