The epitope-independent Parsortix™ system enables isolation, identification and analysis of epithelial and mesenchymal CTCs

Anne-Sophie Pailhes-Jimenez, Senior Scientist

Molecular Diagnostics Europe
24 May 2018 - Lisbon

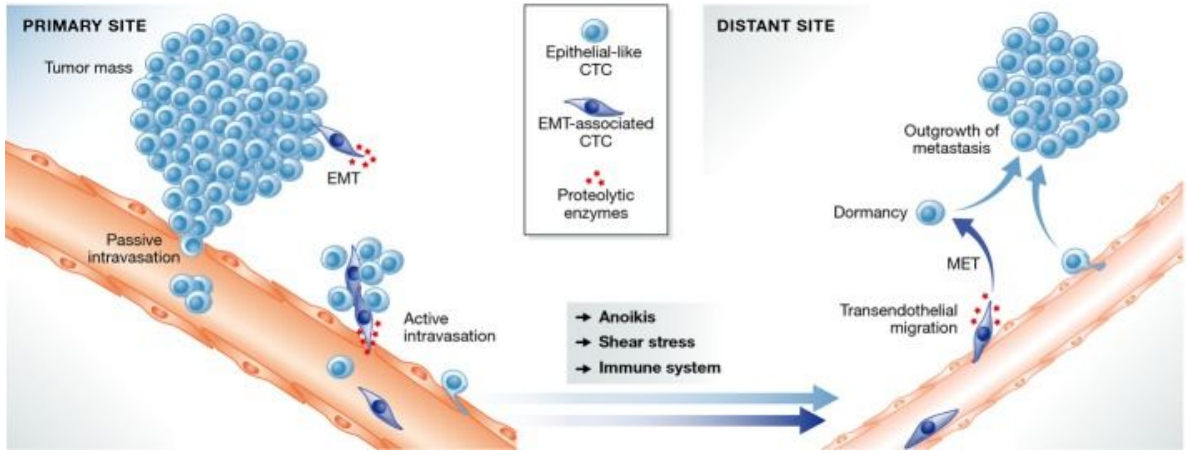
CELLS FOR PRECISION MEDICINE.

INTRODUCTION

Circulating Tumor Cells (CTCs)

Parsortix™ system

Introduction - Circulating Tumor Cells



Biology, detection, and clinical implications of circulating tumor cells. Joosse SA, Gorges TM, Pantel K. EMBO Mol Med. 2015 Jan.

- Reflective of metastatic tumor
- Source of biomarkers
- Non-invasive diagnostics
- Rare event: 1 CTC / 10^8 blood cells

CTCs enrichment

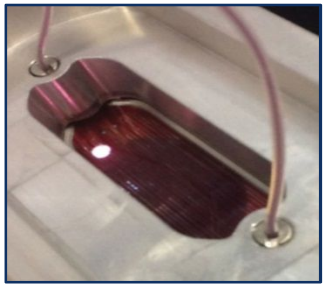
Parsortix™ technology

Platform technology for harvesting rare cells such as CTCs from blood

- based on **physical** cell properties (deformability)
- based on cell **size**
- **epitope-independent** technology

Stepped, microscale cell separators for fluid flow and cell separation

Various cassettes (10, 8, 6.5, 4.5µm) allow customization based on cell type and downstream applications



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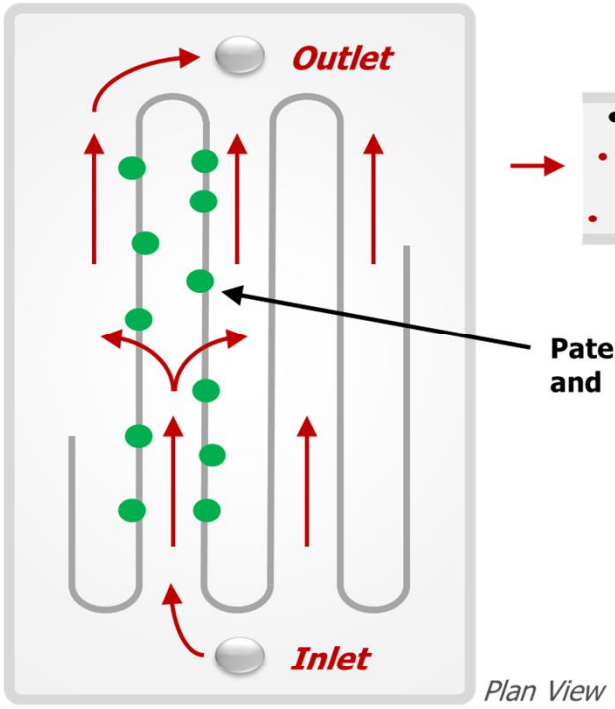
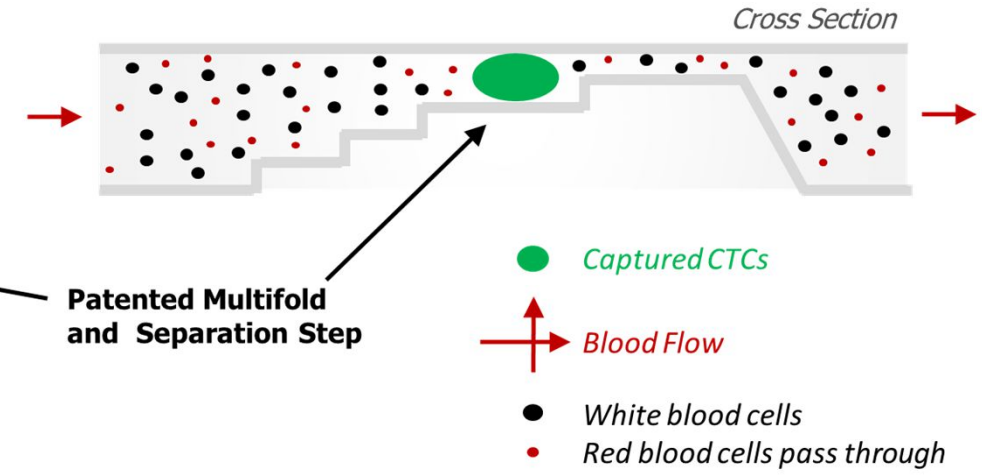
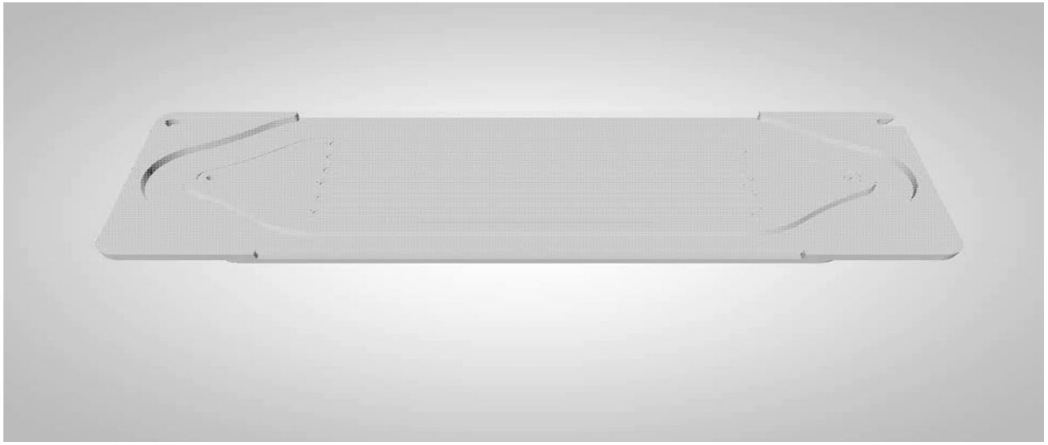
Parsortix cassette





Parsortix™ slide-sized disposable cassette



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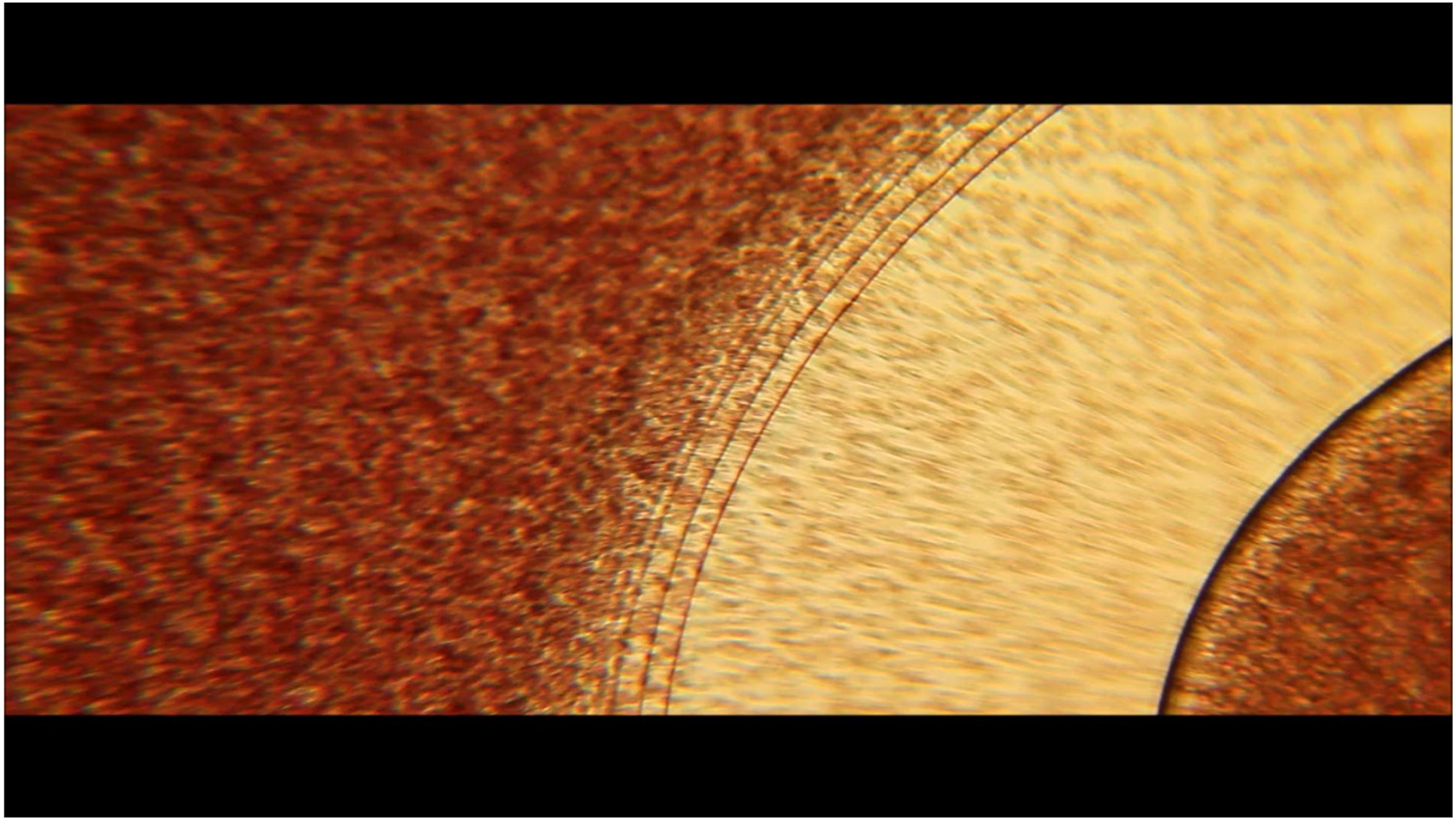
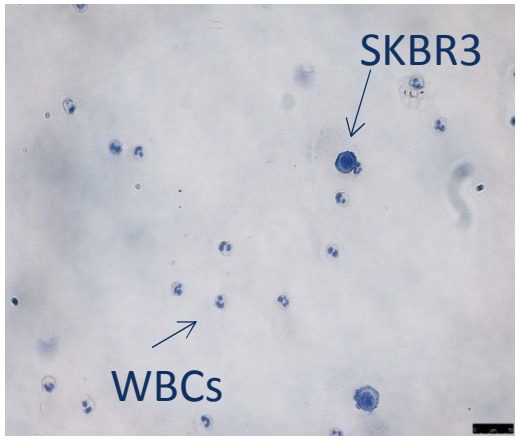
Parsortix microfluidic system



Viable cells captured by Parsortix system

CTCs are captured and enriched by the steps/gap in the Parsortix cassette

- Intact cell morphology



SKBR3 cells enriched by the Parsortix system and stained using Wright's and Giemsa staining

ANGLE's internal data

- Viable cells

→ cell culture and *in vitro* testing

RESEARCH ARTICLE

Optimization and Evaluation of a Novel Size Based Circulating Tumor Cell Isolation System

Lei Xu[1,2], Xueying Mao[1], Ahmet Imrali[1], Ferrial Syed[3], Katherine Mutsvangwa[3,4], Daniel Berney[1], Paul Cathcart[4,5], John Hines[6], Jonathan Shamash[3,4], Yong-Jie Lu[1]*

1 Centre for Molecular Oncology, Barts Cancer Institute, Queen Mary University of London, London, United Kingdom, **2** Department of Urology, Zhongshan Hospital, Fudan University, Shanghai, China, **3** Department of Medical Oncology, Barts Health NHS, London, United Kingdom, **4** Centre for Experimental Medicine, Barts Cancer Institute, Queen Mary University of London, London, United Kingdom, **5** Department of Urology, University College Hospital NHS, London, United Kingdom, **6** Department of Urology, Barts Health NHS, London, United Kingdom

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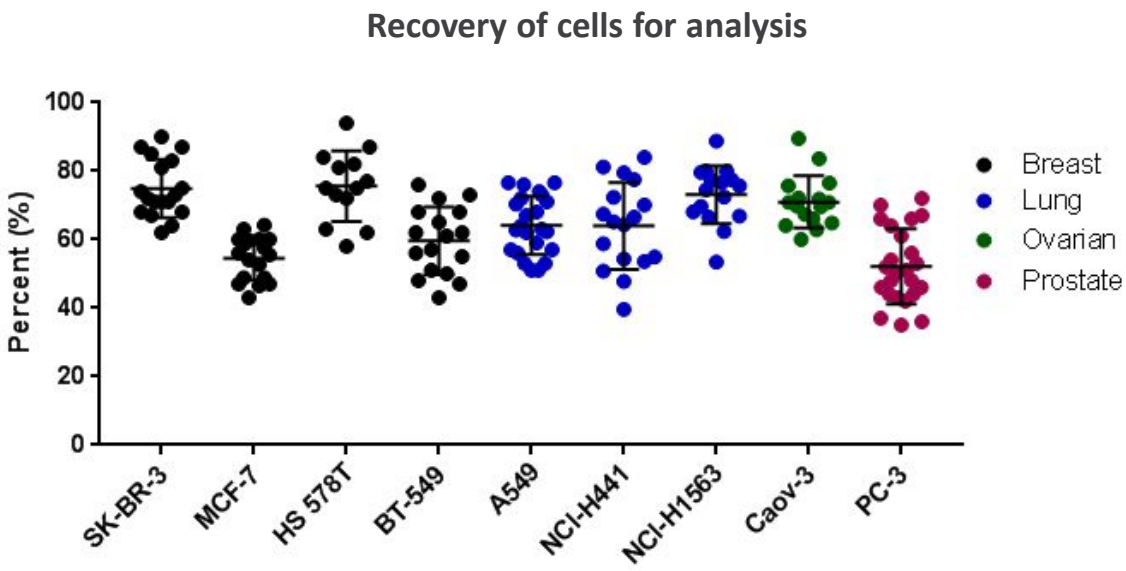
Parsortix system – Recovery of cells

Cancer cells spiked into healthy blood

Separation using **Parsortix** instrument

Cell enrichment



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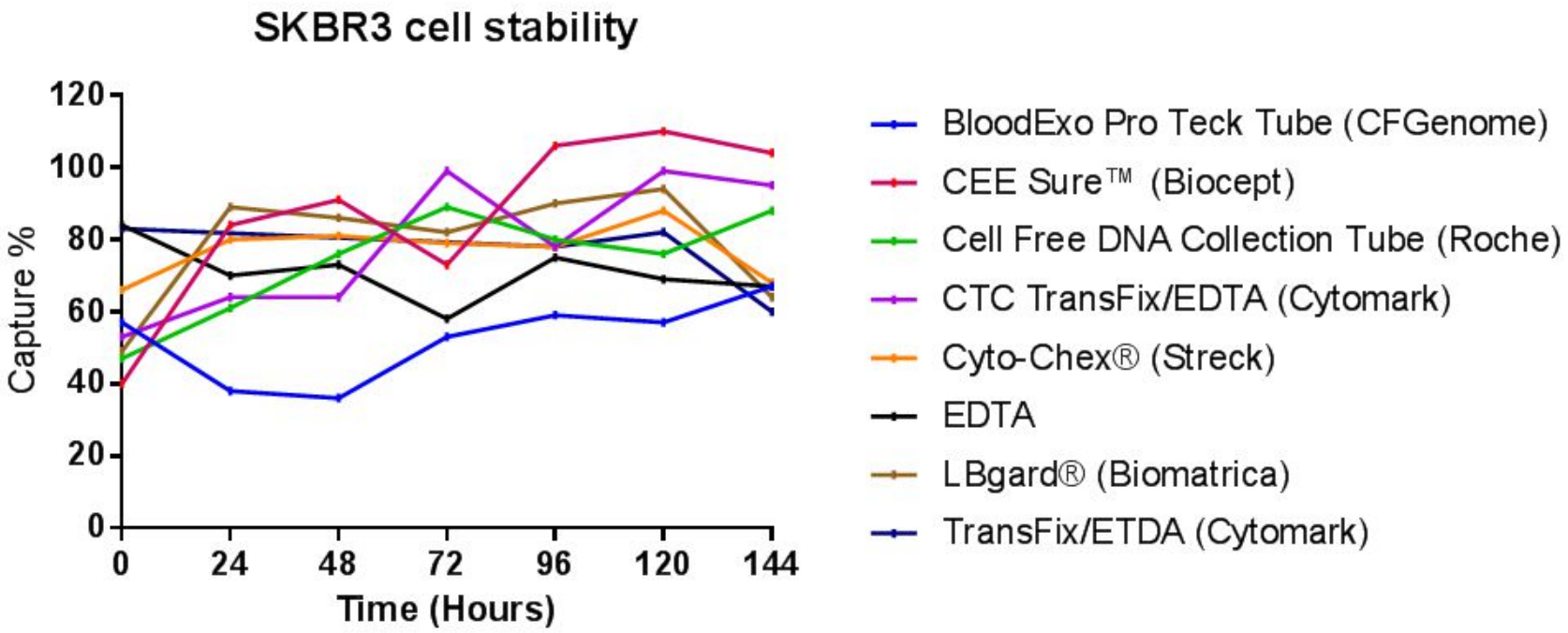
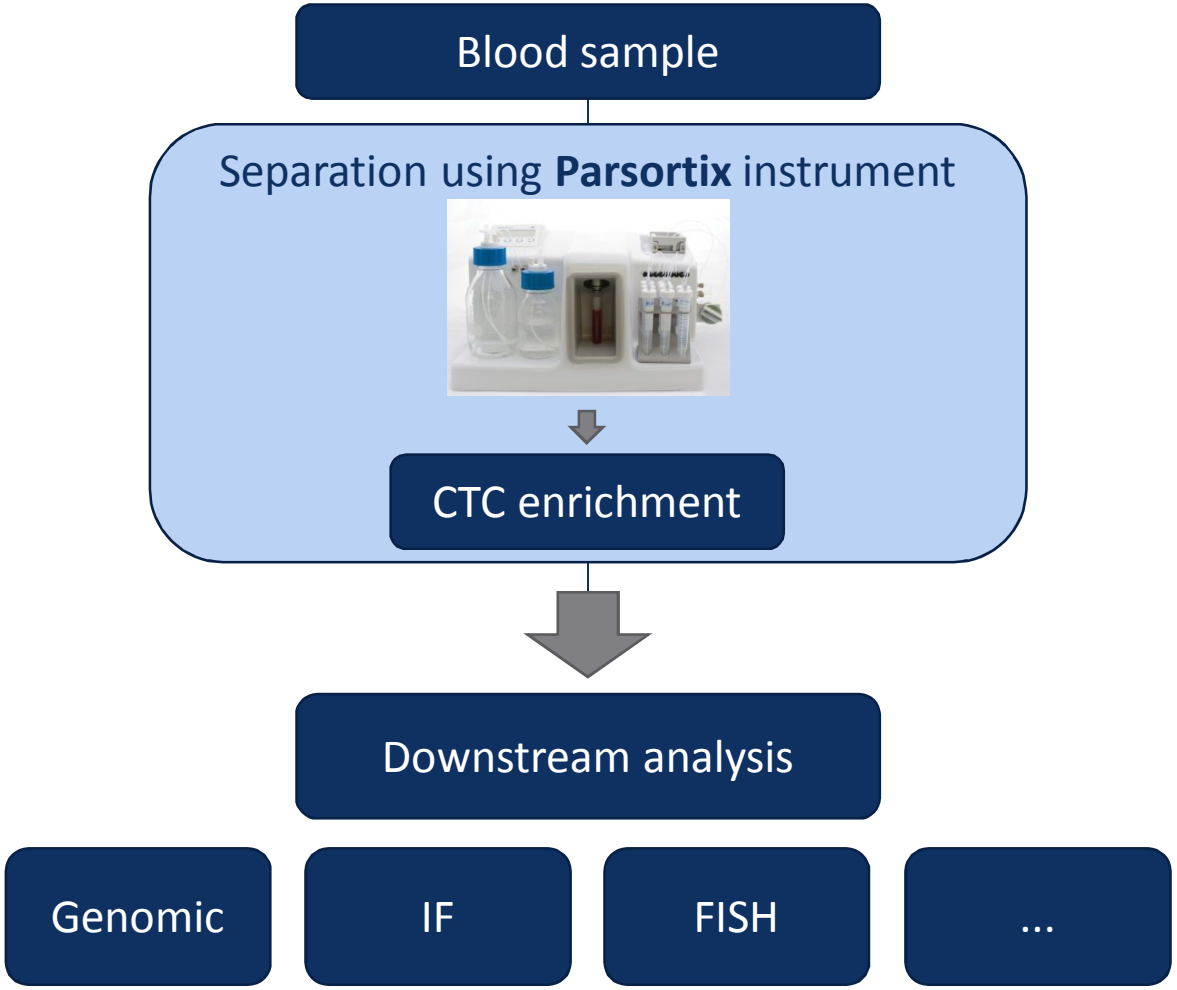
The Parsortix system is able to capture and harvest CTCs <u>independently</u> of cancer type and origin.

Parsortix system – Stability of cells



The Parsortix system is compatible with different preservative blood collection tubes.

Parsortix system - Flexible analyses



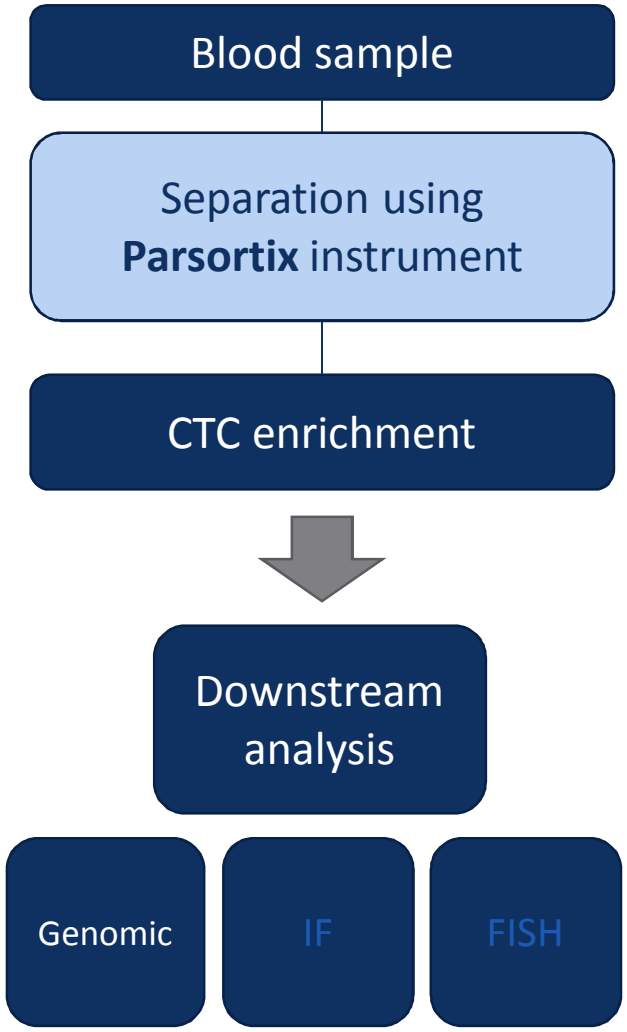
CELLS FOR PRECISION MEDICINE.

ANGLE

DOWNSTREAM ANALYSIS

Some examples

Downstream analysis – Genomic analysis



- Molecular characterisation
- Specific to enriched subpopulations
- NGS/CGH/…
- Compare to ctDNA studies

In Situ Detection and Quantification of AR-V7, AR-FL, PSA, and *KRAS* Point Mutations in Circulating Tumor Cells

Amin El-Heliebi,[1*†] Claudia Hille,[2†] Navya Laxman,[3†] Jessica Svedlund,[3†] Christoph Haudum,[1,4] Erkan Ercan,[1] Thomas Kroneis,[1] Shukun Chen,[1] Maria Smolle,[4] Christopher Rossmann,[5] Tomasz Krzywkowski,[3] Annika Ahlford,[3,6] Evangelia Darai,[3] Gunhild von Amsberg,[7] Winfried Alsdorf,[7] Frank König,[8] Matthias Löhr,[9] Inge de Kruijff,[10] Sabine Riethdorf,[2] Tobias M. Gorges,[2] Klaus Pantel,[2] Thomas Bauernhofer,[4,5] Mats Nilsson,[3] and Peter Sedlmayr[1]

Ziplex® System

International Journal of Molecular Sciences

Article

A Novel Workflow to Enrich and Isolate Patient-Matched EpCAM^{high} and EpCAM$^{low/negative}$ CTCs Enables the Comparative Characterization of the PIK3CA Status in Metastatic Breast Cancer

Rita Lampignano, Liwen Yang, Martin H. D. Neumann, André Franken, Tanja Fehm, Dieter Niederacher and Hans Neubauer *

MDPI

Clinical Chemistry 62:11
000–000 (2016)

Cancer Diagnostics

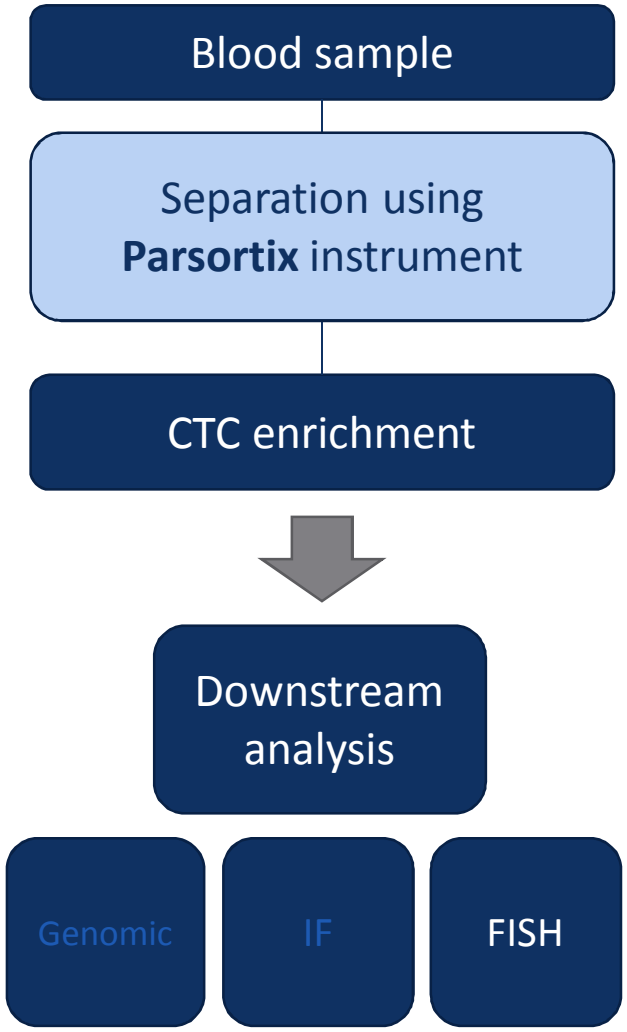
Accession of Tumor Heterogeneity by Multiplex Transcriptome Profiling of Single Circulating Tumor Cells

Tobias M. Gorges,[1*†] Andra Kuske,[1†] Katharina Röck,[1] Oliver Mauermann,[1] Volkmar Müller,[2] Sven Peine,[2] Karl Verpoort,[4] Vendula Novosadova,[5] Mikael Kubista,[5,6] Sabine Riethdorf,[1] and Klaus Pantel[1*]

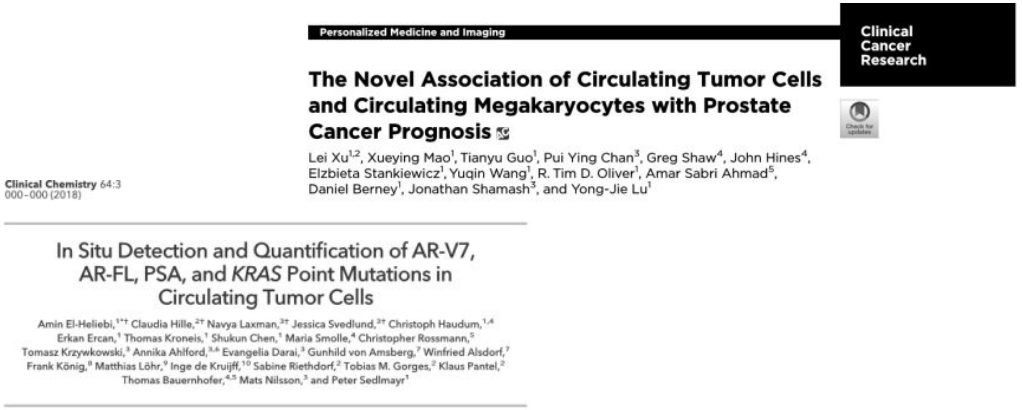
A novel microfluidic platform for size and deformability based separation and the subsequent molecular characterization of viable circulating tumor cells

G.E. Hvichia[1], Z. Parveen[1], C. Wagner[1], M. Janning[2,3,4], J. Quidde[3,4], A. Stein[3,4], V. Müller[5], S. Loges[2,3,4], R.P.L. Neves[6], N.H. Stoecklein[6], H. Wikman[2], S. Riethdorf[2], K. Pantel[2] and T.M. Gorges[2]

Downstream analysis - FISH



➢ Genomic alterations


Personalized Medicine and Imaging

Clinical Cancer Research

The Novel Association of Circulating Tumor Cells and Circulating Megakaryocytes with Prostate Cancer Prognosis

Lei Xu[1,2], Xueying Mao[1], Tianyu Guo[1], Pui Ying Chan[3], Greg Shaw[4], John Hines[4], Elzbieta Stankiewicz[1], Yuqin Wang[1], R. Tim D. Oliver[1], Amar Sabri Ahmad[5], Daniel Berney[1], Jonathan Shamash[3], and Yong-Jie Lu[1]

Clinical Chemistry 64:3 000–000 (2018)

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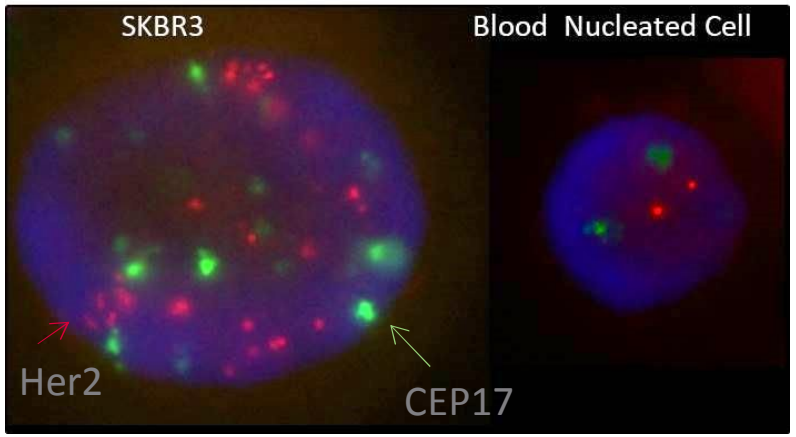
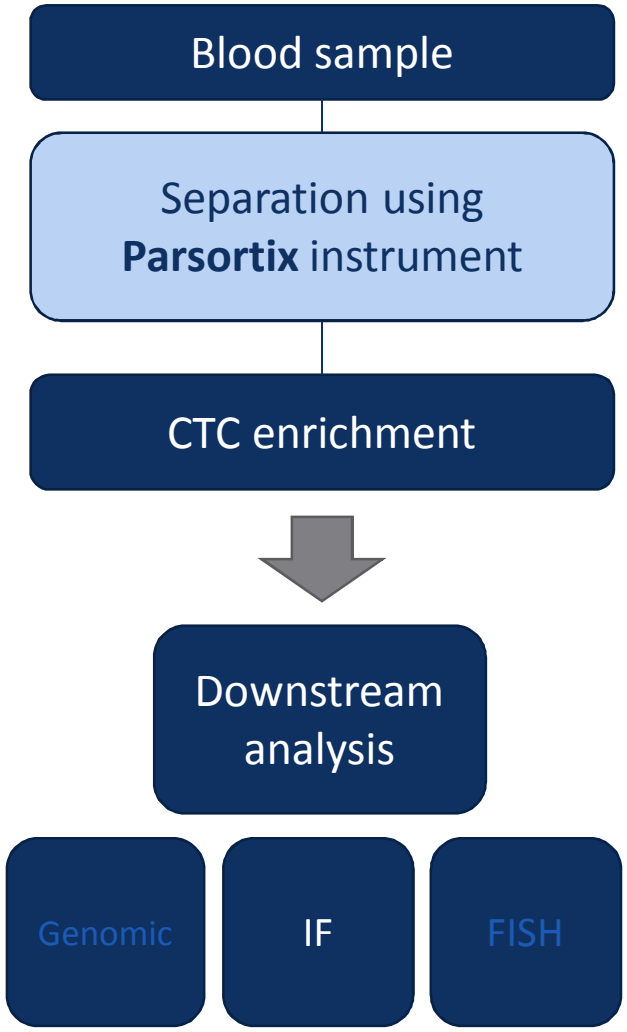


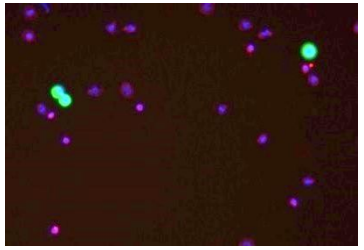
SKBR3 cells enriched by the Parsortix system and hybridized using PathVysion Her-2 DNA Probe Kit

ANGLE's internal data

Downstream analysis - Immunofluorescence



- Morphology
- Real-time visualisation of expressed protein
- Molecular characterisation
- **DAPI^{+}CK^{+}CD45^{-}**



SKBR3 cells enriched by the Parsortix system and stained using CK/EpCAM Ab.
Pailhes-Jimenez et al. manuscript in preparation

RESEARCH ARTICLE

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Analyst. 2016 Jan 21;141(2):669-78. doi: 10.1039/c5an02156a.

Clinical evaluation of a novel microfluidic device for epitope-independent enrichment of circulating tumour cells in patients with small cell lung cancer.

Chudziak J[1], Burt DJ[1], Mohan S[1], Rothwell DG[1], Mesquita B[1], Antonello J[1], Dalby S[1], Ayub M[1], Priest L[1], Carter L[1], Krebs MG[1], Blackhall F[2], Dive C[1], Brady G[1].

www.impactjournals.com/oncotarget/ Oncotarget, Advance Publications 2017

Comparison of isolation platforms for detection of circulating renal cell carcinoma cells

Yvonne Maertens[1], Verena Humberg[1], Franziska Erlmeier[2], Sandra Steffens[1], Julie Steinestel[1], Martin Bögemann[1], Andres Jan Schrader[1] and Christof Bernemann[1]

International Journal of Molecular Sciences

Article

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CTC detection by Immunofluorescence (IF)

Cancer cells spiked into healthy blood

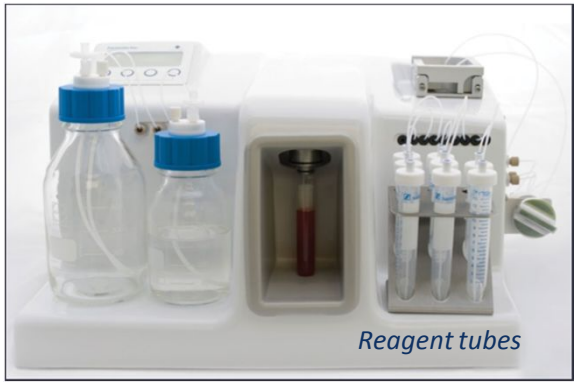
Separation using Parsortix instrument

CTC capture in the cassette

Staining in-cassette

Identification
Enumeration, characterisation

Development of a Parsortix workflow: In-cassette staining



- Automated method
- User-friendly
- Uses a slide-sized cassette
- Minimal cell loss *(spiking experiment)*

CTC detection by Immunofluorescence (IF)

ANGLE

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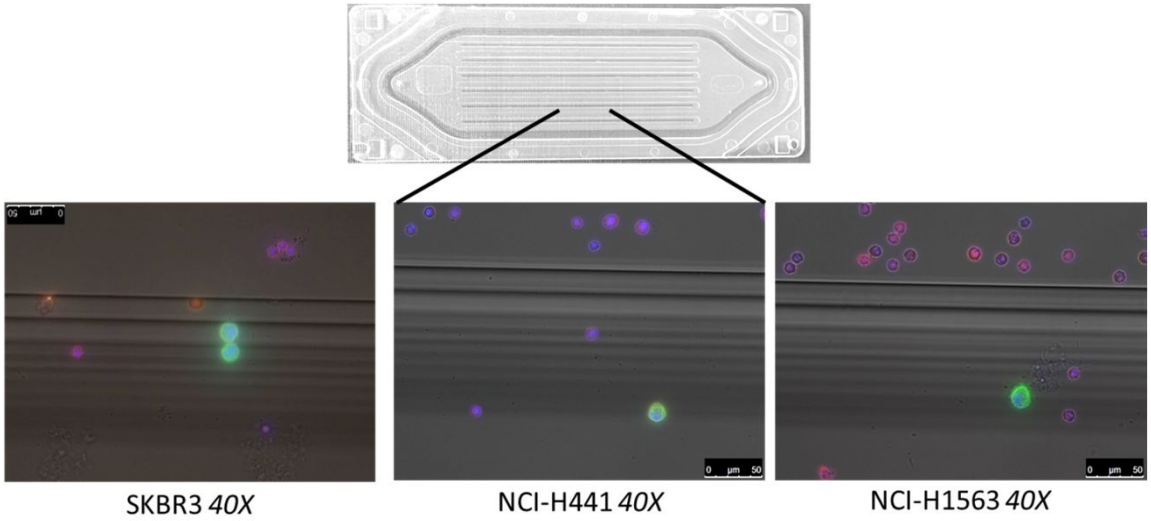

Cancer cells captured by the Parsortix system and stained using CK/EpCAM Abs.

Pailhes-Jimenez et al. manuscript in preparation

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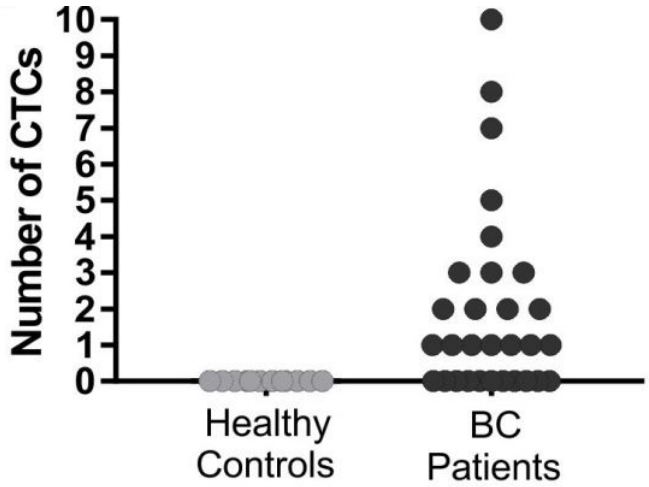
Parsortix in-cassette staining
Patient samples

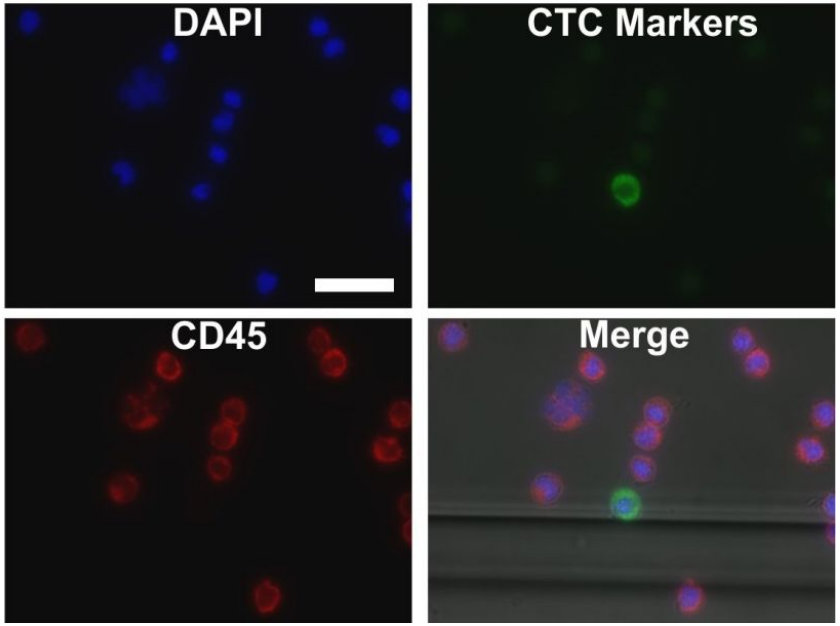
12 healthy donor blood samples
30 **stage I-III** BC patient samples

Separation and staining using **Parsortix** instrument

CTC detection:
0% healthy donor
63% **stage I-III** BC samples



CTCs are detectable in
63% stage I-III BC patient samples.



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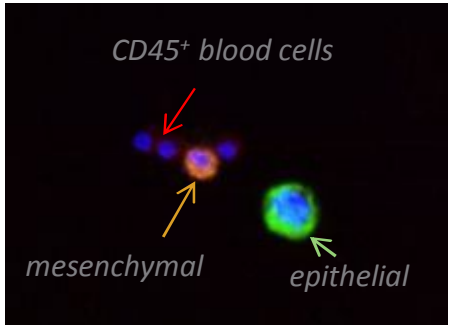
CTC subpopulations detected by Parsortix in-cassette staining

Example: Detection of EMT-associated markers



SKBR3 and Hs578T cells spiked into healthy blood, separated and stained using Parsortix technology.
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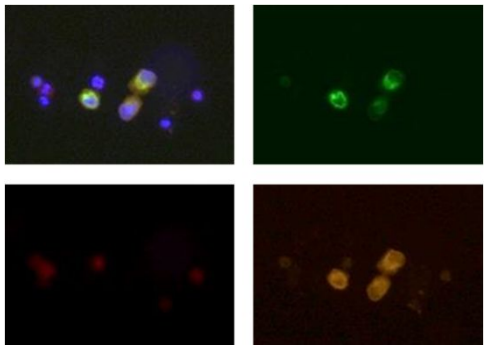
Positive selections:
- Epithelial CTCs (CK/EpCAM based)= green
- Mesenchymal CTCs (CONFIDENTIAL)= orange

Negative selection:
- White Blood Cells (CD45 detection)= red

Example: Detection of phosphorylated proteins



PDBu-treated A549 cells spiked into healthy blood, separated and stained using Parsortix technology.
Pailhes-Jimenez et al. manuscript in preparation

Positive selections:
- Epithelial CTCs (CK/EpCAM based)= green
- Phosphorylated ERK = orange

Negative selection:
- White Blood Cells (CD45 detection)= red

Parsortix in-cassette staining - Flexibility

Parsortix in-cassette staining workflow=
semi-automated and flexible method allowing for the isolation and identification of CTCs by immunofluorescence

- Semi-automated method:
  - In-cassette staining using Parsortix instrument
  - Fast identification of captured cells (few hours)
  - Uses a standard fluorescent microscope
- Flexible method:
  - Use of different blood stability tubes
  - Combination of targets/biomarkers (6 reagent tubes available)
  - Possibility to harvest cells after in-cassette staining

In-cassette staining and single-cell analysis

Stained cells can be harvested and used for further analysis

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- Single cell multiplexing RNA profiling
- *In-situ* mutation detections (AR-V7)
- FISH
- Single cell + sequencing

CELLS FOR PRECISION MEDICINE.

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CONCLUSIONS

Conclusions

Blood sample

Separation using **Parsortix** instrument

CTC enrichment

Downstream analysis

Cell culture

Genomic analysis

Single cell analysis

FISH

IF

CGH

(q)PCR

Cytology
Morphology

NGS

Thank you

ANGLE's R&D team and selected collaborators

Parsortix™ system enables isolation, identification and analysis of CTCs

ANGLE

Questions?

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