

Single tube liquid biopsy for NSCLC

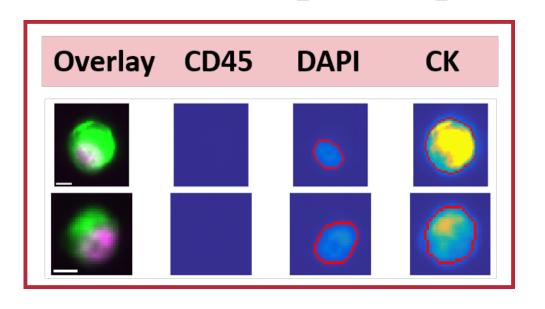
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STUDY 97 Non-small cell lung cancer (NSCLC) patients before treatment were analyzed with:

CellSearch for EpCAM positive CTC



Definition

EpCAM+

DAPI+

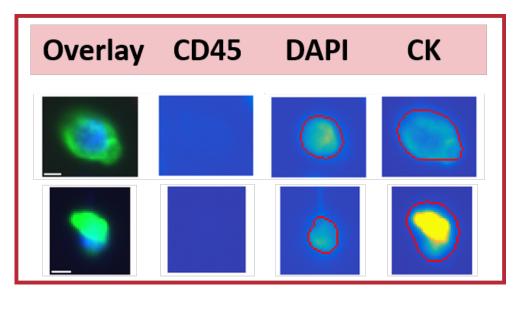
CK+

CD45
Round

> 4µm in size

DAPI-CK overlay >50%

Microsieve filtration for EpCAM negative or low CTC



Definition

EpCAM—/low

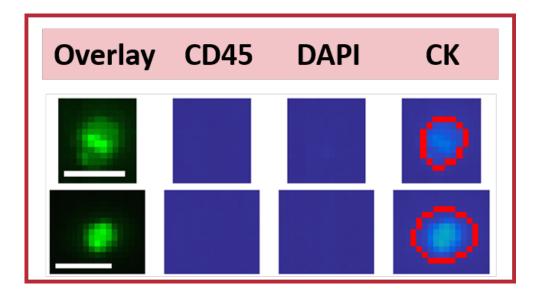
DAPI+

CK+

CD45—

DAPI-CK overlay

❖ ACCEPT software on CellSearch cartridges for tumor derived extra-cellular vesicles (tdEV)



Definition

EpCAM+

DAPI
CK+

CD45
Slightly round

Surface < 150 μm²

Perimeter > 4 μm

❖ Isolation and sequencing of plasma for circulating tumor DNA (ctDNA)

All from a single tube of blood

Liquid biopsy

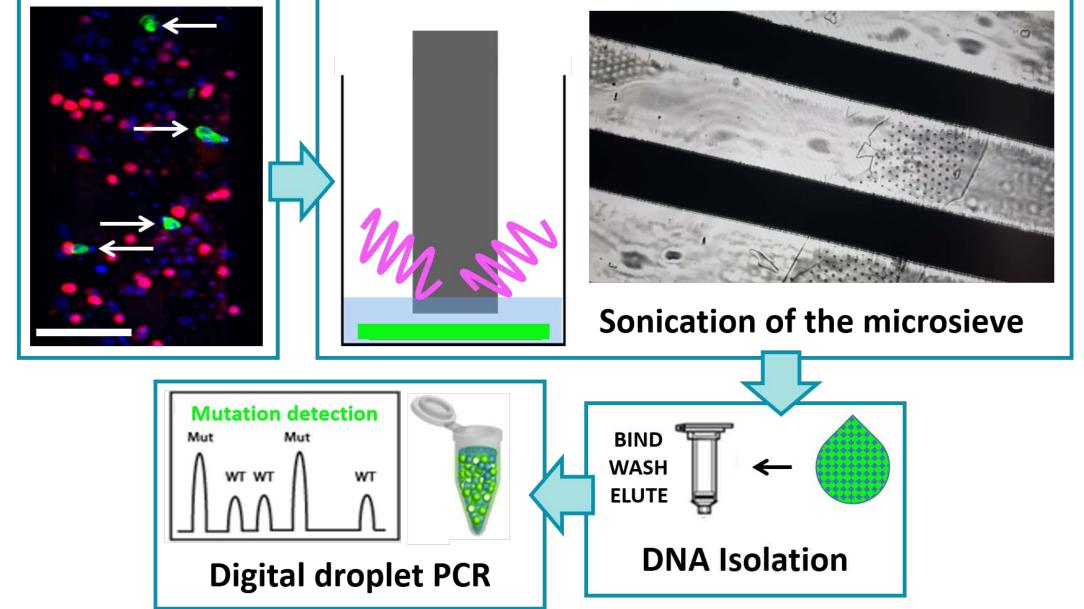
Genetic analysis

ctDNA In CellSave or EDTA plasma samples the presence of >10% mutant alleles in ctDNA was determined with the mFAST-SeqS method.

MUTATIONS IN PLASMA 14 patients were sequenced for mutations detected in primary tumor:

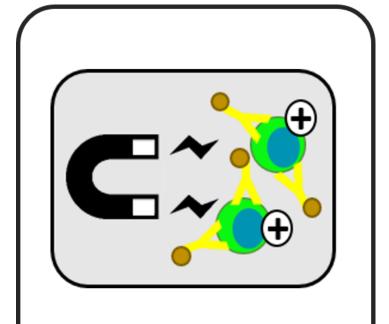
- ❖ All patients had <10% mutant alleles
 - 1 patient with EpCAM+ CTC
 - 5 patients with EpCAM- CTC
 - 1 patient with EpCAM+ and EpCAM- CTC
 - 7 patients without any CTC
- * The mutation was found in all patients with CTC
- In 3 patients without CTC the mutation was found as well

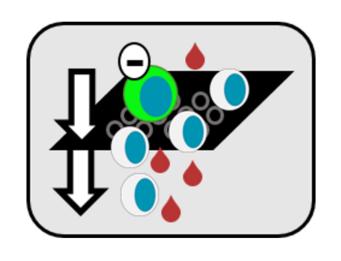
MUTATION DETECTION IN EpCAM-CTC

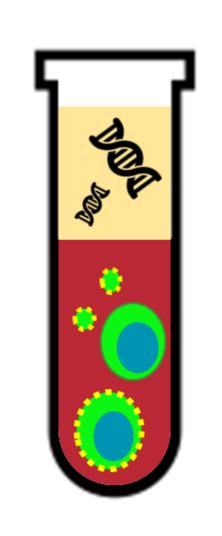


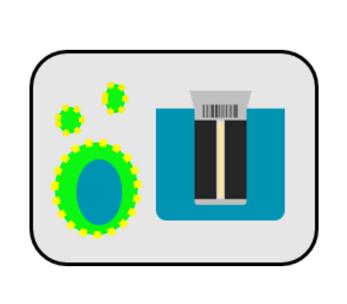
Genetic confirmation of tumorous origin of EpCAM-CTC on the microsieve are ongoing by detecting mutations also found in the primary tumor.

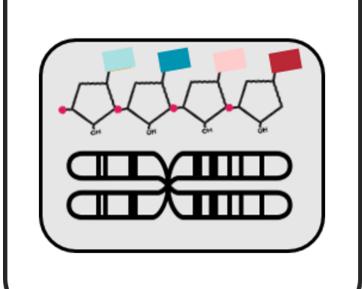
one tube liquid biopsy cancer patients circulating tumor cells mutations liquid biopsy ctc treatment epcam positive circulating tumor cells dna diagnostic biomarkers epcam negative circulating tumor cells liquid biopsy predictive biomarkers tumor derived extracellular vesicles liquid biopsy circulating tumor dna prognostic biomarker liquid biopsy liquid biopsy one tube liquid biopsy cancer patients circulating tumor cells mutations liquid biopsy ctc treatment epcam positive circulating tumor cells dna diagnostic biomarkers epcam negative circulating tumor cells liquid biopsy predictive biomarkers tumor derived extracellular vesicles liquid biopsy circulating tumor dna prognostic biomarker liquid biopsy liquid biopsy one tube liquid biopsy cancer patients circulating tumor cells mutations liquid biopsy ctc treatment epcam diagnostic biomarkers epcam negative circulating tumor cells liquid biopsy extracellular vesicles liquid biopsy circulating tumor dna prognostic biomarker liquid biopsy liquid biopsy one tube liquid biopsy cancer patients circulating tumor cells mutations liquid biopsy ctc treatment epcam positive circulating tumor cells dna diagnostic biomarkers epcam negative circulating tumor cells liquid biopsy





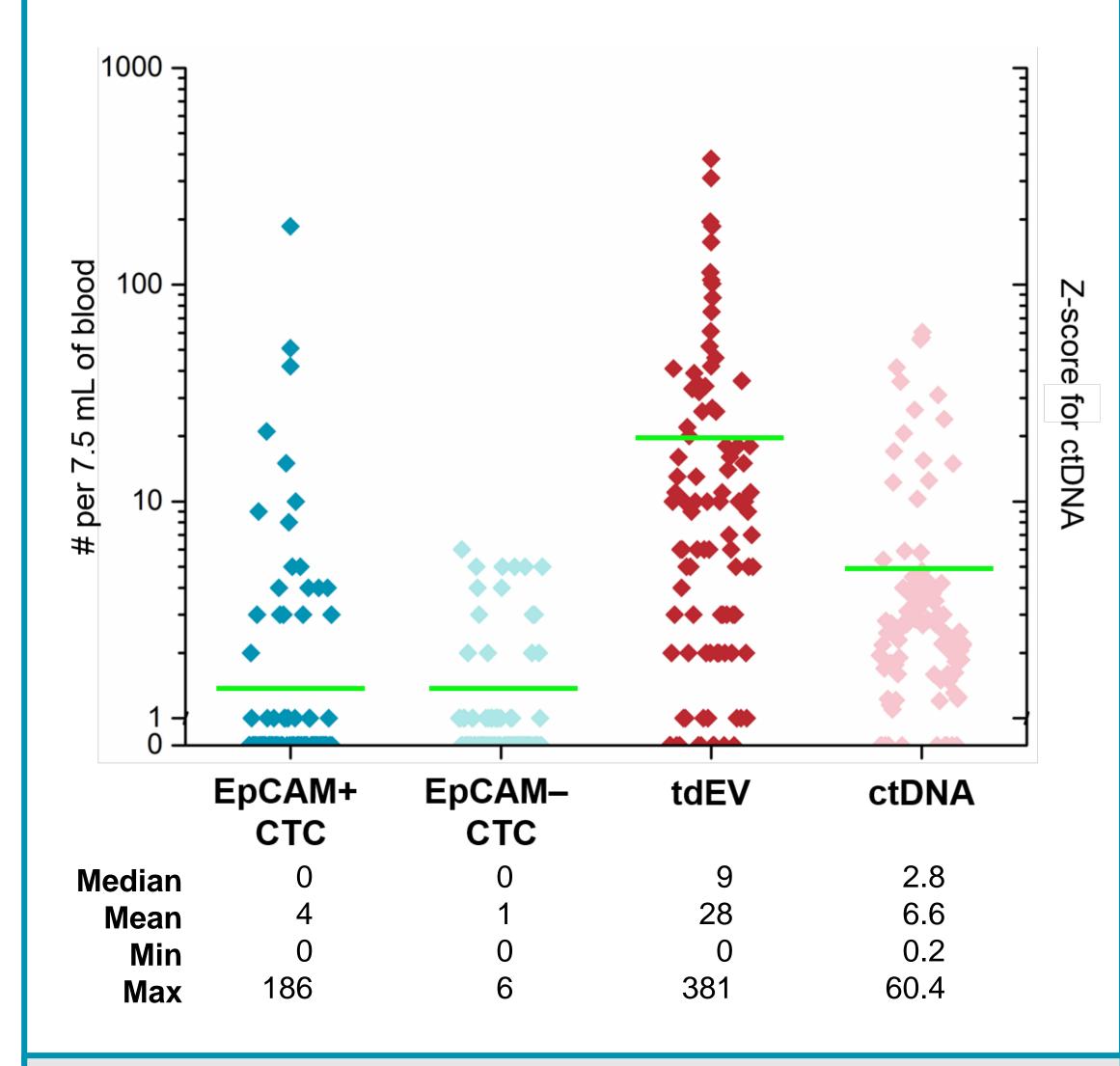






one tube liquid biopsy cancer patients positive circulating tumor cells dna diagnostic biomarkers epcam negative predictive biomarkers tumor derived extracellular vesicles liquid biopsy circulating tumor dna prognostic biomarker liquid biopsy liquid biopsy one tube liquid biopsy cancer patients circulating tumor cells mutations liquid biopsy ctc treatment epcam positive circulating tumor cells dna diagnostic biomarkers epcam negative circulating tumor cells liquid biopsy predictive biomarkers tumor derived extracellular vesicles liquid biopsy circulating tumor dna prognostic biomarker liquid biopsy liquid biopsy one tube liquid biopsy cancer patients circulating tumor cells mutations liquid biopsy ctc treatment epcam positive circulating tumor cells dna diagnostic biomarkers epcam negative circulating tumor cells liquid biopsy predictive biomarkers tumor derived extracellular vesicles liquid biopsy circulating tumor dna prognostic biomarker liquid biopsy liquid biopsy one tube liquid biopsy cancer patients circulating tumor cells mutations liquid biopsy ctc treatment epcam positive circulating tumor cells dna **COUNTING** The presence of biomarkers above the threshold (green bar) was determined for each patient. 43% of the patients have one or more biomarkers present in their blood.

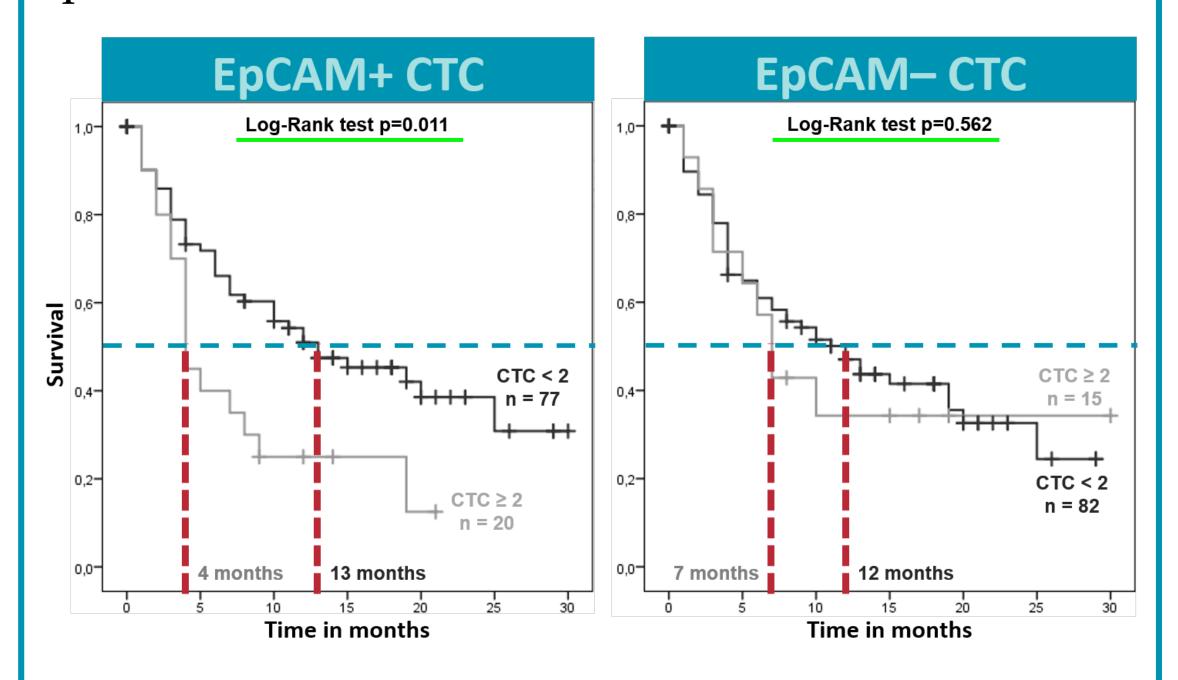
Biomarker present in 97 patients			
≥ 2 EpCAM+ CTC	21%	All 4 biomarkers positive	2%
≥ 2 EpCAM- CTC	15%	3 biomarkers positive	9%
≥ 19 tdEV	27%	2 biomarkers positive	13%
≥ 10% ctDNA	19%	1 biomarker positive	19%

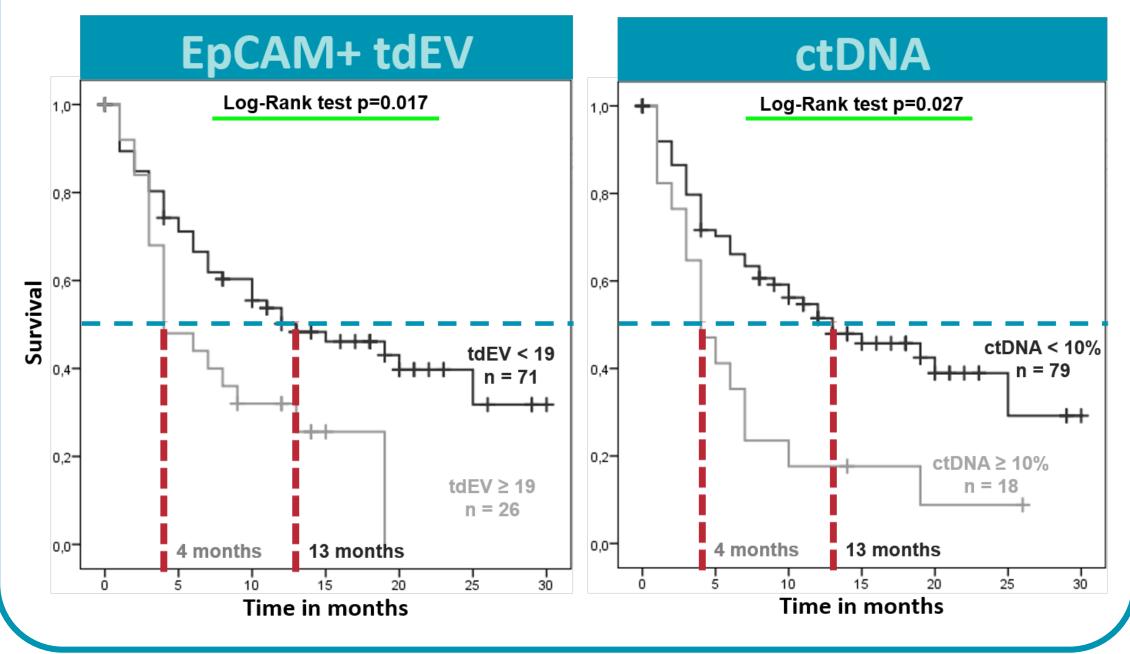


Presence in patients

Overall survival

CONCLUSION Presence of EpCAM+ CTC, tdEV and ctDNA are associated with poor overall survival in NSCLC and are significant predictors for OS, whereas EpCAM-/low CTC are not. What treatment relevant information can be extracted from these predictive biomarkers?





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