SURFACE MARKER INDEPENDENT ENRICHMENT OF CIRCULATING OR DISSEMINATED CANCER CELLS FROM BLOOD OR LYMPH NODES USING A MICRO-FLUIDIC DEVICE

Nataša Stojanović[1]*, Kathrin Weidele[1]*, Sebastian Scheitler[1], Barbara Alberter[1], Michaela Raab[2], Aleksandra Markiewicz[2], Sebastian Haferkamp[3], Philip Renner[4], Christoph A. Klein[1,2], Bernhard Polzer[1]

[1]Project Group "Personalized Tumor Therapy", Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM), Regensburg, Germany; [2]Chair for Experimental Medicine and Therapy Research, University of Regensburg, Regensburg, Germany; [3]Department of Dermatology, University of Regensburg, Regensburg, Germany; [4]Department of Surgery, University Medical Center Regensburg, Regensburg, Germany; *Authors contributed equally

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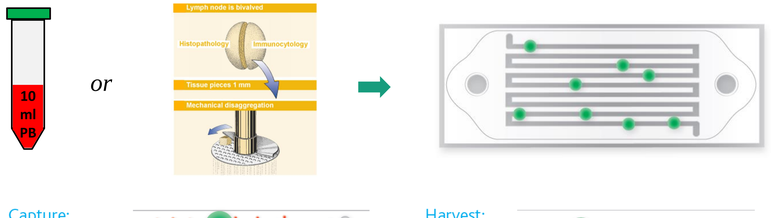
Background

- Detection of disseminated cancer cells (DCCs) in sentinel lymph nodes is important for determining staging and subsequent treatment of melanoma patients.
- We used micro-fluidic device for marker-independent enrichment of tumor cells from whole blood (Parsortix®, ANGLE plc) and adopted it for DCC detection from lymph node cell suspensions.
- Pre-stained MelHo cells were detected with efficiencies of 92.9% (from blood) and 65% from sentinel lymph nodes (SLN).
- Lymphadenectomy suspensions from melanoma patients were stained with MCSP antibody and DCCs were detected with 80% efficiency.
- Single cell DNA was amplified by a deterministic whole genome amplification method (Ampli1TM WGA) and subjected to mutational analysis (BRAF, NRAS).
- Whole transcriptome amplification (WTA) of single cells and transcriptome analysis of selected genes was performed as well as culturing of Parsortix® enriched cells.

Workflow



$$Capture\ rate = \frac{Nr.\,of\ captured\ cells\ on\ the\ cassette}{Nr.\,of\ spiked\ cells} \times 100 \qquad Harvest\ rate = \frac{Nr.\,of\ harvested\ cells}{Nr.\,of\ spiked\ cells} \times 100$$

Adopted from ANGLE plc and Ulmer et al., 2014 PLOS Med.

Conclusions

- Disseminated cancer cells from lymph node samples can be detected with the surface-marker independent Parsortix® system.
- Enriched tumor cells are viable and therefore suited for downstream molecular analysis or in vitro culture, opening new opportunities for translational research.

Contact and Acknowledgements

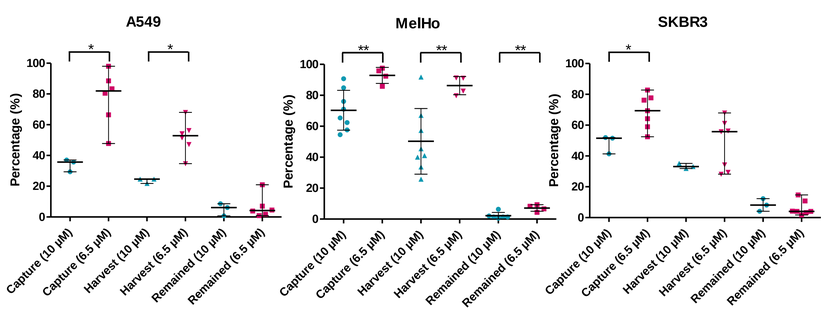
Natasa.Stojanovic@item.fraunhofer.de Kathrin.Weidele@item.fraunhofer.de

Christoph.Klein@ukr.de Bernhard.Michael.Polzer@item.fraunhofer.de

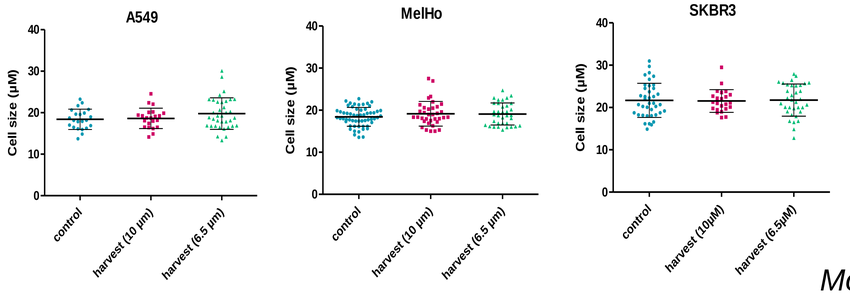
Results I

Isolation of spiked-in cancer cells from blood

Cassette comparison (capture/harvest rates)

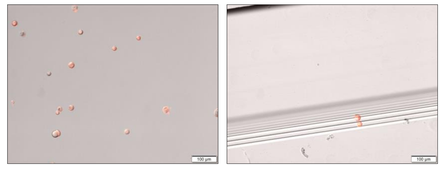


Cell size comparison before and after harvest

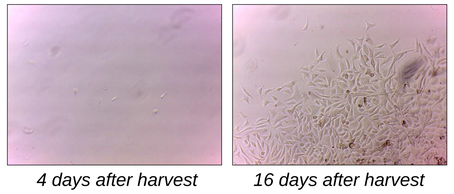


MelHo propagation in culture after Parsortix

control *in cassette*

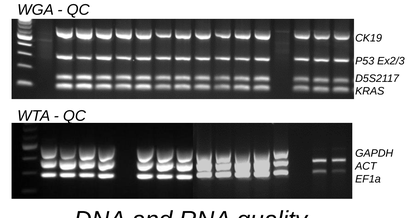


169 harvested cells were propagated

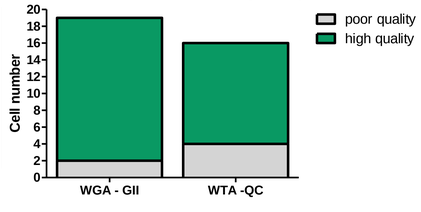


4 days after harvest *16 days after harvest*

Single cell (MelHo) genome and transcriptome analysis



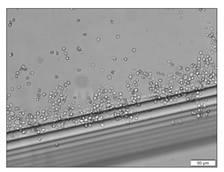
DNA and RNA quality



Results II

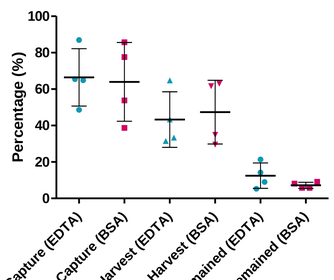
Isolation of DCCs from lymph node

Lymph node processed on Parsortix

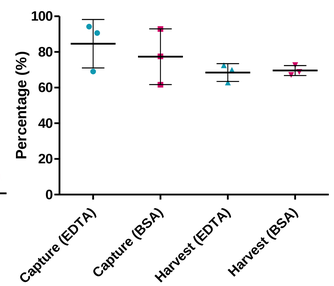


PBS priming

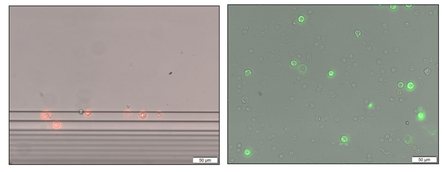
EDTA vs BSA priming (MelHo spiked in LN)



EDTA vs BSA priming (patient LN)

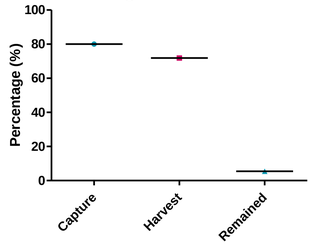


Melanoma patient sentinel lymph node



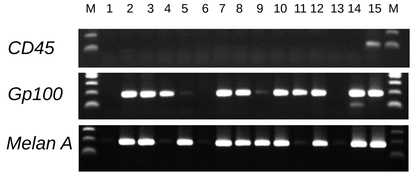
Parsortix (MCSP in cassette staining) *density gradient (MCSP staining)*

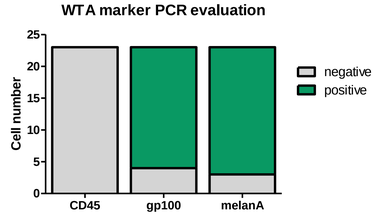
Parsortix vs density gradient



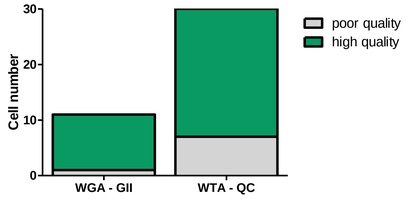
Molecular analysis of DCCs isolated from LN using Parsortix system

Transcriptome analysis





DNA and RNA quality



12/12 cells had hot spot mutation in NRAS exon 2 (p.G12C)