# Analysis of circulating tumor cells displays tumor heterogeneity in advanced prostate cancer

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## Background & objectives

As a result of enduring therapeutic pressure, aggressive variants of prostate cancer (AVPC) can emerge from prostate adenocarcinoma. AVPC are not depending on androgen receptor signaling, can show traits of neuroendocrine (NE) differentiation and are not well detected by prostate specific antigen monitoring. This study aims to evaluate the enumeration of circulating tumor cells (CTCs) in combination with gene expression analysis to identify new biomarkers for AVPC patients with neuroendocrine transdifferentiation.

## Methods

Blood was collected from AVPC patients (n=78) and from patients with hormone-sensitive prostate cancer (HSPC) as controls (n=12). CTC counts were measured by CellSearch. In parallel, the AdnaTest was used to immuno-magnetically enrich CTCs and bulk gene expression of prostate-specific and NE-related transcripts was analyzed by semi-quantitative PCR. CTCs were additionally enriched by Parsortix from a subset of patients to compare gene expression between enrichment strategies.

## Results

CTCs were found in 88.9 % of AVPC patients with a median CellSearch count of 32 CTCs/7.5 ml. Similarly, 81.8 % of NEPC patients were positive for CTCs with a median count of 35 CTCs/7.5 ml. In contrast, CTCs were found in only 50 % of HSPC patients at lower concentrations (median count of 0.5 CTCs/7.5 ml).
Prostate-specific genes such as *KLK3* or *PSMA* were expressed in 93.8 % of AVPC samples. At least one of the NE transcripts was detected in 37.5 % of AVPC samples. In CTCs from NEPC patients, prostate marker detection was significantly reduced to 66.6 % ($p = 0.01$) and the positivity for NE markers was increased to 61.1 %. Using a random Forrest model, HSPC and NEPC samples could be differentiated with an AUC of 79.5 %. Comparison of CTC enrichment strategies in a subset of 13 patients showed similar NE marker detection by size-based enrichment compared to the AdnaTest.

## Conclusion

AVPC and NEPC patients show a high CTC burden that facilitates subsequent molecular analyses and CTC counts allowed a distinction between blood samples from HSPC and NEPC patients. Gene expression analysis revealed a high degree of inter-patient heterogeneity for neuroendocrine-specific transcripts with a reduction of prostate markers in NEPC patients.