

# Smart Bio Surface-CTC platform for identification and characterization of Circulating Tumor Cells in solid tumors

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## 1. INTRODUCTION

- **Detection of early cancer onset and tumor evolution** is the key to cure cancer: **circulating tumor cells (CTCs)** [1][2] represent one of the most informative biomarkers.
- Several methodologies exist to identify CTCs [3] **however sensitivity, specificity and clinical utility may vary** depending on pre-analytical and analytical assay settings [4].
- The development of a clinical test requires **standardization, from sample to procedures: Smart BioSurface-CTC platform (SBS-CTC) has been developed to provide clinically informative samples and standardized pre-analytical procedures to achieve maximum assay sensitivity for CTC detection in tumor diagnostics.**

## 2. GOAL

CTCs identification approaches face the need of optimizing *pre-analytical and analytical parameters*.

We planned to fulfill the following requirements:

- **patient sample integrity**
- **assay sensitivity**

### HOW?

1. Preparation of liquid biopsy samples at point of collection in EDTA (*Time To Analysis (TTA) < 6 hours*) through automation

2. *Avoid enrichment* (only red blood cells lysis) maximizing *sensitivity, specificity and preservation of biological sample integrity*

The SBS-CTC platform has been tested on a small cohort of *locally advanced pancreas cancer patients*

## 3. METHODOLOGICAL PROCEDURES

Blood from pancreas cancer patients (LAPC trial) and healthy donors (HD)

4 h TTA

WBC

Red blood cells lysis

1

SBS-CTC slide

proprietary

2

\*Cell seeding through CellSeed

proprietary

Automated

3

Immunofluorescent (IF) staining

(PK, CD45, DAPI)

Automated

4

Automated microscopy and imaging analysis

proprietary algorithms

Automated

5

Selection of putative CTCs

PK+ CD45- DAPI+

6a

FISH (CEP8)

6b

Microdissection and molecular analysis

I.

II.

Wild type

Mutation

nucleus CEP8

CEP8 amplification

Wild type

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SBS-CTC platform

Main features of SBS-CTC platform

## 4. RESULTS

Activity

Location

Instruments

PRE-ANALYTICAL: PATIENT SAMPLE PREPARATION



Blood sampling



CellSeed, for automated patient slides preparation



PATIENT SLIDES TRANSPORTATION



Shipment of patient slides, according to hospital need

Tethis Hub-lab

SLIDES STORAGE AND SAMPLE PROCESSING



High automation & throughput level

Immunological characterization



Imaging analysis



Microdissection platform



Genetic analysis platform



Proprietary SBS-CTC production



SmartBioSurface®

## SBS-CTC slide



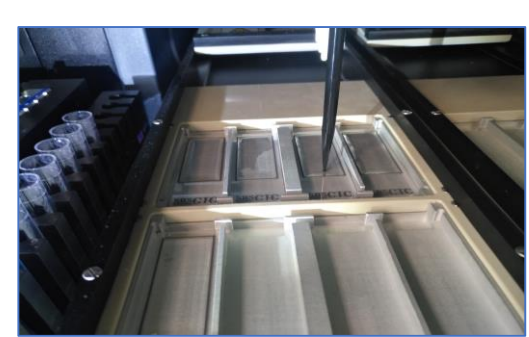
### Nanotechnology

- nano-coating with high biobinding activity [5]
- biocompatibility with primary and cancer cells [6]
- no autofluorescence background
- transparency
- suitable for automation

### Biology

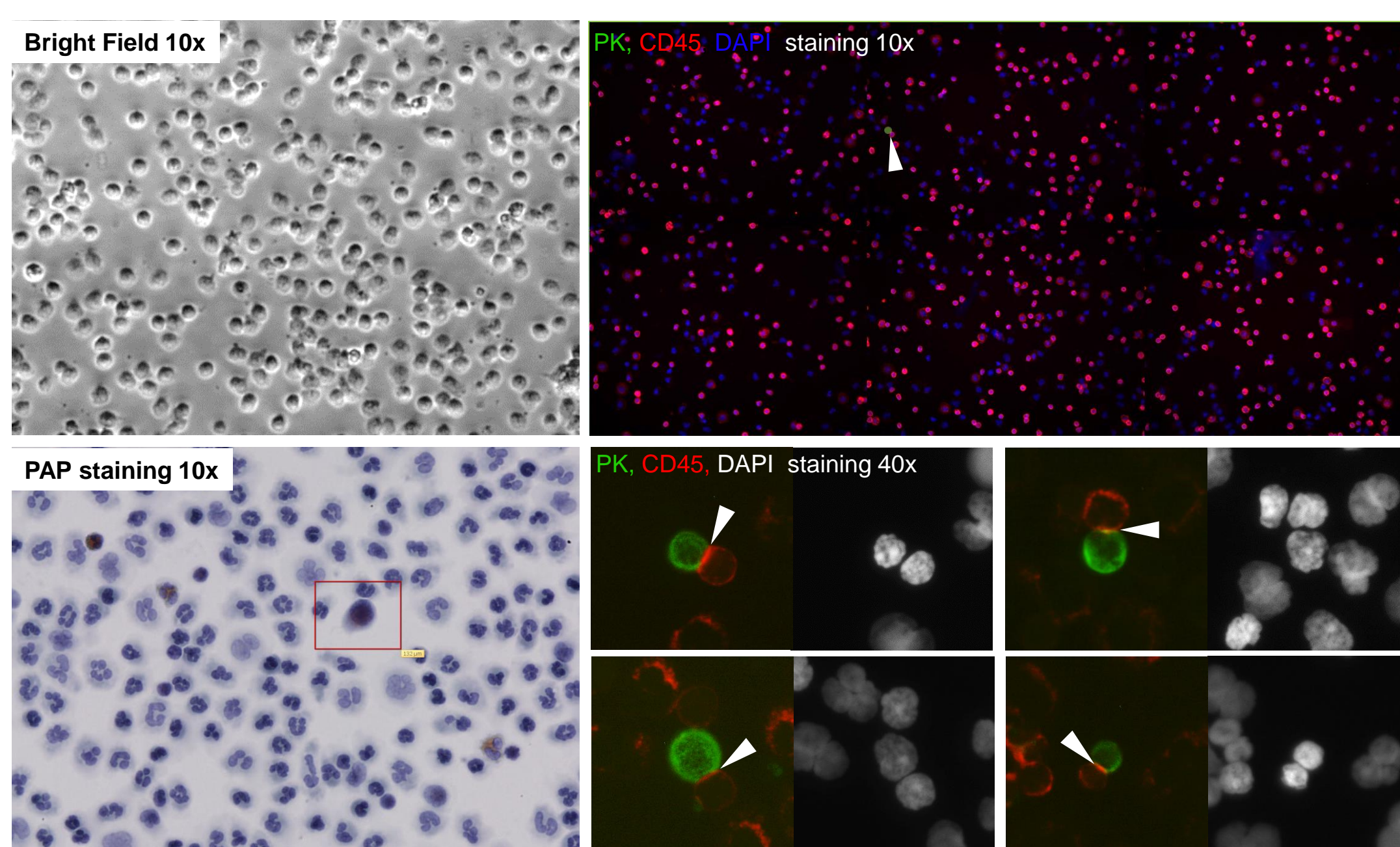
- adhesion of any **spontaneously non-adherent living cell** with no selection
- **99% efficiency of cell immobilization** within 20 min at room temperature (*paper in preparation*)
- **perfect preservation of cell morphology**, gentle adhesion
- suited for IF, ImmunoFISH, IHC staining and automated analysis as well as microdissection and further molecular analysis

## CELLSEED- CUSTOMIZED LIQUID HANDLING FOR AUTOMATED WBC SEEDING AND FIXING



- standardization and reproducibility
- automated processing from cell adhesion, through fixation up to the washings
- minimal reagents use

Examples of patient samples on SBS-CTC slides: bright field, PAP staining and IF staining



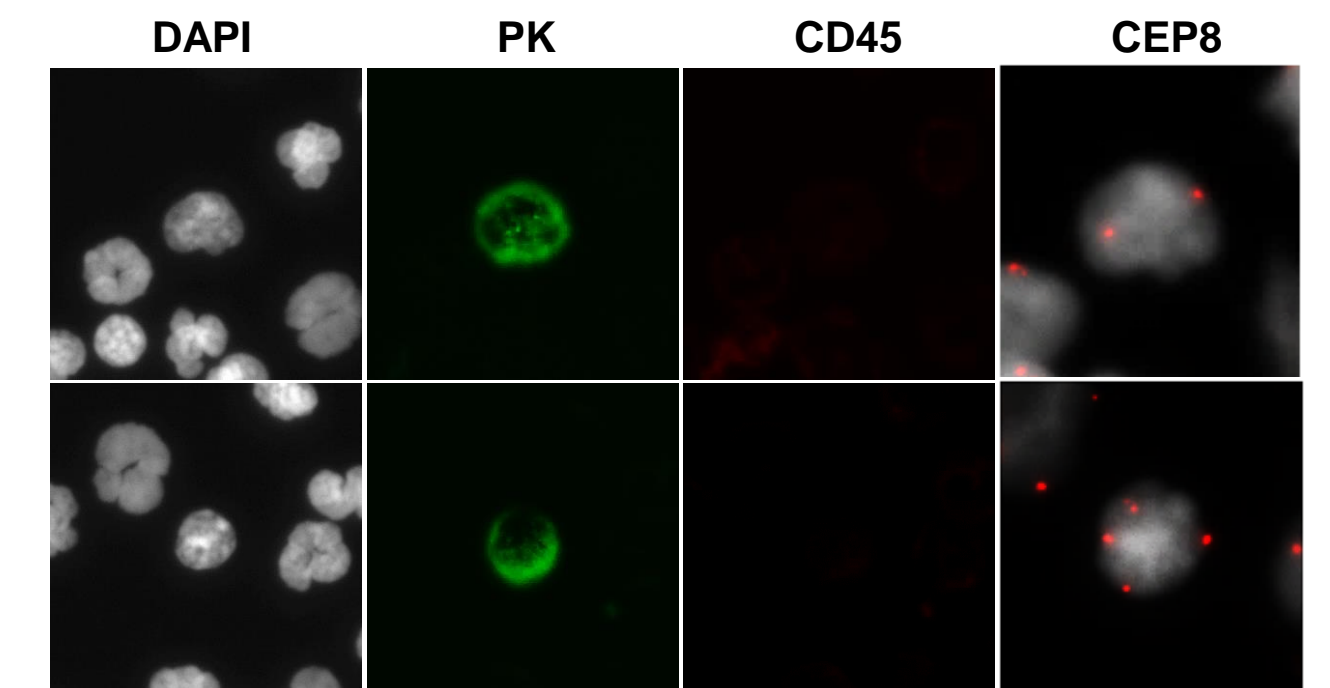
As a **proof of principle of CTCs identification in clinical samples** 5 locally advanced pancreatic cancer (LAPC) blood samples (PNC) and 5 healthy donor blood samples (HD) were tested for **putative CTC associated aneuploidy or KRAS mutation\***

Sample ID	Type of analysis	Number of cells with CEP8 aneuploidy	KRAS mutation
HD01	FISH	0	-
HD02	FISH	0	-
HD03	FISH	0	-
HD04	Molecular analysis	-	WT
HD05	Molecular analysis	-	WT
PNC01	FISH	2	-
PNC02	FISH	2	-
PNC03	FISH	18	-
PNC04	Molecular analysis	-	GGC > AGC G13S codon 13 Gly>Ser
PNC05	Molecular analysis	-	GGT > CCT G12P codone 12 Gly>Pro

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Examples of the analyses:

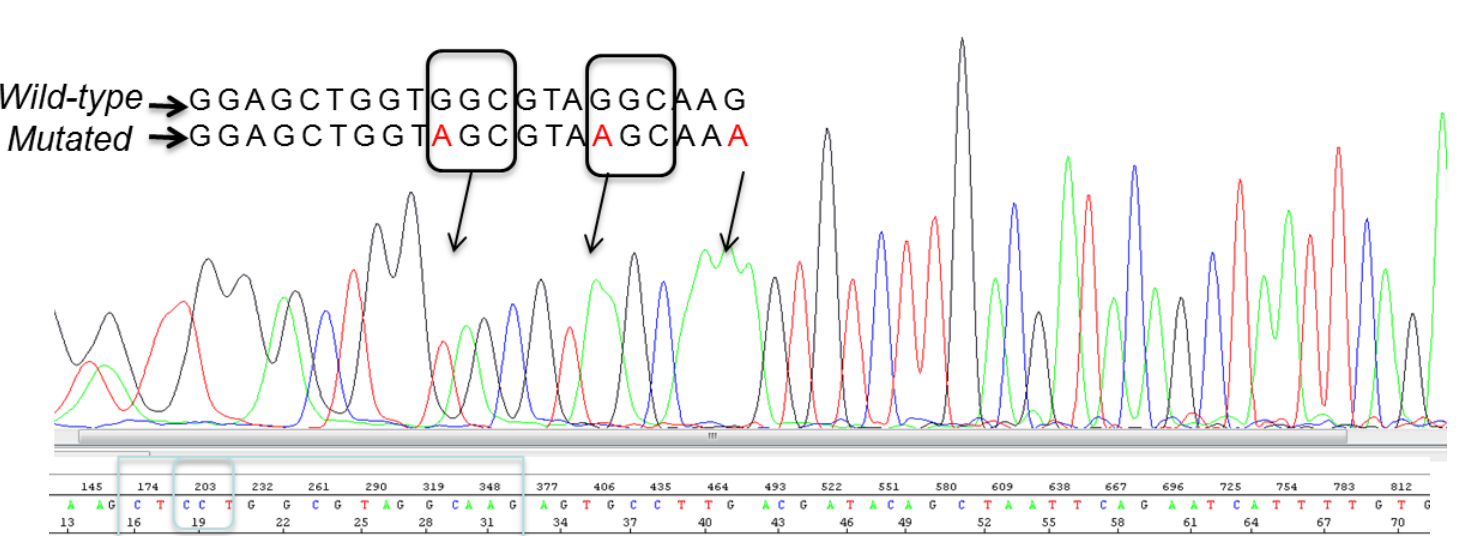
HD01  
WT CEP8 hybridization



PNC01  
CEP8 aneuploidy

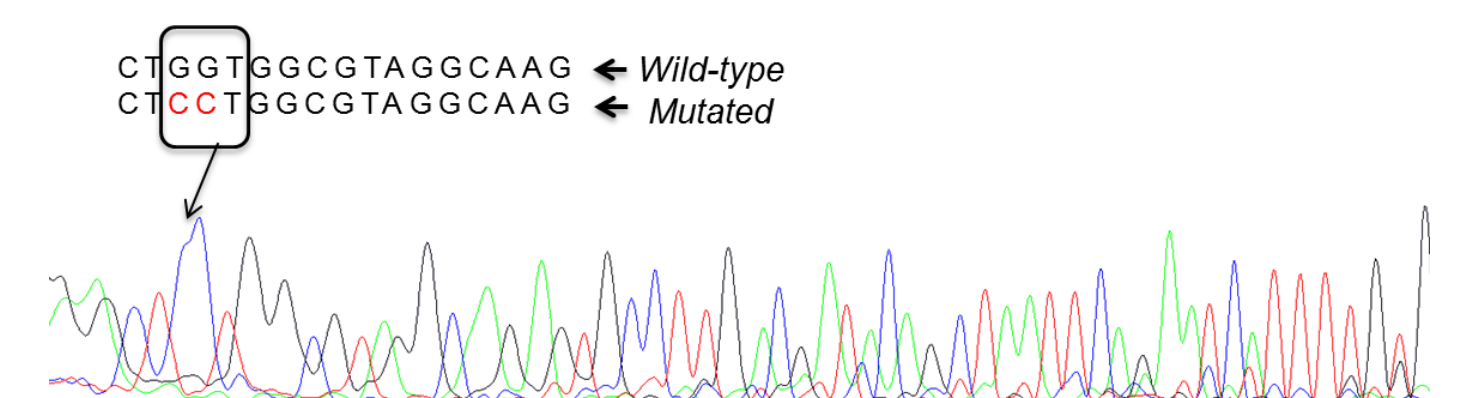
PNC04

Mutation in codon 13: GGC > AGC  
G13S Substitution: Gly>Ser



PNC05

Mutation in codon 12: GGT > CCT  
G12P Substitution: Gly>Pro



## 5. CONCLUSIONS

- **SBS-CTC platform, thanks to short TTA and sample handling automation**, enables CTCs analysis from a **fresh blood sample**, with minimal manipulation from cells to DNA, with the aim to **preserve sample biological integrity, standardized pre-analytical conditions therefore maximizing assay sensitivity**
- **Proof of principle study** on LAPC patients demonstrates the feasibility of SBS-CTC platform in **CTCs identification and characterization**
- **The sensitivity and the specificity of SBS-CTC platform is currently under validation** in different clinical settings, mainly lung and breast, **from early to metastatic disease**

### Bibliography

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