# Circulating tumor cell enumeration and ultradeep T-cell receptor-sequencing in a Phase I clinical trial of metastatic prostate cancer

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## Background & objectives

Metastatic castration-resistant prostate cancer (mCRPC) has a dismal prognosis. In this Phase I clinical trial, patients with mCRPC were treated with dendritic cell (DC)-based cryoimmunotherapy (CryoIT). The primary endpoint was safety and patient acceptability. It was examined if circulating tumor cell (CTC) enumeration and ultradeep T-cell receptor sequencing (TCR-seq) could be helpful biomarkers to indicate therapeutic effects or immune activation, respectively.

## Methods

Patients with mCRPC (n=18) progressing on standard treatment were included into the trial according to Regional Ethical Committee approval (ClinicalTrials.gov ID: NCT02423928). Autologous immature DCs were injected into cryoablated prostate cancer tissue. DC doses were escalated in a 3 + 3 design (n=9) combined with either single-dosed intratumoral ipilimumab (n=6) or intravenous pembrolizumab (n=3). Disease burden was assessed by PET-CT, MRI and bone scintigraphy prior to, and 14, 22 and 46 weeks after CryoIT and progression evaluated according to iRECISTv1.1 and PSA, lactate dehydrogenase (LD) and alkaline phosphatase (ALP) laboratory tests. CTCs collected in 7.5 ml Cellsave tubes were enumerated using the CellSearch platform. Ultradeep TCR-seq of peripheral blood lymphocyte beta-chain DNA (performed by HS Diagnomics (Berlin, Germany) quantified T-lymphocyte clonotypes before treatment and during follow-up.

## Results

The patients had aggressive cancer, with ISUP grade group 4 or 5 in 72% (13/18) at diagnosis and in 100% at inclusion. A low incidence of adverse events and good patient tolerability were recorded. Median Overall Survival and Progression Free Survival were 40.7 and 10.5 months, respectively. Of the 8 patients still alive at 43 to 85 months post-CryoIT, 7 had progressed and received other treatment. All patients with pre-CTCs showed either transient CTC decreases (pre-CryoIT CTC≥5, n=7), or complete CTC disappearance (pre-CryoIT CTC=1-4, n=4). None without CTCs prior to CryoIT (n=7) developed CTCs during follow-up. Ultradeep TCR-sequencing revealed a high incidence of new and more than 5-fold expanded T-cell clonotypes among the top 200 largest clonotypes in all patients post-CryoIT, suggesting immune activation, but without clear association to survival benefits.

## Conclusion

CTC enumeration confirmed its prognostic strength and suggested CryoIT treatment effects. Ultradeep TCR-seq suggested extensive immune activation. Safety data and indications of therapeutic effects encourage the planned next stage clinical trial.