Establishment and evaluation of a novel method for size separation of disseminated tumor cells from cancer patient bone marrow by a microfluidic platform

André Koch, Florin-Andrei Taran, Sara Y. Brucker, Andreas D. Hartkopf

Department of Women's Health, Universityclinic Tuebingen, Calwerstrasse 7, 72076 , Tuebingen, Germany

UNIVERSITÄTS KLINIKUM TÜBINGEN

Department für **Frauengesundheit**

3rd ACTC Advances in Circulating Tumour Cells
Liquid Biopsy in Clinical Practice
October 4th - 7th, 2017, Rhodes, Greece

1 ABSTRACT

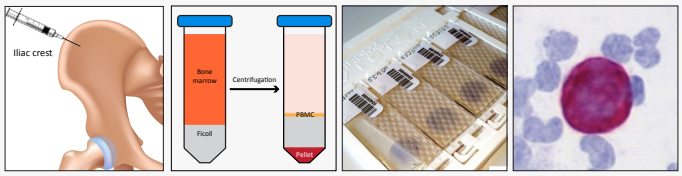
Background: The dissemination of individual tumor cells is a common phenomenon in solid epithelial cancers. In primary breast cancer these disseminated tumor cells (DTCs) have been found in the bone marrow (BM) of around 20-30% of the patients. The detection of DTCs has been correlated not only to metastatic relapse but also to locoregional relapse. Characterizing DTCs is often difficult due to their general rarity in BM aspirates. The method of choice for detecting DTCs is an antibody-based staining for various cytokeratins after density gradient centrifugation of the BM to enrich for mononuclear cells. In experiments with spiked cancer cells we show that this procedure is accompanied by a tremendous reduction of target cells with an average of up to 80% cell loss.

Methods: Here we provide a fast and easy method for bone marrow preparation to enable the use of the size based cell enrichment system, Parsortix™. The system has already been established as a tool for capturing circulating tumor cells (CTCs) from cancer patient blood. The here established and evaluated method helps to increase capture rate of initially spiked cancer cells in patient bone marrow to 75% on average.

Results and Conclusion: The Parsortix™ microfluidic platform has recently been established for the size based enrichment of CTCs from cancer patient blood. This is the first report on a successful establishment of a protocol for detecting DTCs using cancer patient bone marrow with this device. The method is easy to use and can presumably be applied to other body fluids for detection of tumor cells in liquid biopsies.

2 Standard DTC-detection

Standard procedure of DTC-detection in bone marrow form cancer patients

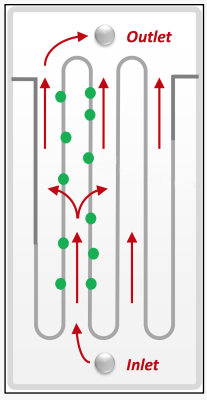


Bone marrow aspiration | PBMC isolation | Immunohistochemistry | Analysis

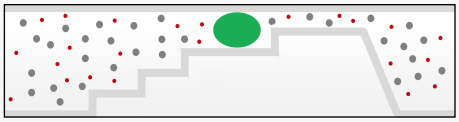
≈20% of cancer patients are DTC-positive (detected by Pan-Cytokeratin-antibody)

3 Parsortix

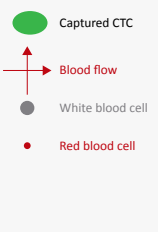
Principle of marker-independent enrichment of tumor cells by a microfluidic platform

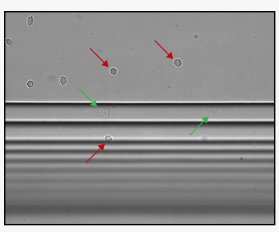


Plan view



Cross section through cassette



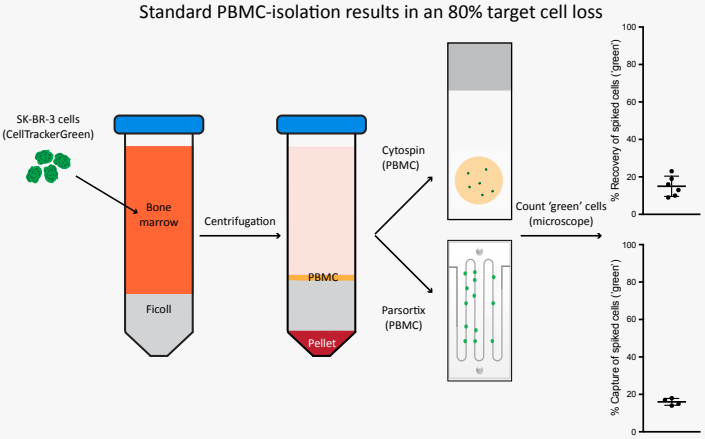


Example of captured CTCs

- Size-based capture of tumor cells
- Blood cells can pass through
- Various step sizes available (6/10 µm)
- Automated in-cassette antibody staining
- Harvest of cells out of the cassette for downstream analysis

4 Results I

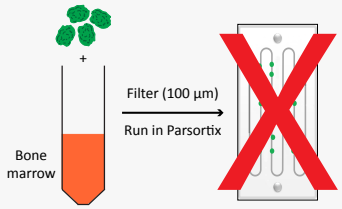
Standard PBMC-isolation results in an 80% target cell loss



-> Parsortix seems to capture a high amount of available cells in the PBMC-fraction

5 Results II

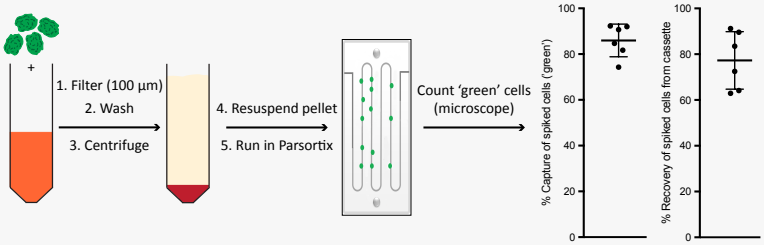
Establishment of a new work protocol without PBMC-isolation needed



-> Running filtered bone marrow clogs the cassettes

6 Results III

Establishment of a protocol for processing patient bone marrow before running through Parsortix



-> The newly established protocol allows a recovery of about 80% of input target cells

-> 75% of captured cells can be harvested for downstream analyses

-> Low pressure run drastically reduces the amount of cells during harvesting process

7 Summary

- Spiked tumor cells in bone marrow can be isolated by Parsortix
- Newly established protocol increases the amount of recovered cells by 60%

Outlook

-> 1st experiments for isolation of patient DTCs look promising

-> Establish workflow for downstream analyses of captured DTCs

Acknowledgements

We would like to thank Angle PLC for giving us the opportunity to develop this protocol by using the Parsortix system. Special thanks goes to Sabine Hofmeister and the team of the immunocytology laboratory of the University Women's Clinic in Tübingen and to all patients for donating bone marrow.