Concordance of CTCs and cfDNA with Tissue Biopsy using See.d® and SmartBioSurface® Slides: A Feasibility Study in Metastatic Breast Cancer

Luise C.[1], Senic-Matuglia F.[1], De Marni M.L.[1], Moretti V.[1], Stepien G.E.[1], Gole L.[1], Chen S.[1], Picocci S.[1], Mazzarella L.[2] and Bascetta L.[1]

[1]Tethis S.p.A., Via Olgiati 5, 20143 Milan, Italy; [2]Istituto Europeo di Oncologia, Via Adamello 16, 20139 Milan, Italy

Introduction

CTCs and cfDNA capture tumor heterogeneity and evolution beyond single-site tissue biopsies[1].
Our study evaluates a novel liquid biopsy workflow in Metastatic Breast Cancer (MBC) by comparing their biomarker profiles with matched metastatic tissue.

See.d® is a liquid handler that automates pre-analytical processing of blood samples[2] collected in EDTA, providing:

- **nucleated cell fraction**, white blood cells (WBCs) and rare CTCs, seeded onto Tethis' **SmartBioSurface® (SBS) slides**[3-4-5]
- **plasma** collected for molecular analysis

Matched **plasma samples** prepared with **See.d®** and **Cell-Free DNA BCT® tubes** were also analyzed using a commercial NGS panel to compare cfDNA mutations (Thermo Fisher).

Methods

Patients	**10 MBC patients** (40% ER+, 20% ER+/HER2+, 40% TNBC)[6]
Blood Processing	- **Streck** Cell-Free DNA BCT®: plasma collected using standard protocol - **See.d®** for plasma in **EDTA** and **SBS slides** (cytology slides)
CTC Analysis[7-8]	- **IF staining** (PanCK/EpCAM + ER + CD45/CD66b) - **H&E staining** for morphological evaluation - ***ERBB2* FISH** (for ER+/HER2+ and TNBC histotypes)
DNA Profiling	- **Plasma**: Oncomine™ Dx Express panel (Thermo Fisher) - **Tissue**: OCA Plus panel (Thermo Fisher)
Definitions	- **Multimodal tumor informativity** : ≥1 CTC (identified by IF and confirmed by cytologist) - **Concordance with Tissue Biopsy**: **Biomarker expression:** ≥1 CTC with ER expression or *ERBB2* amplification **Mutation profiles identified by NGS**: Full concordance: plasma shares all or additional mutation with tissue Partial concordance: plasma shares only some mutation with tissue

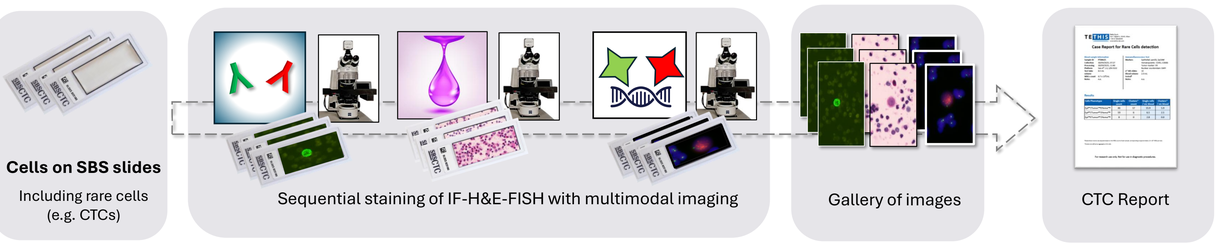


FIGURE 1: Liquid Biopsy workflow for Sequential Staining and Multimodal Imaging.
SBS slides allow the use of multiple staining modalities: Immunofluorescence (IF), Hematoxylin and eosin (H&E), and Fluorescence In Situ Hybridization (FISH) on a single slide: after multimodal staining, a gallery of images is provided for analysis.

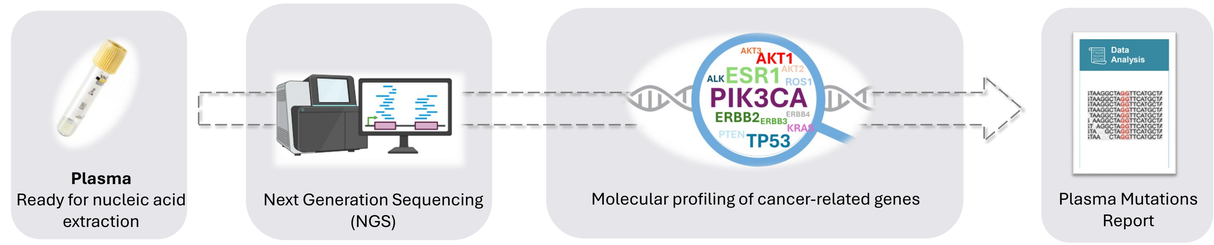


FIGURE 2: Plasma in EDTA collected by See.d®.
Upon See.d® processing, plasma is stored at -80°C for cfDNA extraction, quality and NGS analysis with commercial panels

Results

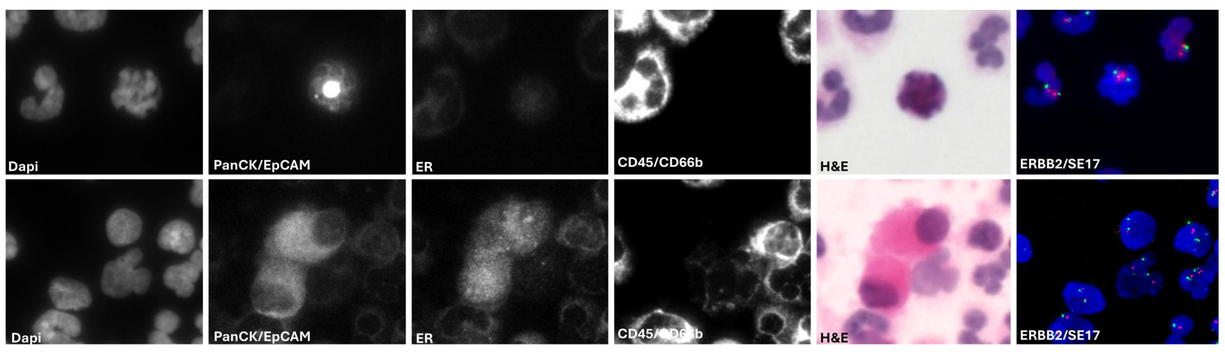


FIGURE 3: Example of multimodal staining in 2 different patients.
Upper panel: patient PT02 with tissue histotype ER+/HER2+ shows no ER expression at single cell level, malignant chromatin features evidenced H&E staini and *ERBB2* FISH amplification;
Lower panel: patient PT10 with tissue histotype TNBC shows a cluster of two cells with ER expression, malignant morphology revealed by H&E staining and no *ERBB2* FISH amplification.

a)

Case ID	Tissue Biopsy Histology	Blood volume analyzed (mL)	single CTC confirmed /mL blood	CTC cluster confirmed /mL blood	Multimodal tumor informativity	CTC confirmed ER^{pos} by IF	CTC confirmed HER2 amplified by FISH
PT01	ER+/HER2+	2.3	1.8	0.0	**yes**	**yes**	**no**
PT02	ER+/HER2+	2.3	3.1	0.0	**yes**	**yes**	**yes**
PT03	ER+	2.3	1.3	0.0	**yes**	**yes**	-
PT06	ER+	5.8	3.1	0.0	**yes**	**yes**	-
PT07	ER+	5.1	0.2	0.0	**yes**	**no**	-
PT08	ER+	3.4	4.7	0.6	**yes**	**yes**	-
PT04	TNBC	2.3	3.1	0.0	**yes**	**yes**	**no**
PT05	TNBC	2.3	3.9	0.0	**yes**	**yes**	**no**
PT09	TNBC	5.8	0.0	0.0	**no**	-	-
PT10	TNBC	4.3	10.2	3.5	**yes**	**yes**	**no**

b)

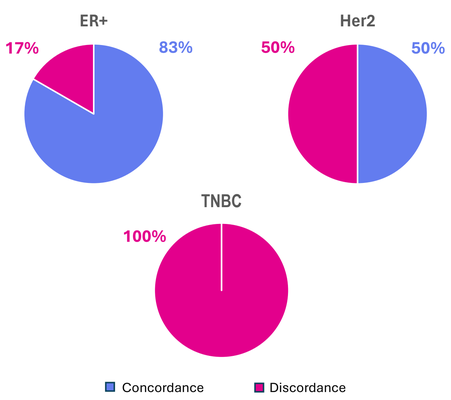
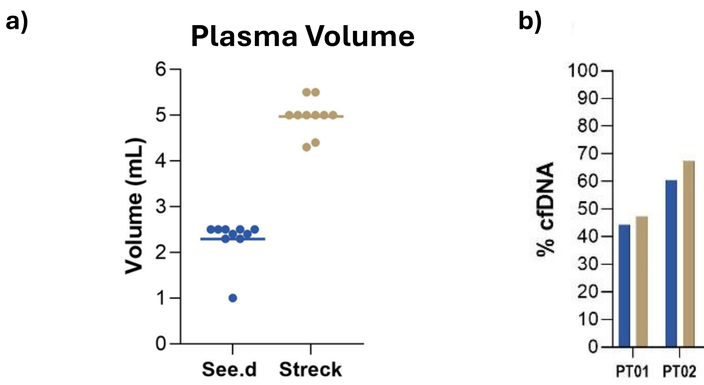


FIGURE 4: a) CTC enumeration and characterization: the table summarizes, for each enrolled patient, the tissue biopsy histology, blood volume analyzed, tumor-informativeness status and enumeration, and biomarker expression of cytologist-confirmed single cells and cluster CTCs;
b) Concordance of biomarker expression between tissue biopsy and CTCs. Pie charts illustrate the proportion of matched and unmatched expression profiles.

CTC Analysis and Biomarkers Concordance with Tissue Biopsies

- **CTCs were identified in 90%** of cases using the multimodal analytical approach
- **Concordance** of CTCs biomarkers characterization with matched tissue was **83% for ER and 50% for HER2**
- No concordance was observed in TNBC samples

a)



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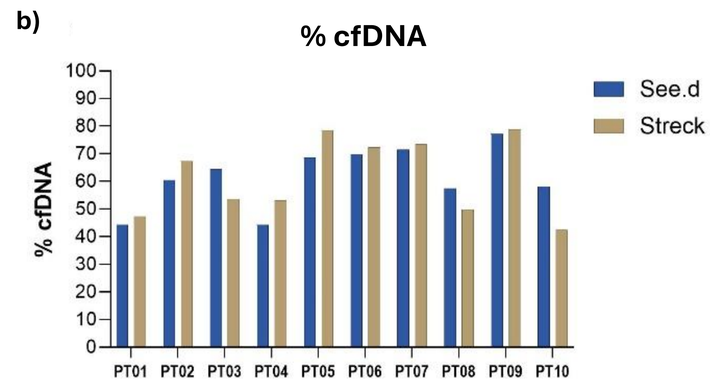

FIGURE 5: Comparison of plasma yield and cfDNA quality.

a) Graph showing the **volume of plasma recovered** after See.d® and manual standard processing (Streck).

b) Graph showing the **percentage of cfDNA** (range 50-300 bp) in samples processed on Tape Station run (size range 50-700 bp) by See.d® and with manual standard processing (Streck).

a)

b)

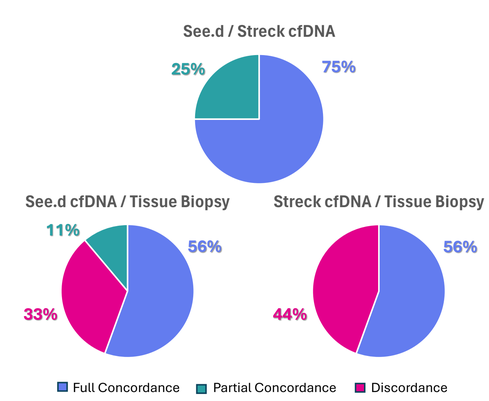

FIGURE 6: a) Oncoplot showing somatic variants detected by sequencing of cfDNA from plasma (processed with See.d® or using the standard manual protocol in Streck tubes) and gDNA from tissue biopsies. Rows represent genes; columns represent See.d®, Streck, and Tissue samples grouped by patient (PT01–10). Mutation types are color-coded: missense (cyan) and multi-hit (yellow).
b) Concordance of mutation profiles. Pie charts illustrate the proportion of matched and unmatched genetic alterations between See.d® and Streck samples and See.d®, or Streck, and tissue biopsy.

cfDNA Concordance between See.d® and Streck (8 evaluable cases)

- Full concordance: **75%**; partial concordance: **25%**

cfDNA vs. Tissue Biopsy Comparison (9 evaluable cases):

- See.d®: full concordance in **55.5%** (PT02 excluded for sequencing failure)
- Streck: full concordance in **55.5%** (PT10 excluded for insufficient material)
- See.d® and Streck: **partial or no concordance** (4 cases), with **clinically relevant variants** (in ESR1 and PIK3CA genes) **exclusively in cfDNA**

Conclusion

In this feasibility study in MBC we show that:

- **See.d® + SmartBioSurface® workflow** is a cutting-edge platform enabling high-efficiency CTC detection and enhanced multimodal analysis
- **See.d® plasma cfDNA:** delivers mutation profiles with clinical relevance fully aligned with standard methods of analysis
- **CTC–cfDNA–tissue concordance:** highlights the power of liquid biomarkers in revealing complementary tumor insights

This **integrated analysis** propels biomarker-driven precision oncology toward broader clinical validation.

Acknowledgements

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CONTACT INFORMATION
contact@tethis-lab.com
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