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

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Methods
The Autoprep Sample Collection Device (ASCD) 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 80 μm2 microfiltration silicon microcircuit containing 60,000 pores of 5 μm in diameter.

Cell lines
The performance of the ASCD and microsieve filtration was tested using four pre-stained EpCAM+ and EpCAM− cell lines: Colo320, T24, SW480 and SKBR3. The staining protocol and image analysis was tested with healthy donor samples. 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 in a manual count. The preliminary results showed that these CTC – not detected by the original CellSearch approach – are also of clinical relevance.

Table 2
Table 2: Samples from healthy donors: not spiked and spiked with 10 7 CTC (μm3) of the EpCAM− non-small lung cancer cell line NCI-H1650.

Staining
Image processing steps to determine the expression of extra markers. A threshold for DAPI and PE is determined for all images in a cartridge. Each event above the DAPI threshold is evaluated in all channels. If an overlapping PE/DAPI is found, this is used to measure additional markers.

Patient study
Patients with NSCLC (enrollment is ongoing) were processed on the CellSearch and filtration system between 24 and 96 hours of collection. The cells on the microsieves were stained with a nucleic acid dye and antibodies recognizing leukocytes and all cytokeratins. Additional antibodies were added to the CellSearch test to cover all cytokeratins and broaden the coverage of leukocytes.

Conclusion
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.

Figure 2
Figure 2: Threshold gallery showing a CTC and white blood cells after filtration on a sieve.

Figure 3
Table 2: Image analysis of CTC found in NSCLC patients. *Some CTC/FITC+ events are due to debris which would be discarded in a manual count.