Circulating tumor cells as a source of information about castration-resistant prostate cancer

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Introduction

Circulating tumour cells (CTCs) are emerging tumour biomarkers. Essentially, they represent a "liquid biopsy" sample and well-defined targets for the study and understanding of tumour biology and dissemination of tumour cells. Detection and characterization of CTCs offers an exciting approach to better understand the development of metastases in cancer patients. Beyond the enumeration and profiling of CTCs, they have the potential to provide predictive information to guide the selection of the therapy and to monitor the therapy efficacy. The significance of CTCs in cancer patients was reported. In the men population, the second leading cause of cancer-related death is the prostate cancer (PC). Approximately 10 – 20% of PC patients with androgen blockade during the five-year monitoring enter the most serious stage of castration-resistant PC (CRPC). At the time of diagnosis, almost 85% of CRPC patients suffer from metastases preferentially in to the skeleton. In this project, we aimed at the detection of CTCs in the blood of patients with CRPC (Figure 1) together with their characterization and the study of their behaviour during in vitro cultivation.

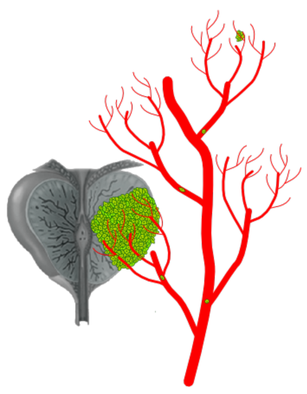


Figure 1: Castration-resistant prostate cancer

Materials and Methods

CTCs Detection

Five millilitres of peripheral blood of patients with CRPC were collected twice: at the time of CRPC diagnosis and after first four cycles of docetaxel chemotherapy. CTCs were immunomagnetically enriched using magnetic beads conjugated with epithelial and tumour-associated antigens (Adnagen Germany). Separated CTCs were lysed and released mRNA was isolated. CTCs mRNA was reverse transcribed into cDNA serving as a template for CTCs detection and characterization by multiplex PCR. Three tumour associated markers and one control gene were amplified (PSA, PSMA, EGFR and Actin). PCR fragments were evaluated on Bioanalyzer 2100 (Agilent). If at least one of three markers was determined in required concentration, the test result was considered as positive (Figure 2).

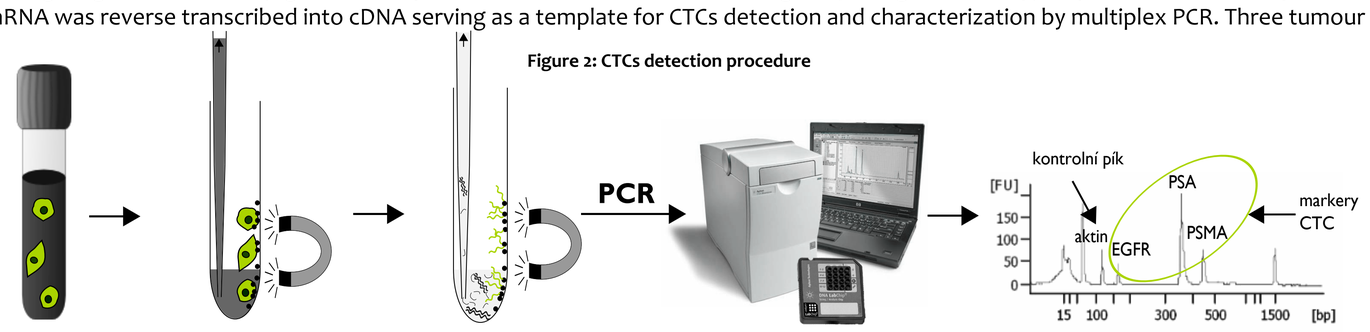


Figure 2: CTCs detection procedure

CTCs cultivation

To verify if in vitro cultivation of CTCs from the mononuclear cell´s layer is possible the cells of LNCaP cell mine were added into the blood of voluntary donor in 5 different concentrations from 1 to 10000 per ml. Samples were processed by gradient centrifugation using Leucosep® tubes to isolate the layer of mononuclear cells. Isolated fraction was placed into the cultivation flask with RPMI 1650 medium with fetal calf serum. The samples were incubated at 37°C temperature and in 5% CO_2 atmosphere. Samples were macroscopically and microscopically inspected. Immunofluorescence staining was performed (pan-cytokeratin/FITC, CD-45/PE). (Figure 3).

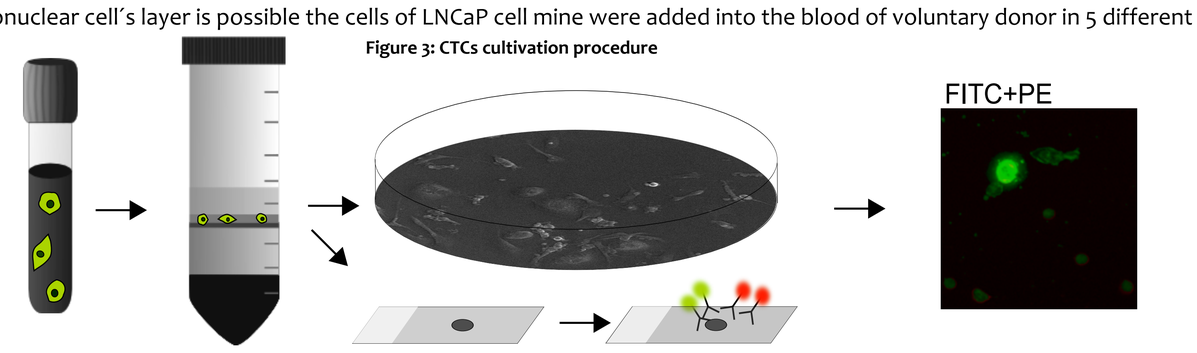
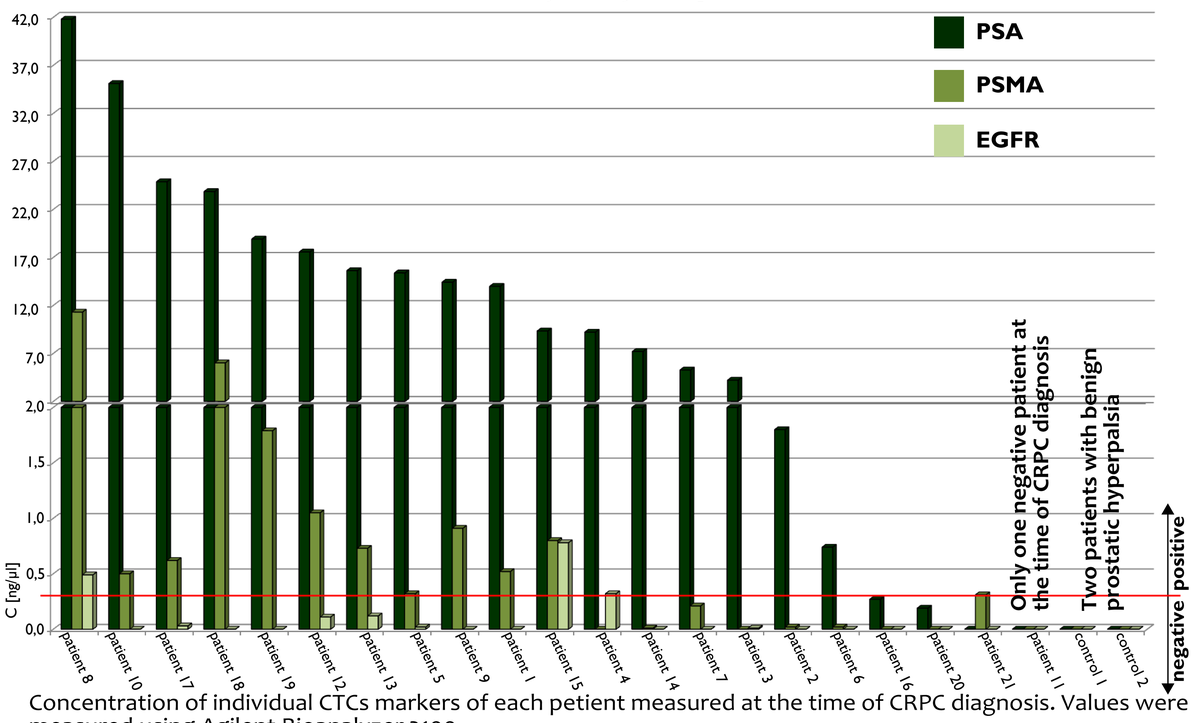


Figure 3: CTCs cultivation procedure

Results

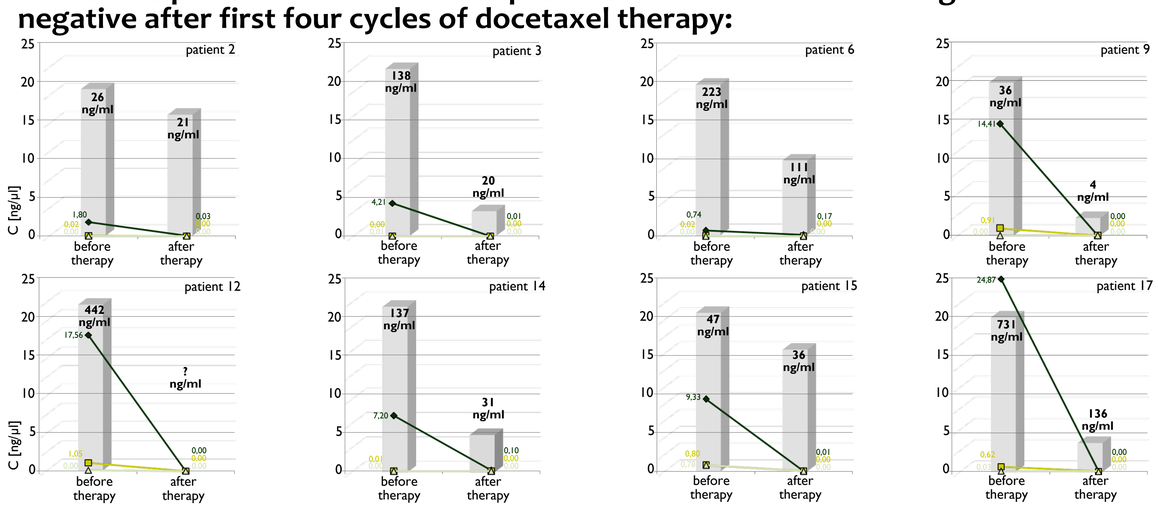
CTCs were detected in the majority of patients with CRPC.



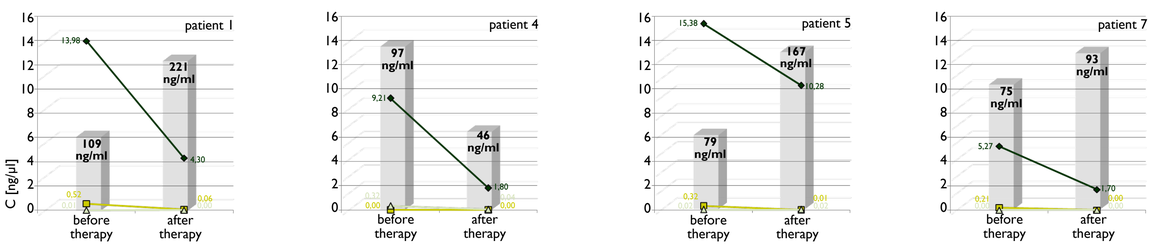
Concentration of individual CTCs markers of each petient measured at the time of CRPC diagnosis. Values were measured using Agilent Bioanalyzer 2100.

CTCs and serum PSA changes before and during therapy.

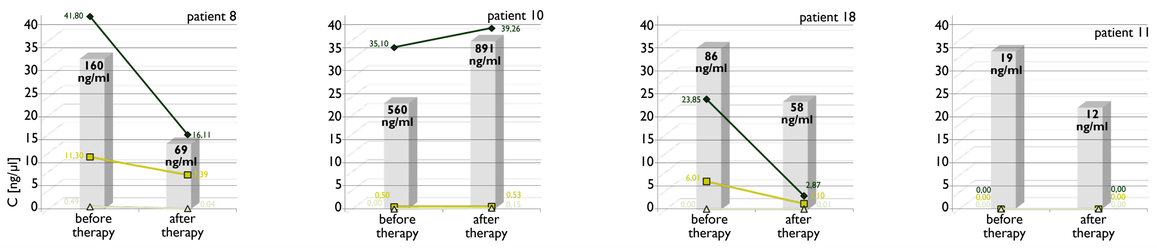
- **Half of the patients who were CTCs positive at the time of CRPC diagnosis became CTCs negative after first four cycles of docetaxel therapy:**



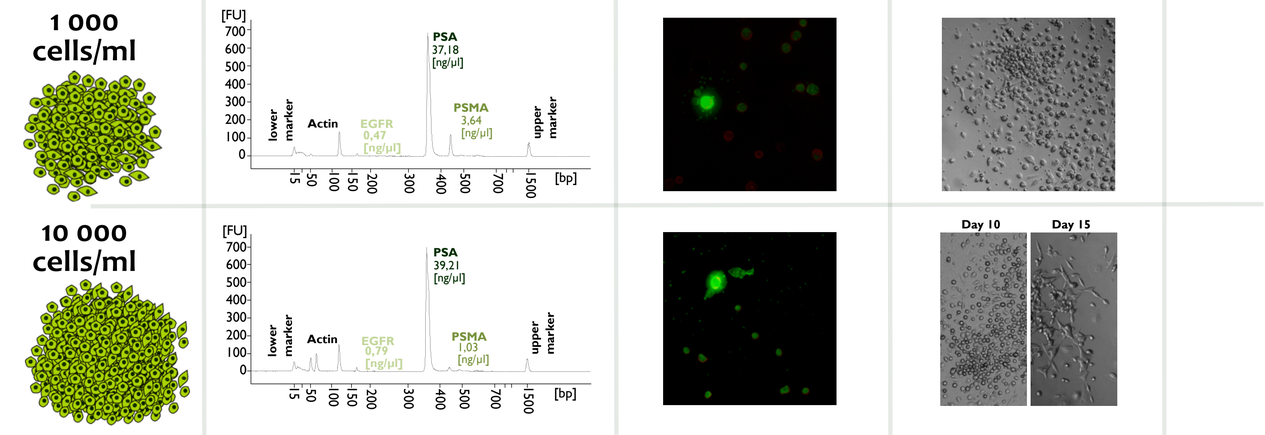
- **Four patients stayed CTCs positive showing significant decrease in the CTCs markers:**



- **Some patients remained CTCs positive in more than one marker:**
- **CTCs negative patient:**



Optimization of CTCs separation and cultivation processes.



We verified that we are able to detect tumor cells from concentration of 1 cell per 1 ml of blood. The presence of tumor cells has been demonstrated both by AdnaTest and immuno-fluorescence staining of cells isolated from the mononuclear layer. Cultivation conditions need to improve, but we achieved tumor-cells growth in the sample with the highest concentration.

Conclusions

Our results indicate that most of the patients with CRPC have CTCs present in their blood at the time of diagnosis. Changes in CTCs do not correspond very well with changes in serum PSA levels. In our opinion the level of CTCs and its changes during the therapy could serve as another prognostic and therapy efficiency marker for the clinicians. Moreover, the expression of the tumour-associated genes in CTCs differs between the patients. For this reason, we are estimating that CTCs could give useful information about differences between the patients with CRPC regarding to specific antigens expression for targeted therapies or chemotherapy sensitivity for personalized medicine. Successful cultivation of the CTCs should serve as a source of information about biological behaviour of tumour cells.

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