Circulating tumor cells as liquid biopsy for castration resistant prostate cancer patients treated with cabazitaxel

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Background.

Among several novel chemotherapeutic drugs targeting non-AR signaling, cabazitaxel has been demonstrated to improve overall survival in CRPC and to overcome resistance to docetaxel. Circulating tumor cells (CTC) enumeration and characterization have been demonstrated to be useful tool for precision medicine in prostate cancer: CTCs profiling after and during cabazitaxel treatment could help to establish novel predictive biomarkers and tools to monitor cancer evolution during the treatment.

Methods.

CTCs enrichment was assessed with Adna Test EMT/STEM Select/ Detect.Test specificity was analyzed using healthy donor blood samples as well as VCap and PC3 cell spiked samples. mRNA was isolated by Oligo (dT)25 Dynabeads and DNA was recovered from supernatant. Expression analyses were performed for: *AHR*; *AKR1C3*; *AKT2*; *ALDH1*; *AR*; *EPCAM*; *FOLH1*; *MDK*; *PARP*; *MRP1*; *PI3KCA*; *POU5F1*; *PSCA*; *TUBB3*; *VIM*; *ACTB*; *HPRT1*,*TWIST1* using real time or conventional PCR approaches. DNA was isolated using QiaAmp mini kit (Qiagen) and a whole genome amplification (WGA) was performed by *Ampli1* WGA kit (Silicon Biosystem). WGA DNA was tested by a specific quality control PCR (*Ampli1* QC kit, Silicon Biosystem) for 4 targets (chromosome 12p; 5q; 17p; 6q) and different amplicon length (91bp; 108-166bp; 299bp; 614bp, respectively).

Results.

We enrolled 18 CRPC patients treated with cabazitaxel. Fourteen patients were evaluated for presence of CTCs at baseline of treatment. A sample was considered “CTC positive” when one or more CTCs expression biomarker among *AKT*, *AR*, *EPCAM*, *PSMA*, *PSCA*, *PI3KCA* was positive. Eight (57%) out of 14 patients showed a positivity of CTCs. Higher median baseline PSA level was found in patients with CTCs positive compared to patients without CTCs (171 ng/mL [25-944.4] *vs*. 40 ng/mL [2.7-146.2] , respectively) (Table 1). The PSA response was evaluable in 12 patients, a PSA decline >50% was observed in 10 /12 (83%). At baseline of treatment, CTCs were present in all non responsive patients and showed expression of various markers (Table 2) compared to responder cases.
Moreover, preliminary data on CTCs in few patients at radiological evaluation and at disease progression time were obtained. We showed that the loss of CTCs with EMT/STEM characteristics

during treatment suggests a treatment response while a constant positivity to CTCs suggests a disease progression.

Conclusions.

We detected CTCs in about 60% of CRPC patients prior to cabazitaxel treatment with a positive correlation between PSA and CTCs presence. We have also performed a feasibility study on DNA from adnatest selection of CTCs and we obtained a good quality DNA to perform subsequent analyses with conventional PCR or digital PCR approaches. We are now collecting follow up data and CTCs samples during treatment and at disease progression, to complete and enlarge the case series.

Table 1

Case series (14 pts)	**CTC positive at baseline**	
	Yes	**No**
	No (%)	**No (%)**
Patients	**8 (57)**	**6 (43)**
Baseline PSA, median	**171 ng/mL**	**40 ng/mL**
	[25-944.4]	**[2.7-146.2]**