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**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

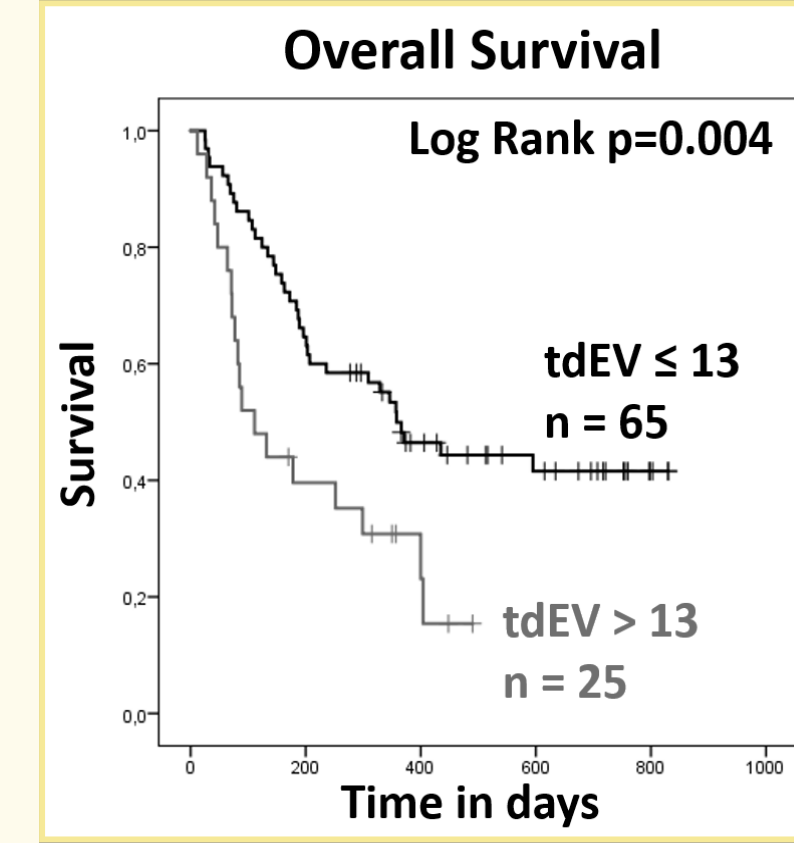
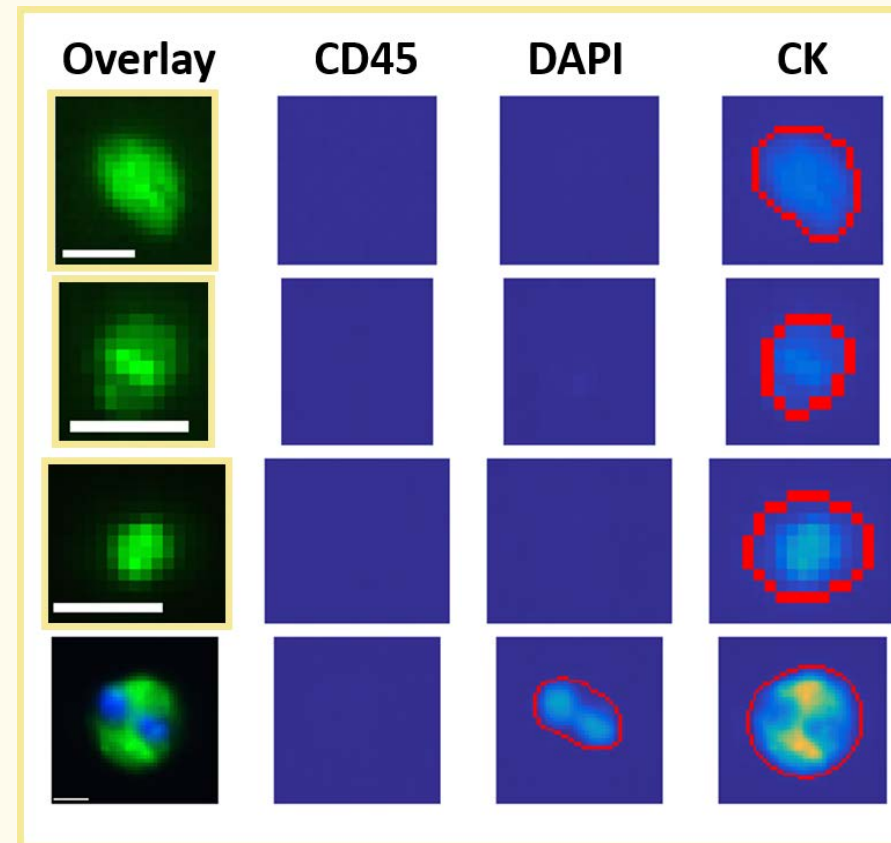
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**DEFINITION** tdEV are EpCAM+, DAPI-, cytokeratin+, CD45-, slightly round, surface < 150 μm<sup>2</sup>, perimeter > 4μm.

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



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

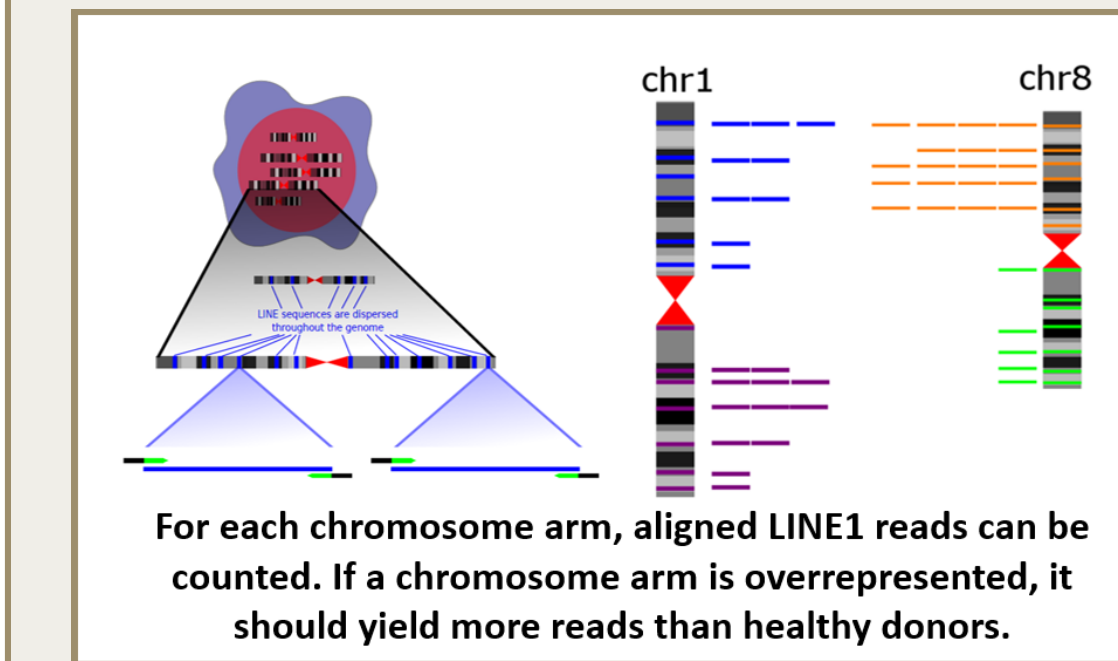
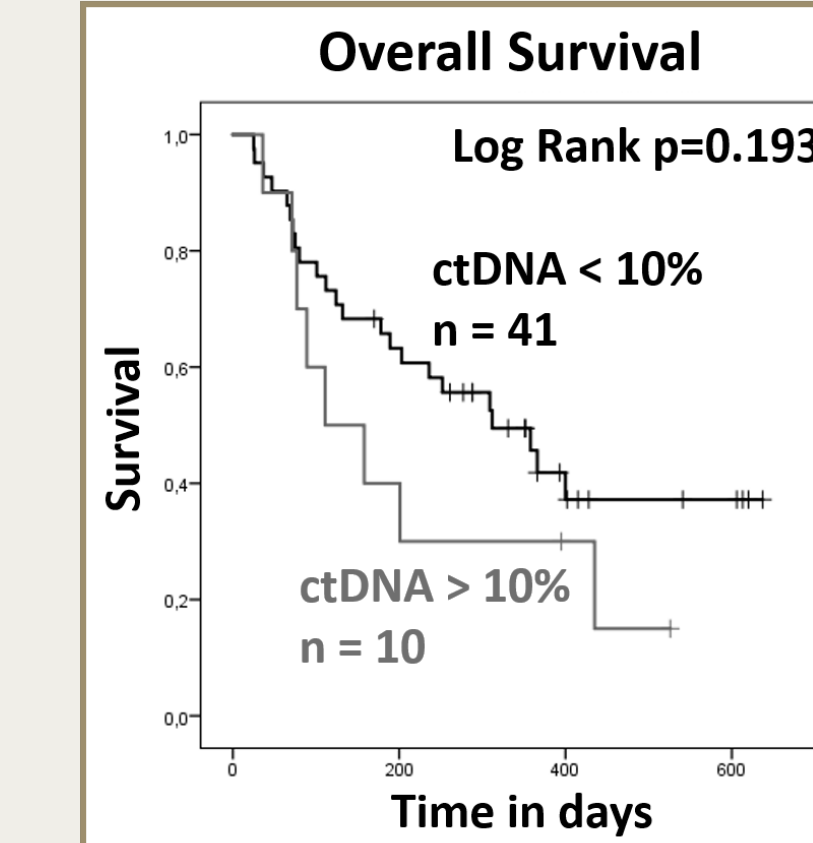
**WHAT** Four biomarkers are explored for their potential to represent a liquid biopsy.

**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.

**DEFINITION** DNA present in plasma originating from the tumor

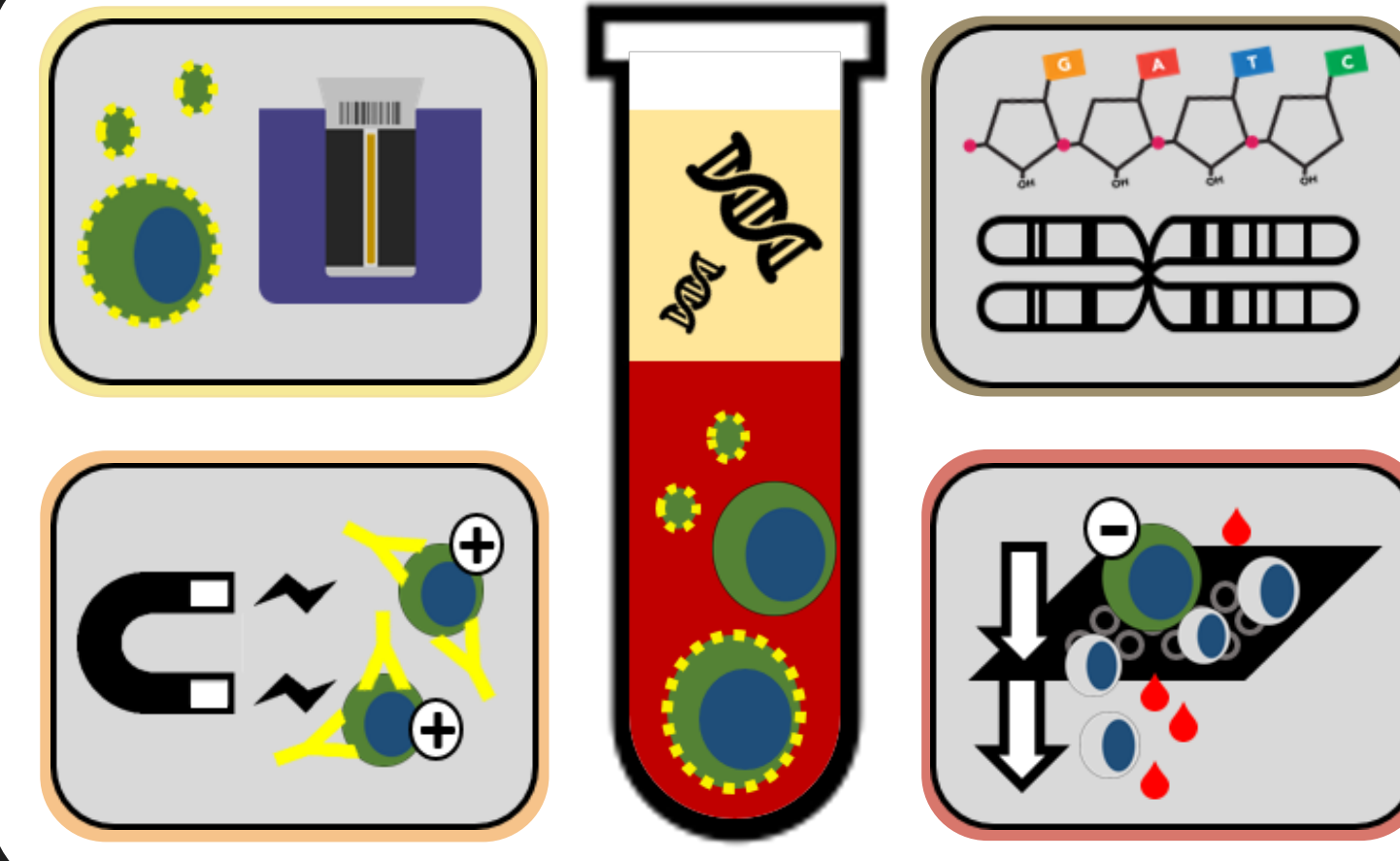
**METHOD** Plasma was collected from the CellSave tube and ctDNA concentration was measured with the mFAST-SeqS approach (n=51). This approach relies on the amplification of uniquely mappable LINE1-sequences across the genome and can be used as a general measure of aneuploidy in a plasma sample. Detection 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)	HC
ctDNA < 10%	74%
ctDNA > 10%	26%
Min	0.8
Max	66.7
Median	2.3

## LIQUID



## Tumor Derived Extracellular Vesicles

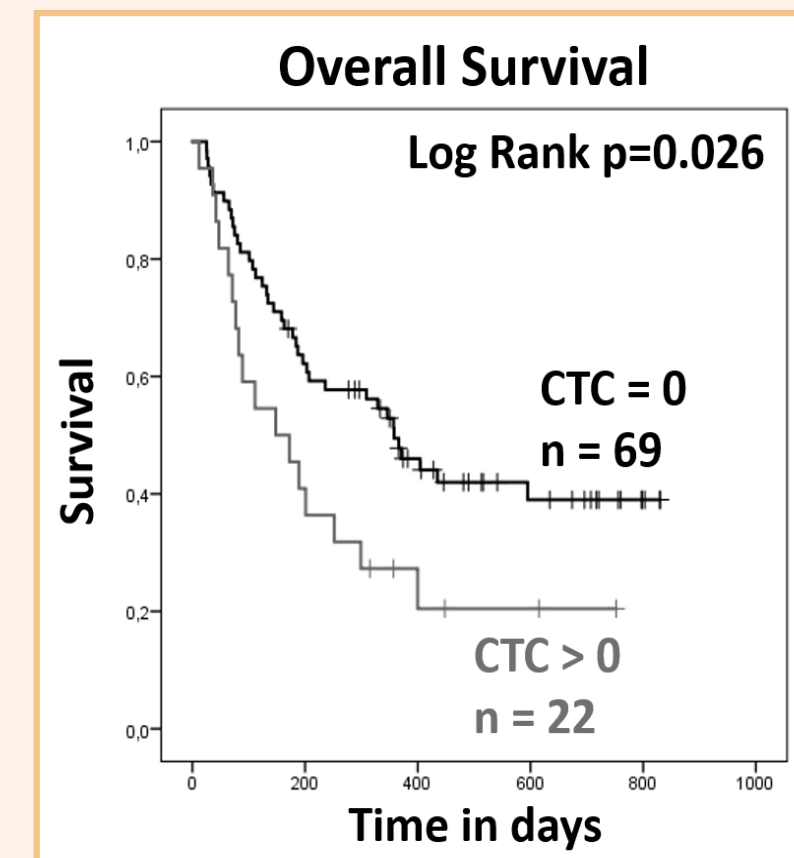
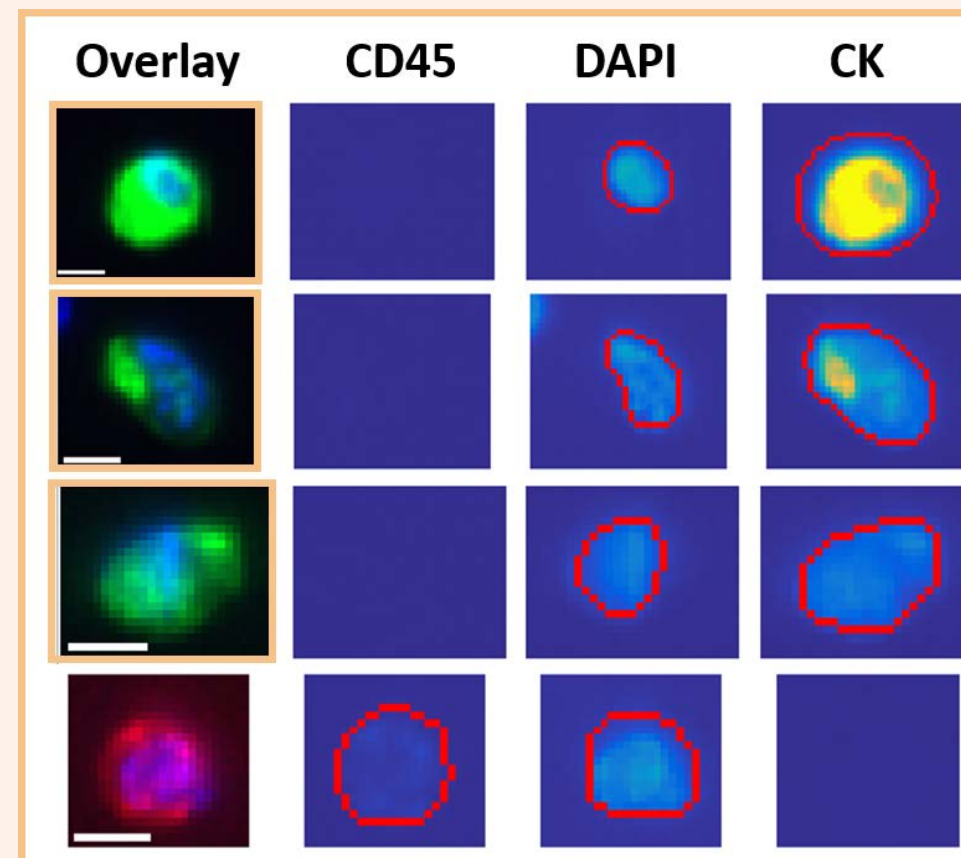
### EpCAM+ Circulating Tumor Cells

**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



## BIOPSY

### 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

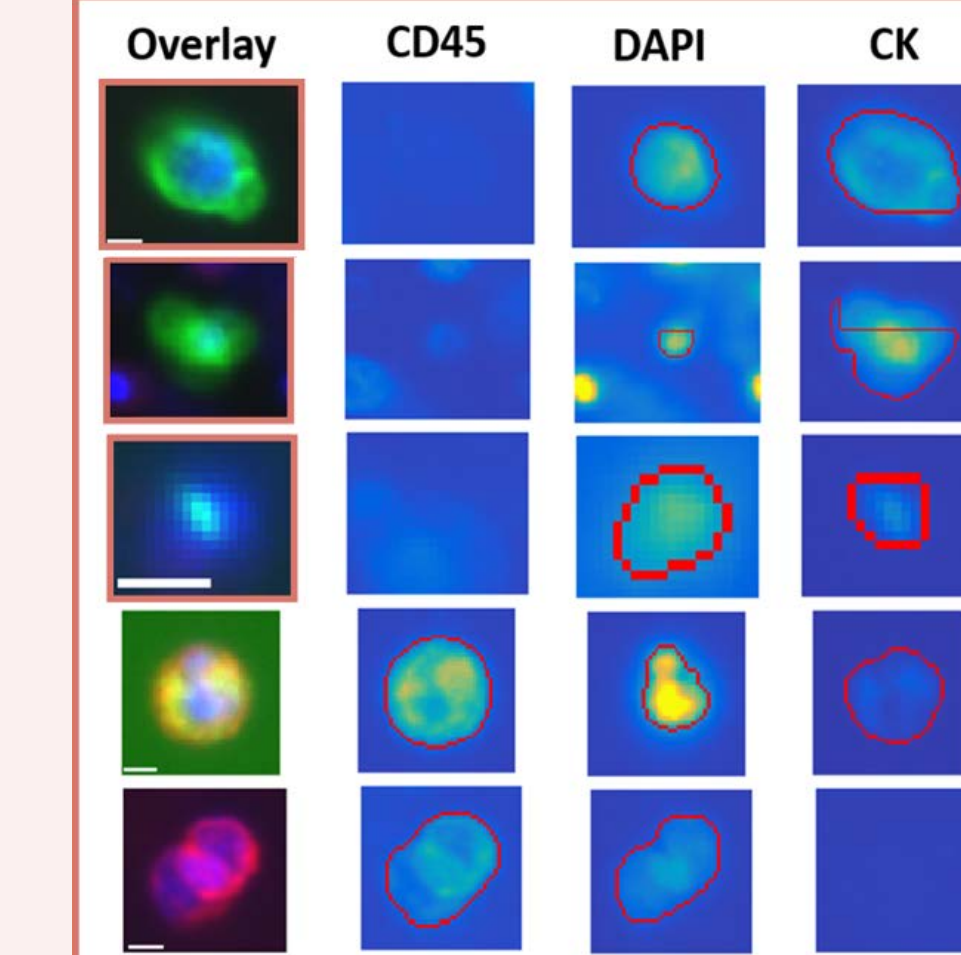
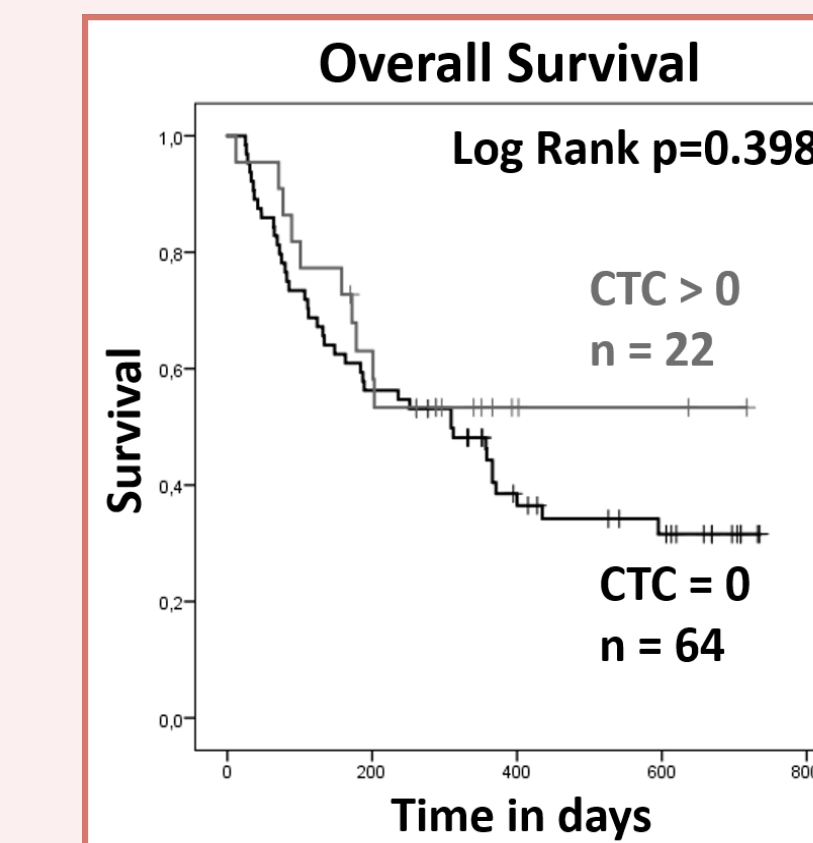
tdEV showed the strongest association with overall survival. Addition of any combination of the biomarkers did not increase this association. Remaining question is what the efficiency is to extract treatment relevant information from these biomarkers.

## Circulating Tumor DNA

### 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 (1.4x10<sup>2</sup> 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