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

Sanne de Wit[1*], Guus van Dalum[1#], Joost van Dalum[1], Aufried T.M. Lenferink[1], Arjan G.J. Tibbe[2], Cees J.M. van Rijn[3], T. Jeroen N. Hiltermann[4], Harry J.M. Groen[4], Leon W.M.M. Terstappen[1]

s.dewit@utwente.nl

[1] Department of Medical Cell BioPhysics, MIRA institute, University of Twente, Enschede, The Netherlands; [2] VyCAP, Deventer, The Netherlands;
[3] University of Wageningen, Wageningen the Netherlands; [4] Department of Pulmonary diseases, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

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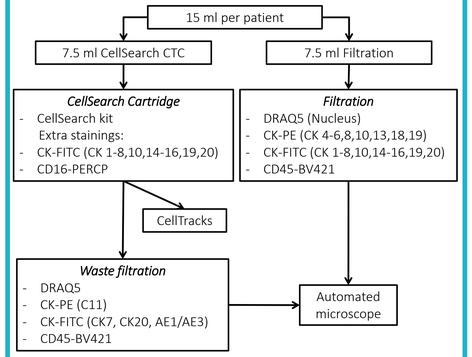
Background

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 .

Study design



Study design *From each patient 7.5 ml is prepped by CellSearch and direct filtration. CellSearch waste is collected, filtered and separately stained.*

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 8x8 mm² microfabricated silicon microsieve containing 60,000 pores of 5 µm in diameter.

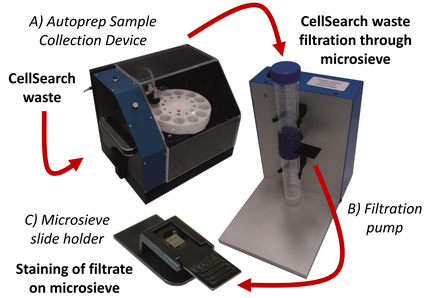


Figure 1 *The Autoprep Sample Collection Device (ASCD) collects the waste from patient samples (A). A pump with disposable filtration unit filters the cells collected by the ASCD (B). The staining holder with micrsosieve slide is for staining and detection of CTC captured on the microsieve after filtration (C).*

Staining

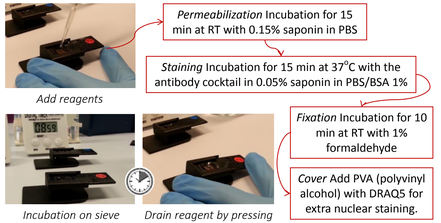


Image analysis

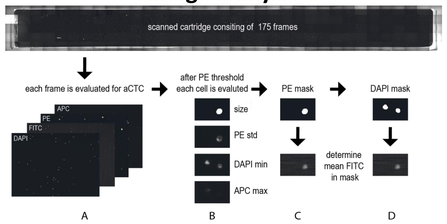


Figure 2 *Image processing steps to determine the expression of extra markers. A threshold for DAPI and PE is determined for all images in a cartridge. Each event above the DAPI threshold is evaluated in all channels. If an overlapping PE Mask is found, this is used to measure additional markers.*

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.

Healthy Donor (n=5)	Spike A "Big Cells"		Spike B "Small Cells"	
	T24 EpCAM –	SK-BR-3 EpCAM +	Colo-320 EpCAM –	SW480 EpCAM +
CellTracks	2% (±1)	87% (±12)	2% (±2)	91% (±13)
Sieve	59% (±9)	2% (±1)	18% (±6)	6% (±7)

Table 1 *Cells are spiked in 7.5 mL of blood collected in CellSave tubes from healthy volunteers.*

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

#	Healthy control: not spiked		Healthy control: spiked	
	CellSearch	Filtration	CellSearch	Filtration
1.	0	0	0	80
2.	0	0	0	8
3.	0	0	0	5
4.	0	1	0	88
5.	0	0	0	21
6.	0	1	0	33

Table 2 *Samples from healthy donors: not spiked and spiked with 10 to 100 cells of the EpCAM– non-small lung cancer cell line NCI-H1650.*

Preliminary results

Patients with NCSLC (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.

Patient #	CellSearch			Whole blood Filtration	CellSearch Automated Analysis			
	CTC CellTracks	CTC Waste	Total CTC	CTC	CK PE+	CK PE+ CK FITC+	CK *FITC+	Total
1.	0	0	0	0	0	0	2	2
2.	0	0	0	0	0	0	1	1
3.	2	0	2	0	3	2	10	13
4.	0	0	0	0	0	0	11	11
5.	0	0	0	0	0	0	9	9
6.	11	0	11	NA	4	1	5	9
7.	0	NA	0	0	0	0	2	2
8.	2	0	2	2	2	1	1	3
9.	1	30	31	14	0	0	6	6
10.	29	14	43	11	38	13	3	41
11.	AB	29	29	3	NA	NA	NA	NA
12.	2	14	16	0	0	0	10	10
13.	0	6	6	38	0	0	1	1
14.	0	1	1	12	1	0	18	19
15.	0	3	3	3	1	0	9	10
16.	2	4	6	9	2	1	16	18
≥1 CTC (%)	*38*	*50*	*69*	*50*	*44*	*25*	*94*	*94*
≥10 CTC (%)	*13*	*25*	*31*	*25*	*6*	*6*	*31*	*44*

Table 3 *Overview of CTC found in NSCLC patients. *Some CK-FITC+ events are due to debris which would be discarded in a manual count.*

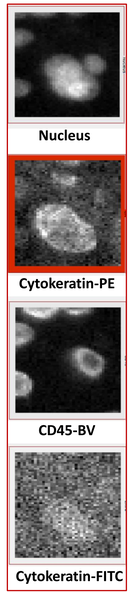


Figure 3 *Thumbnail gallary showing a CTC and white blood cells after filtration on a sieve.*

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.

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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 mm^2 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, ~1,616 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 NCSLC (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

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