



New platform for Liquid Biopsy enables
precise cancer diagnosis
and CTC clusters detection in early-stage breast cancer

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Fighting against cancer

- Diagnosing cancer early dramatically improves patients outcomes
- **Non invasive, cost effective, highly specific and reproducible** diagnostic tools are strongly needed to detect cancer at earlier stage and to better guide treatment
- **Liquid biopsies**, i.e. detecting and characterizing cancer from a body fluid, hold the promise to provide a **paradigm shift in cancer management**

17 mln

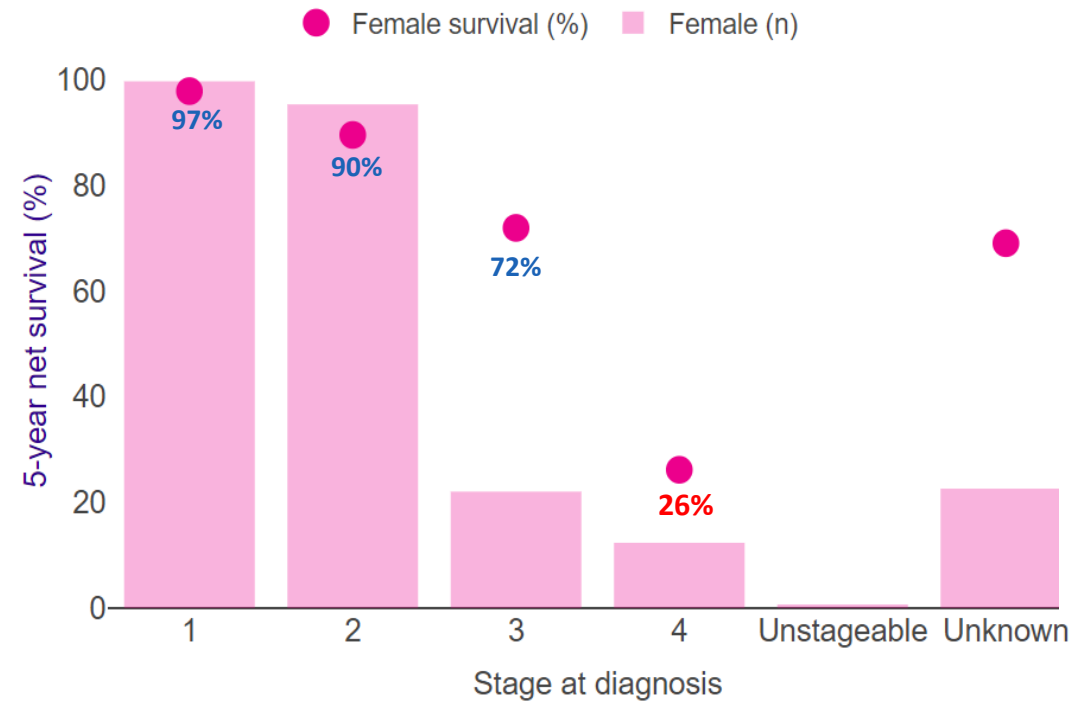
new cases worldwide in 2018

9.6 mln

deaths worldwide in 2018

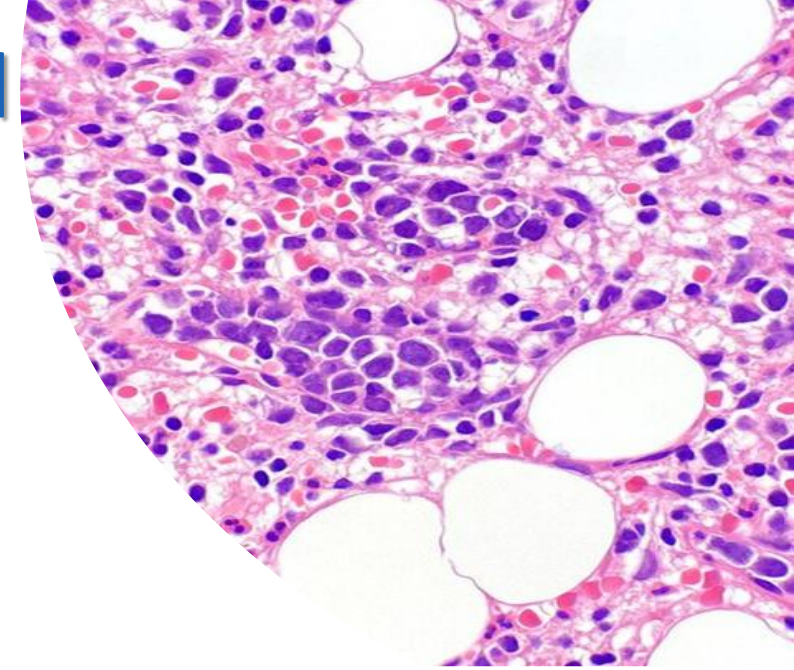
Source: www.cancerresearchuk.org/health-professional/cancer-statistics/worldwide-cancer

Breast cancer five-year net survival by stage, with incidence by stage (2013-2017)



<https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/breast-cancer/survival#heading-Three>

Tissue biopsy: today gold standard



Tissue biopsy is today's gold standard for confirmatory diagnosis, staging, prognosis and therapy selection in almost all cancer patients

Through a tissue biopsy, pathologists and oncologists can acquire information from:



Tumor analysis under the microscope from its visual characterization



Protein expression (IHC) for further characterization and therapy selection



Downstream molecular analysis (FISH, PCR, NGS) for genetic picture of the tumor

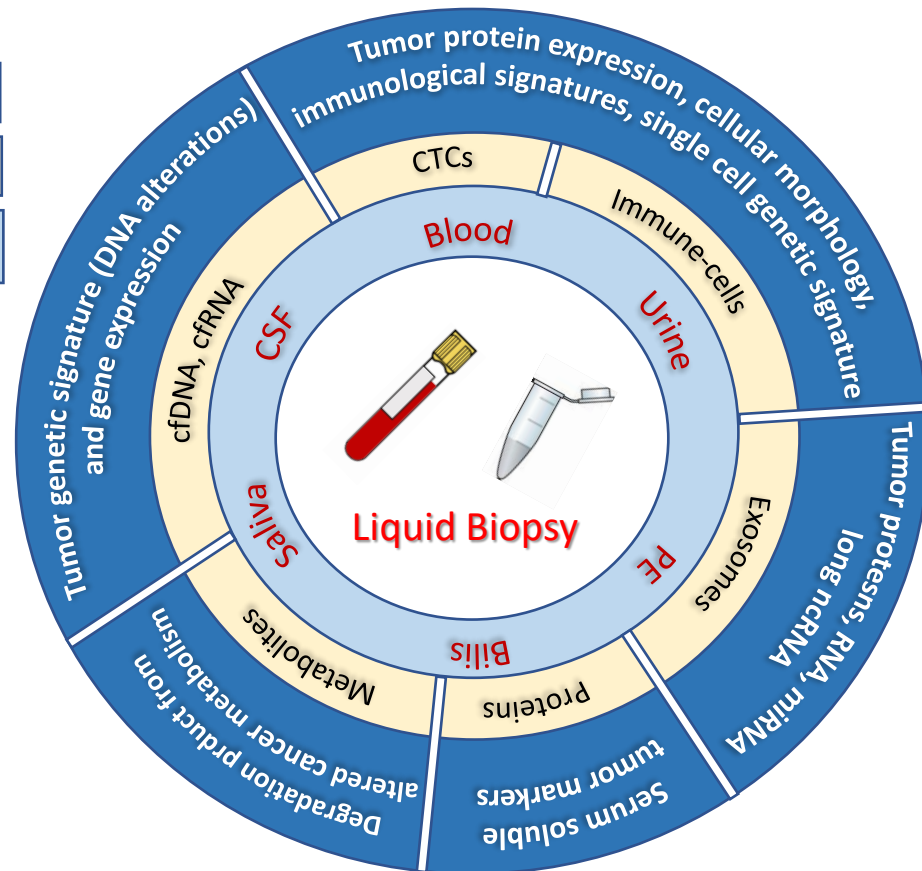
Problems and limitations

- ✗ Invasive procedure (needle, endoscopy, surgery)
- ✗ Limited tissue availability
- ✗ Feasible only when tumor is visible and accessible
- ✗ Cannot be repeated with frequency to assess changes in tumor profile
- ✗ Can miss tumor genetic heterogeneity

Liquid Biopsy

A liquid biopsy should and could provide most, if not all or even more, the information of a tissue biopsy, while overcoming its limitations

Biomarkers
Analytes
Body Fluids



Advantages

- Non invasive procedure
- Sample availability
- Always feasible
- Enable to catch tumor genetic heterogeneity
- Can be repeated with frequency to assess changes in tumor profile

Liquid Biopsy today in clinical settings

A liquid biopsy should and could provide most, if not all or more, the information of a tissue biopsy, while overcoming its limitations



But which information clinicians can derive from current approved and clinically available blood based liquid biopsies?



Tumor analysis under the microscope from its visual characterization



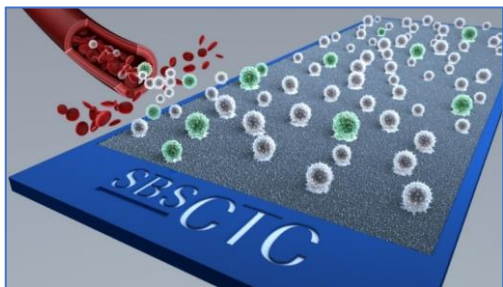
Protein expression (IHC) for further characterization and therapy selection



Downstream molecular analysis (FISH, PCR, NGS) for genetic picture of the tumor

Current “liquid biopsies” are mostly **blood-based genetic analysis**, offering a very precious but partial view of all the clinical information provided by a biopsy

Our approach to Liquid Biopsy : preserve sample integrity and clinical content



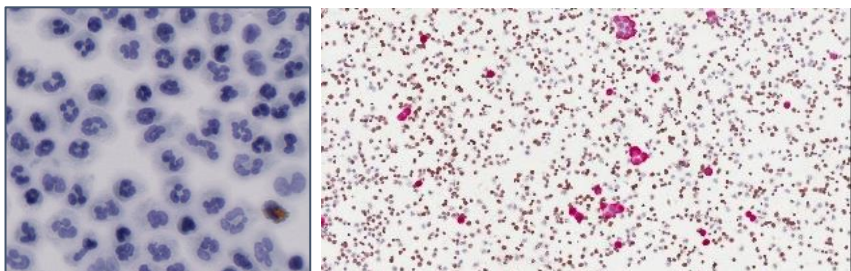
- EDTA collection tube
- Collection in *shear stress free of the whole WBC fraction* on SBS proprietary slide as a monolayer, in short time at RT



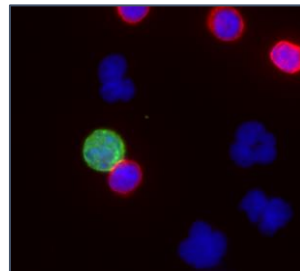
- A proprietary instrument, CellSeed, to standardize blood sample preparation (seeding and fixing) on the slides at the point of collection
- Enable blood processing immediately from withdrawal

A Liquid Biopsy with the clinical content of Tissue Biopsy

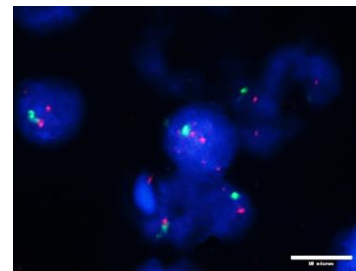
Cytology and ImmunoCytoChemistry



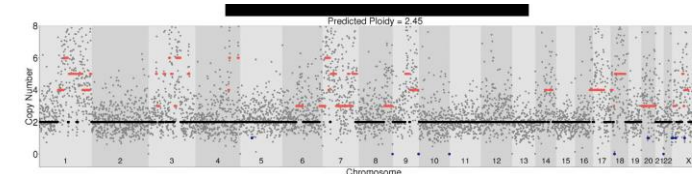
ImmunoFluorescence



FISH



(Single cell) Molecular Analysis



SBS Liquid Biopsy platform for cancer detection by CTCs analysis

PATIENT SAMPLE PREPARATION



Blood sampling in
EDTA Tubes



CellSeed,
for automated patient slides
preparation directly
from fresh blood

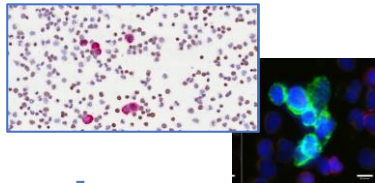


PATIENT SLIDES TRANSPORTATION

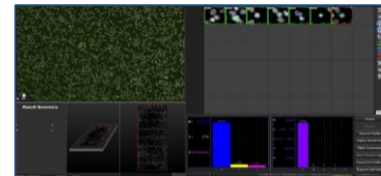


Standard shipment
of patient slides, according to
hospital need

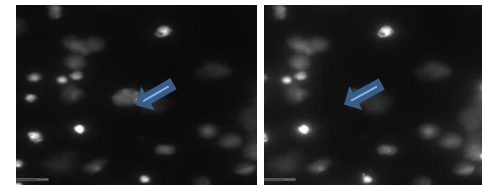
✓ Immunophenotypic
characterization



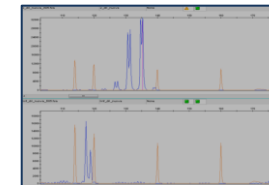
✓ Automated digital imaging
analysis



✓ Laser Microdissection

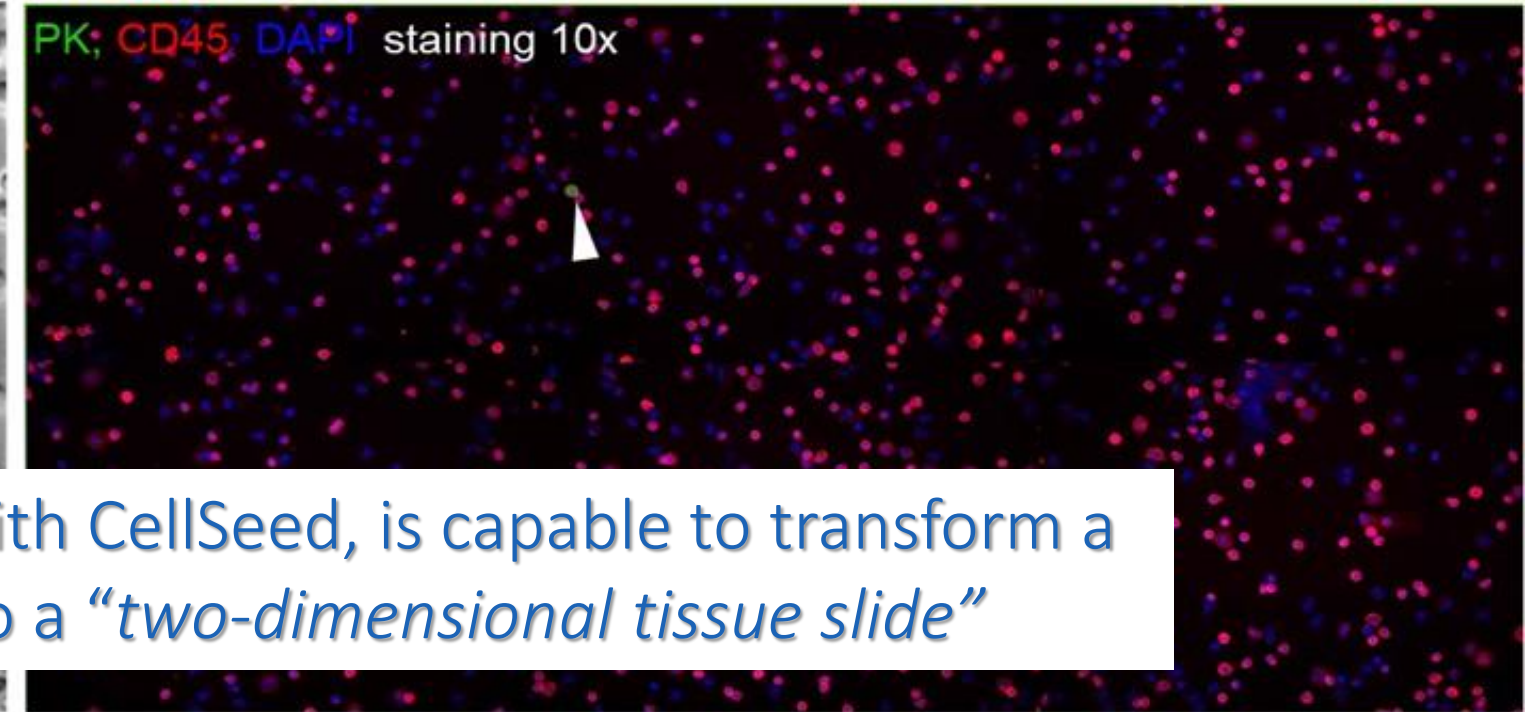
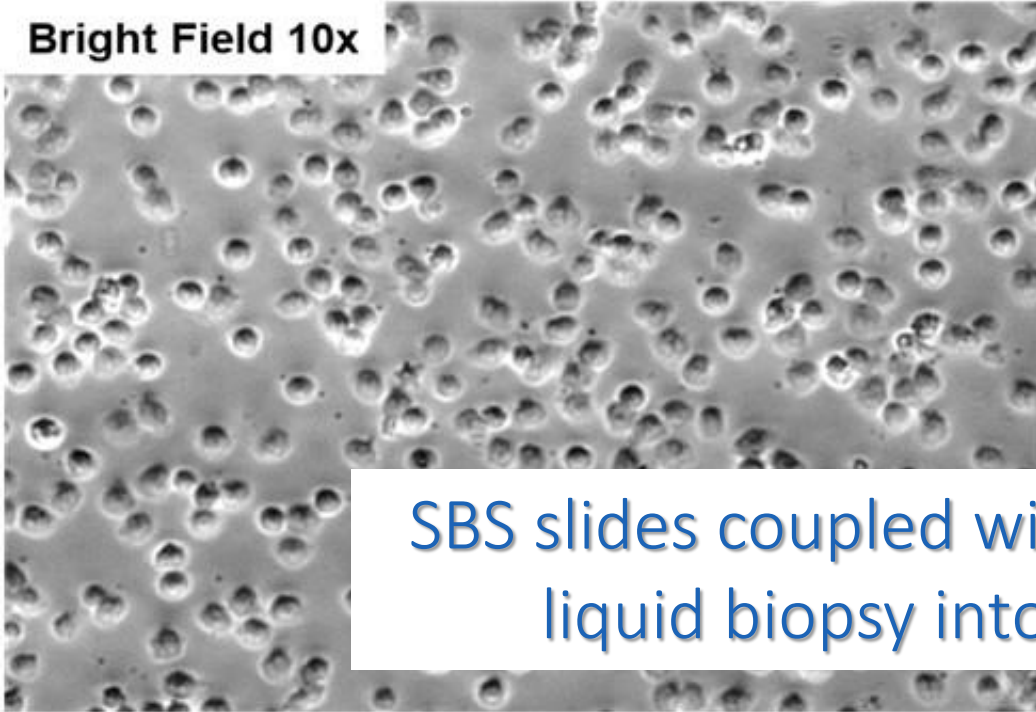


✓ Molecular analysis

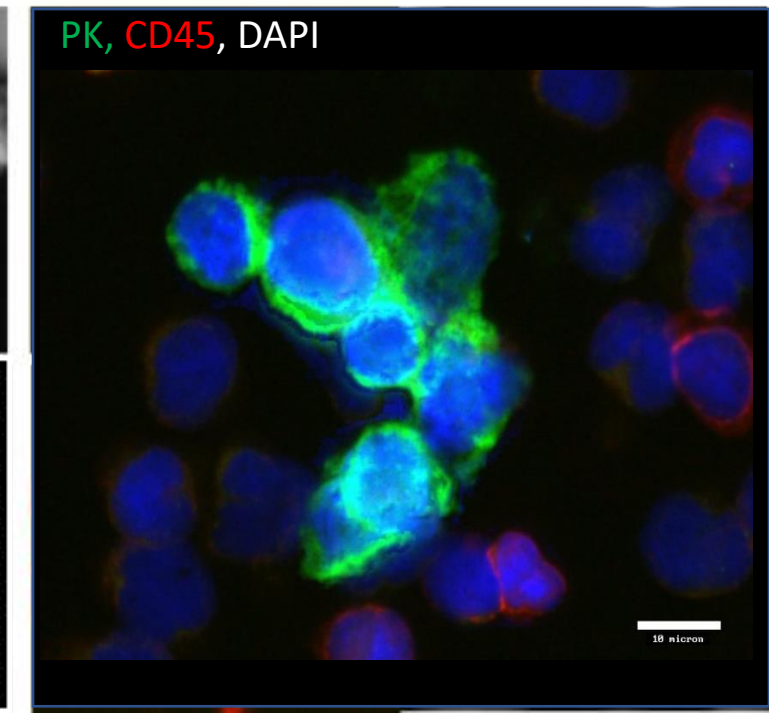
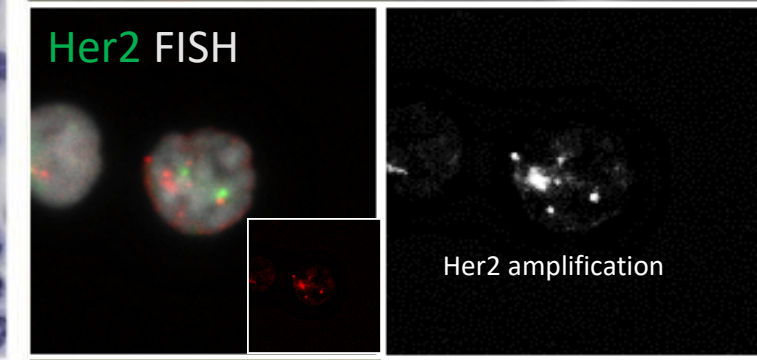
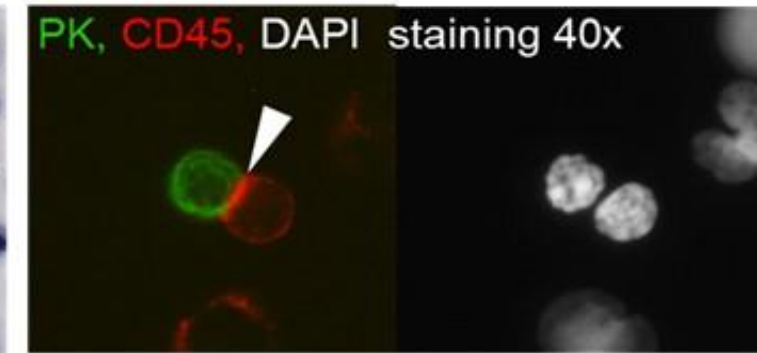
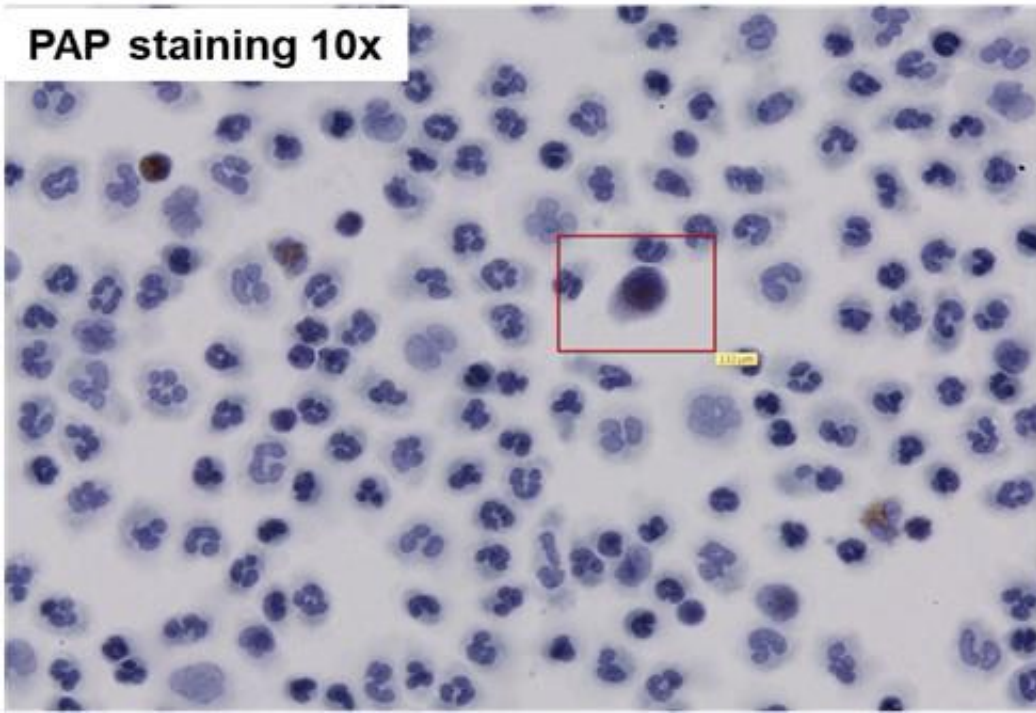


Clinical report of *CTC status*, and
tumour/hematopoietic-biomarkers positivity

Clinical report of *CTC single cell analysis* for specific
molecular biomarker (SNV, CNV, etc..)



SBS slides coupled with CellSeed, is capable to transform a liquid biopsy into a “two-dimensional tissue slide”



Cancer diagnosis and CTCs cluster detection in early breast cancer

Early Breast Cancer Cohort: pilot study

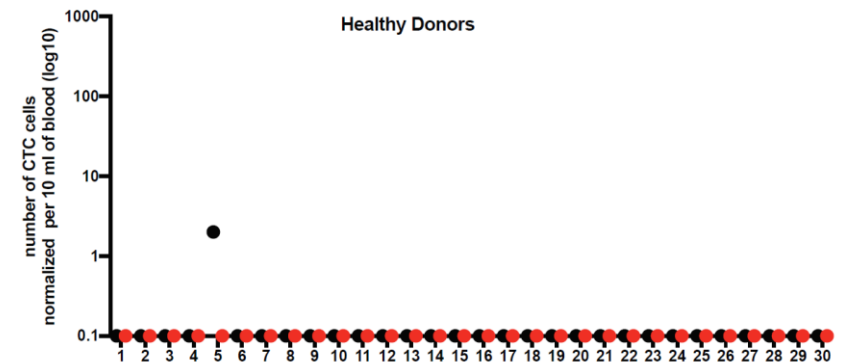
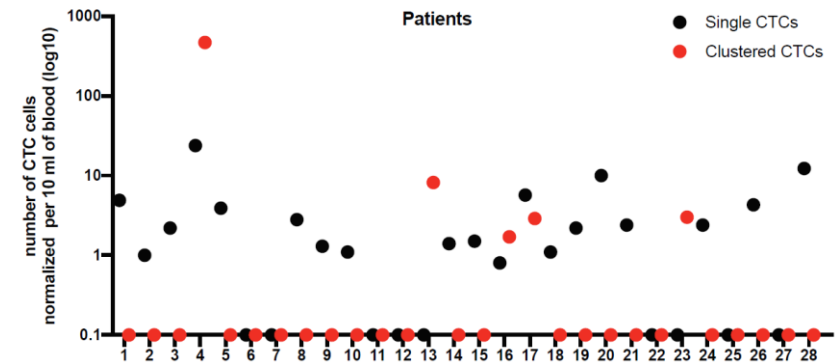
- 28 early stage breast cancer stage I and II (no distant metastasis): patient cohort was characterized by all the different histological subtypes namely ER/PR, Her2 and TNBC tumors
- 30 Healthy Donors
- EDTA collection tubes
- SBS Slides prepared with CellSeed, stored at 4°C then shipped blinded to Tethis Lab

Criteria for CTC positivity:

- cytokeratin (CK)- positivity, CD45-negativity and cancer-related morphological features (e.g. large nucleolus, irregular size and shape, scarce cytoplasm)

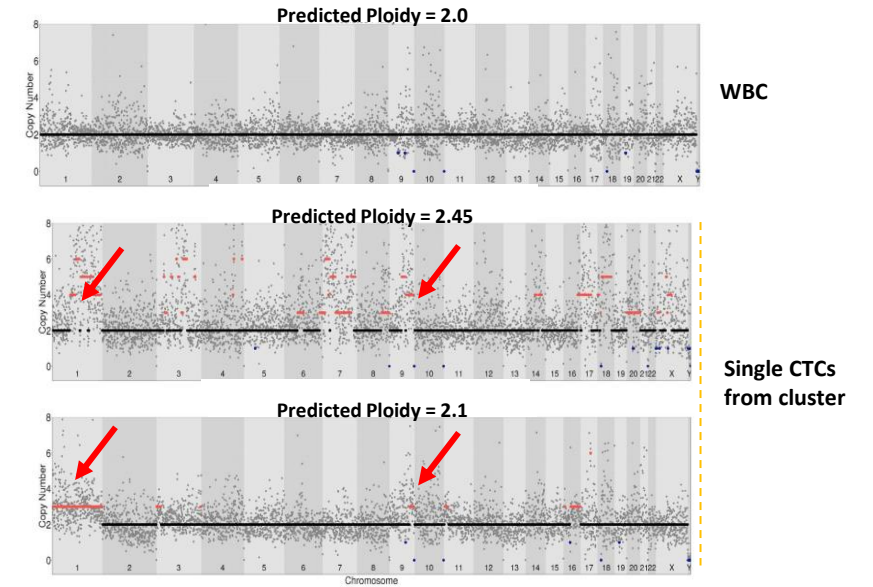
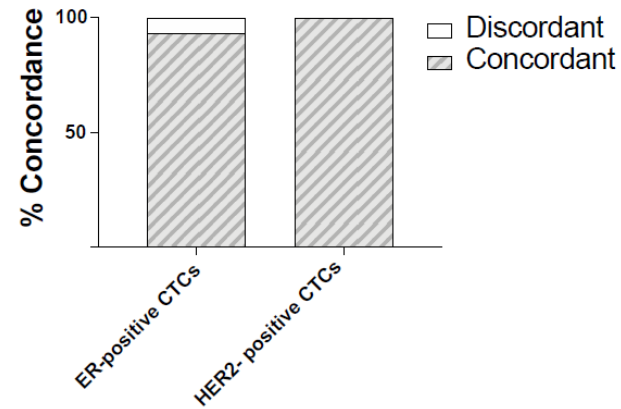
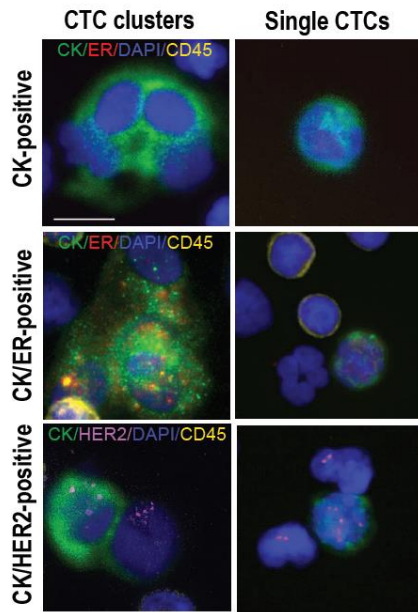
AND:

- presence of multicellular aggregates of CK-positive cells (at least two cells), positivity for the estrogen receptor (ER) or gene amplification of the tyrosine kinase receptor ErbB2 (HER2, evaluated by fluorescence in situ hybridization)



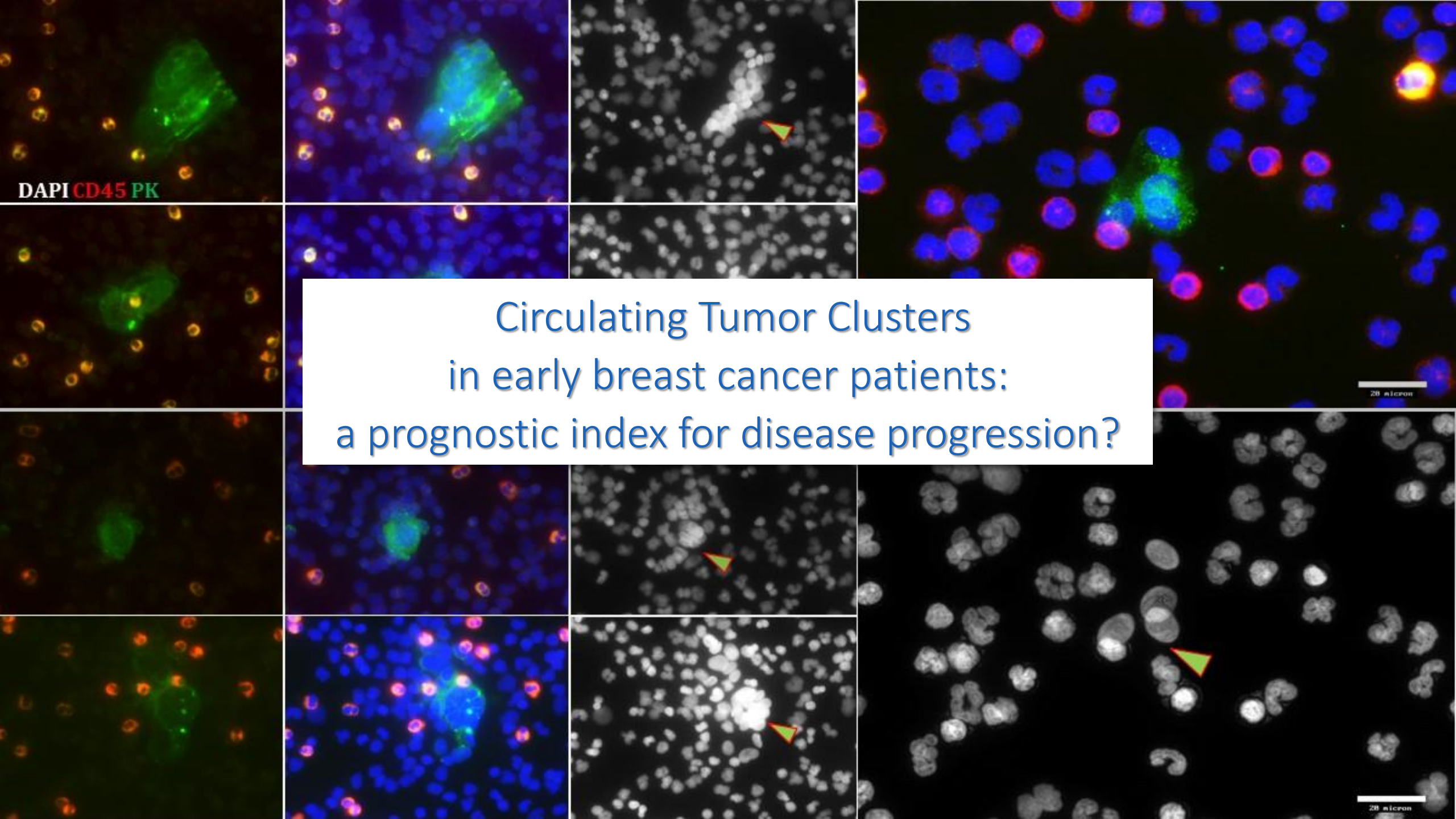
CTC cluster identification in five patients with early breast cancer (three with stage IA and two with stage IIA)

Cancer diagnosis and CTCs cluster detection in early breast cancer



UNPUBLISHED DATA
Copy number profiles of white blood cell controls and CTC clusters

75%	sensitivity all histologies
80%	sensitivity except TNBC
18%	CTC clusters
96.7% specificity	



Circulating Tumor Clusters
in early breast cancer patients:
a prognostic index for disease progression?

DAPI CD45 PK

20 micron

20 micron

Take home message and next steps

- SBS slides, coupled with CellSeed at point of blood collection, is a new liquid biopsy technology achieving highly efficient CTC capture and preservation of morphological features of cells, thus enabling subsequent non-disruptive CTC analysis in clinical samples with multiple analytical assays, at single cell level
- In a blind pilot early breast cancer study of 58 cases CTC clusters have been detected in 18% of patients (with 100% specificity), suggesting that they are not exclusive features of metastatic disease but possibly a general phenomenon of breast cancer, independently of stage and that the metastatic process can be intercepted at its onset
- The possibility that CTC clusters at this stage could represent a risk factor for disease progression still need to be assessed by larger clinical trials that could correctly address this point
- SBS technology provided very high sensitivity and specificity in detecting early breast cancer, opening the perspective for the evaluation of this liquid biopsy approach in many different clinical applications

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