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**
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.

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**Tumor Derived Extracellular Vesicles**

**EpCAM+ Circulating Tumor Cells**

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

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

**EpCAM- Circulating Tumor Cells**

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

**CONCLUSION**
Presence of EpCAM- CTC are not correlated with overall survival.

**Circulating Tumor DNA**

**DEFINITION**
EpCAM- circulating Tumor Cells

**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 (1.4x10^2 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.

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**LIQUID**

**CONCLUSIONS**
dEV 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.