s.dewit@utwente.nl

LB-250

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Liquid biopsy in advanced NSCLC: EpCAM+ and EpCAM- circulating tumor cells, tumor derived extracellular vesicles and cell-free circulating tumor DNA

Sanne de Wit[1], Menno Tamminga[2], Ellen Heitzer[3], Joost F Swennenhuis[1], Ed Schuuring[2], Leonie L Zeune[1], Michael R Speicher[3], T Jeroen N Hiltermann[2], Leon WMM Terstappen[1], Harry JM Groen[2]

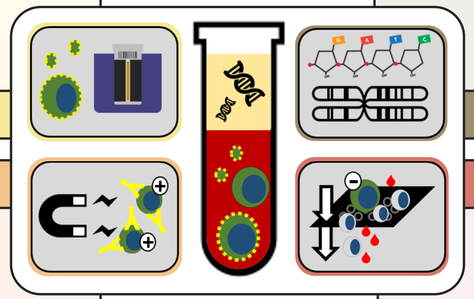
[1] Department of Medical Cell BioPhysics, MIRA institute, University of Twente, The Netherlands, [2] Department of Pulmonology, University Medical Center Groningen, the Netherlands, [3] Institute of Human Genetics, Medical University of Graz, Austria

WHY The need for a liquid biopsy in non-small cell lung cancer (NSCLC) patients is rapidly increasing as more targeted therapies become available.

WHAT Four biomarkers are explored for their potential to represent a liquid biopsy.

WHO In metastatic NSCLC patients before treatment we investigate the biomarkers in relation with overall survival.

HOW In just one 7.5 mL CellSave tube of blood.



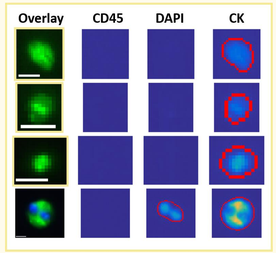
Tumor Derived Extracellular Vesicles

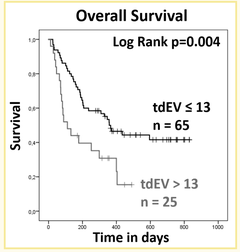
DEFINITION tdEV are EpCAM+, DAPI-, cytokeratin+, CD45-, slightly round, surface < 150 µm², perimeter > 4µm.

METHOD Analysis of the CellSearch cartridge (n=90) with open source program ACCEPT after processing 7.5 mL blood for detection of CTC. Cut-off value >13 is based on mean+1SD of 127 healthy controls (HC).

CONCLUSION Presence of tdEV is significantly associated with poor overall survival.

Patients (n=90)		*HC*
tdEV ≤ 13	72%	12%
tdEV > 13	28%	88%
Mean	21.6	6.7 (±6.3)
Min	0	0
Max	381	37
Median	7	5

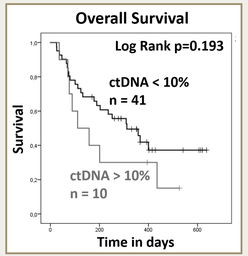


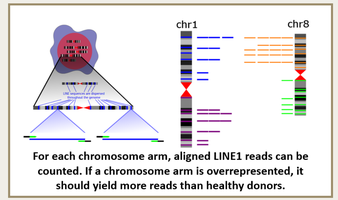


Circulating Tumor DNA

DEFINITION DNA present in plasma originating from the tumor

METHOD Plasma was collected from the CellSave tube and ctDNA concentration was measured with the mFAST-SeqS approach (n=51). This approach relies on the amplification of uniquely mappable *LINE1*-sequences across the genome and can be used as a general measure of aneuploidy in a plasma sample. Detection limit of ctDNA concentration is ≥10% mutant alleles.





CONCLUSION ctDNA concentration did not significantly correlate to overall survival, but might be reached by increasing the number of patients.

Patients (n=51)	
ctDNA < 10%	74%
ctDNA > 10%	26%
Min	0.8
Max	66.7
Median	2.3

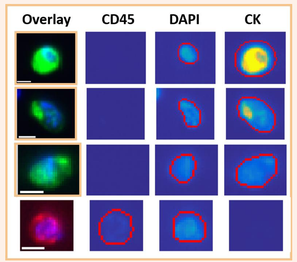
EpCAM+ Circulating Tumor Cells

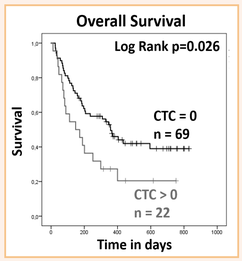
DEFINITION CTC are EpCAM+, DAPI+, cytokeratin+, CD45-, round, >4µm in size, DAPI-CK overlay >50%.

METHOD 7.5 mL blood measured with CellSearch for detection of CTC by immunomagnetic selection (n=91 and HC n=39).

CONCLUSION Presence of EpCAM+ CTC is significantly associated with poor overall survival.

Patients (n=91)		*HC*
CTC = 0	76%	97%
CTC ≥ 1	24%	3%
CTC ≥ 3	13%	0%
CTC ≥ 5	5%	0%
Min	0	0
Max	186	1
Median	0	0

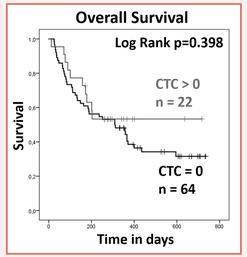


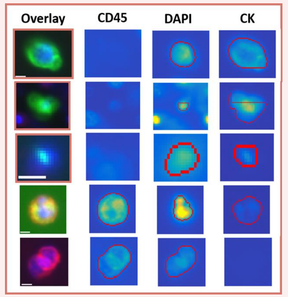


EpCAM- Circulating Tumor Cells

DEFINITION CTC are EpCAM-, DAPI+, cytokeratin+, CD45-, DAPI-CK overlay.

METHOD Blood discarded by CellSearch after immuno-magnetic isolation was filtered through 5µm pores and stained with a CK-antibody cocktail (n=86). HC (n=27) spiked with ~300 EpCAM- NSCLC cell line NCI-H1650 cells (1.4x10² EpCAM antigens and size 12µm): mean recovery = 31% [min 11-max 350].





CONCLUSION Blocking of the filter influences CTC recovery. Presence of EpCAM- CTC are not correlated with overall survival.

Patients (n=86)		*HC*
CTC = 0	76%	76%
CTC ≥ 1	24%	24%
CTC ≥ 3	13%	6%
CTC ≥ 5	5%	6%
Min	0	0
Max	186	12
Median	0	0

CONCLUSIONS

Patients (n=50)	
All 4 biomarkers positive	6%
3 biomarkers positive	12%
2 biomarkers positive	20%
1 biomarker positive	58%
Correlation EpCAM+ CTC	p=0.019
Correlation EpCAM- CTC	p=0.571
Correlation tdEV	p=0.134
Correlation ctDNA	p=0.082

tdEV showed the strongest association with overall survival. Addition of any combination of the biomarkers did not increase this association. Remaining question is what the efficiency is to extract treatment relevant information from these biomarkers.

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