Circulating Tumor Cells in metastatic lung cancer enriched by EpCAM expression and physical characteristics


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Background

Circulating tumor cells (CTC) measured with the CellSearch system in patients with metastatic carcinomas are associated with poor survival. The frequency of CTC detected by the CellSearch system in non-small cell lung cancer (NSCLC) patients is relatively low, raising the question: are some CTC not detected by the CellSearch system?

To investigate this, a device was designed that collects the sample material of the individual samples that are discarded by CellSearch. This collected waste is filtered for CTC isolation based on physical characteristics and the CTC are stained with a cocktail of antibodies. Also, additional antibodies were added to the CellSearch system to broaden the coverage of cytokeratins and leukocytes.

Methods

The Autoprep Sample Collection Device (ASCD) uses optical sensing to detect the presence of blood in the waste tube of the CellSearch system. It collects the waste of individual samples in a 50 ml conical tube. After collection, the blood is passed with 100 mbar pressure through a 8 μm² microfiltrated silicone microcircuit containing 60,000 pores of 5 μm in diameter.

Figure 2 The Autoprep Sample Collection Device (ASCD) collects the waste from sample patients (A). A pump with disposable filtration unit filters the cells collected by the ASCD (B).

Staining

The staining protocol and image analysis was tested with healthy donor samples.

Conclusions

We combined the CellSearch system with a device for collecting and filtering the CellSearch waste. On cell lines this demonstrated that low EpCAM expression results in the presence of CTC in the waste, that otherwise would not be detected by the CellSearch system. In NSCLC additional CTC can be detected but it still remains to be determined whether these CTC – not detected by the original CellSearch approach – are also of clinical relevance.
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ABSTRACT 4825
AACR 2014

Introduction
Circulating tumor cells (CTC) measured with the CellSearch system in patients with metastatic carcinomas are associated with poor survival. The frequency of CTC detected by the CellSearch system in non-small cell lung cancer (NSCLC) patients is relatively low, raising the question whether some CTC are not detected by the CellSearch system. To investigate this, a device was designed that collects the sample material of the individual samples that are discarded by CellSearch. This collected waste is filtered for CTC isolation based on physical characteristics and the CTC are stained with a cocktail of antibodies. Also, additional antibodies were added to the CellSearch system to broaden the coverage of cytokeratins and leukocytes.

Results
The recovery percentage of the CellSearch system for the different cell lines used was: 2% of COLO320, 91% of SW480, 2% of T24 and 87% of SKBR3. Additional recovery on the microsieves after filtration of the CellSearch waste was: 18% of COLO320, 6% of SW480, 59% of T24 and 2% of SKBR3. The combined recovery accounts for 20% of COLO320, 97% of SW480, 61% of T24 cells and 89% of SKBR3. In patients with NSCLC and SCLC either no CTC were detected at all, or in various proportions in the CellSearch cartridge, on the microsieves after filtration of the CellSearch waste or with the additional antibodies that were added.

Conclusions
We combined the CellSearch system with a device for collecting and filtering the CellSearch waste. On cell lines this demonstrated that a low EpCAM expression results in the presence of CTC in the waste that would not be detected by the CellSearch system. Additional CTC can be detected in NSCLC, but it still remains to be determined whether the CTC not detected by the original CellSearch approach are also of clinical relevance.

Methods
A device was designed that uses optical sensing to detect the presence of blood in the waste tube of the CellTracks Autoprep. It collects the waste of individual samples in a 50 mL conical tube. After collection, the blood is passed with 100 mbar pressure through a 8x8 mm2 microfabricated silicon microsieve containing 300,000 pores of 5 μm in diameter (VyCAP, Deventer, The Netherlands). The performance was tested using four pre-stained cell lines: Colo320 (size 11 μm, ~1,161 EpCAM antigens), SW480 (size 11 μm, ~63,233 EpCAM antigens), T24 (size 16 μm, ~2,167 EpCAM antigens) and SKBR3 (size 16 μm, ~445,000 EpCAM antigens). Cells are spiked in 7.5 mL of blood collected in CellSave tubes from healthy volunteers. Spiked blood samples from healthy donors and patients with NSCLC (enrollment is ongoing) were processed on the CellSearch and filtration system between 24 and 96 hours of collection. Additional antibodies were added to the CellSearch test to cover all cytokeratins and broaden the coverage of leukocytes. The cells on the microsieves were stained with a nucleic acid dye, antibodies recognizing leukocytes and antibodies recognizing all cytokeratins.

ABSTRACT CATEGORY AND SUBCLASSIFICATION
TB04 Tumor Metastasis    TB04-08 CTC/DTC detection
RESEARCH TYPE CLASSIFICATION    Translational research
KEYWORDS    CTC NSCLC EpCAM

* Research funded by the EU FP7 CTCTrap Project
# Research funded by Janssen Diagnostics