Circulating cell-free DNA is a potential prognostic biomarker of metastatic castration-resistant prostate cancer for taxane therapy

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Prostate cancer (PC) is the most frequently diagnosed cancer and a major cause of cancer deaths among men in the developed countries (1). Metastatic prostate cancer remains the major challenge in PC treatment. Despite standard treatment with androgen deprivation therapy (ADT), patients with metastatic PC will inevitably progress to metastatic castration-resistant prostate cancer (mCRPC). In recent years, a number of therapeutic options for mCRPC have been developed, including taxane-based chemotherapy and androgen receptor (AR)-targeting therapy with either abiraterone or enzalutamide (2,3). Although not curative, these treatments prolong patient survival. In this regard, the selection of appropriate treatment plan to maximize survival benefit becomes important. Of note, extensive research effort has been devoted in exploring prognostic biomarkers to assess disease progression under second line therapies in men with mCRPC. Using the CALGB-90401 phase III clinical trial (n=1,050) and validation with a subgroup (n=942) in the ENTHUSE 33 trial, a prognostic model was recently updated to assess overall survival (OS) in mCRPC patients receiving the first line chemotherapy with docetaxel. The model consists of eight baseline clinical factors: opioid analgesic use, lactate dehydrogenase (LDH), disease site, Eastern Cooperative Oncology Group (ECOG) performance status, albumin, hemoglobin, alkaline phosphatase (ALP), and prostate-specific antigen (PSA) (4). Recently, an even more powerful risk estimate model, the ensemble of penalized Cox regression (ePCR) model, has been reported to predict OS in mCRPC patients receiving docetaxel treatment. This model was generated by a massive collaborative effort through the Dialogue for Reverse Engineering Assessments and Methods (DREAM) challenge platform and involved five phase III clinical trials (n=2,336). In addition to the parameters described above, this model also include many other factors related to kidney function, haematology, and others (5,6).

In an effort to develop additional prognostic biomarkers of mCRPC for taxane-based chemotherapy, Mehra *et al.* performed a post-hoc analysis of two phase III clinical trials to estimate the biomarker values of plasma cell-free DNA (pcfDNA) for disease progression on taxane-based chemotherapy (7). Chemotherapy-naïve patients with mCRPC in the FIRSTANA trial were receiving either cabazitaxel or docetaxel (8); post-docetaxel mCRPC patients in the PROSELICA trial were treated with cabazitaxel (9). Drugs were administered intravenously once every three weeks (Q3W) for multiple cycles; blood was collected prior to initiating cycles 1, 2, 4, and at the end of therapy (*Figure 1*). A total of 571 patients with 315 from FIRSTANA (n=1,168) and 256 from PROSELICA (n=1,200) had blood collected and analyzed in this study (7). Cell-free DNA in plasma was subsequently isolated and quantified. The pcfDNA concentrations from baseline, cycle 2 (C2), and C4 were analyzed for associations with PSA response using logistic regression, radiological progression free survival (rPFS), and OS using Cox models (*Figure 1*). Patients from individual trials were

analyzed separately and in combination; in the latter setting, a two-stage individual patient meta-analysis was performed and heterogeneity was controlled with I^2 statistics (7).

Baseline pcfDNA concentration was not associated with PSA response (defined by the Prostate Cancer Working Group 2 as ≥50% of PSA reductions) and radiological response in FIRSTANA, PROSELICA, and the combination populations (*Table 1*) (7). Nonetheless, longitudinal pcfDNA concentrations were significantly lower per cycle treatment in patients with PSA response in comparison to those without PSA response (7). Additionally, C2 and C4 pcfDNA concentrations were significantly associated with PSA response in FIRSTANA, PROSELICA, and combined patient populations (*Table 1*) (7). Collectively, baseline pcfDNA concentration does not have a clear predictive value for PSA response in patients with mCRPC receiving chemotherapy, while pcfDNA concentrations during treatment are predictive of PSA response. However, it should be noticed that the reduction of pcfDNA concentration were only transiently observed in patients with PSA response in cycles 2 and 4 treatment and that the C2 concentration of pcfDNA did not associate with PSA response in FIRSTANA patients (*Table 1*) (7).

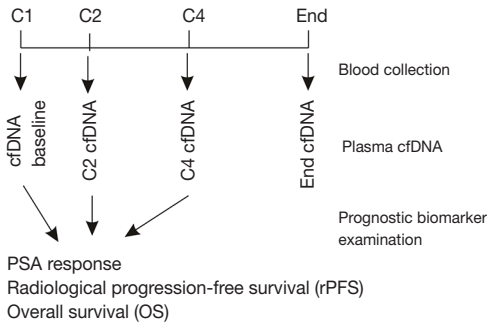

Figure 1 Study design. Patients with mCRPC were receiving the designed taxane drugs in the indicated cycles (C1 and so on) intravenously every three weeks. Blood was collected prior to the initiation of individual cycle treatment. Plasma cell free DNA (pcfDNA) were isolated, quantified and analyzed for biomarker potential for PSA response, rPFS, and OS.

Unlike PSA response, baseline pcfDNA concentration was significantly associated with rPFS with adjusted hazard ratio (aHR 1.54), 95% confidence interval (95% CI, 1.15–2.08),

Table 1 Associations of plasma cfDNA with mCRPC progression on chemotherapy

pcfDNA	Outcome	Study patients	Association status
Baseline	PSA response[i] and radiological response[i]	FIRSTANA	No
		PROSELICA	No
		Combined	No
	rPFS[ii]	FIRSTANA	Yes (HR =1.82/95% CI, 1.13–2.95)
		PROSELICA	No (1.26/0.72–2.20)
		Combined	Yes (1.56/1.08–2.95)
	OS[ii]	FIRSTANA	Yes (1.80/1.16–2.79)
		PROSELICA	No (1.36/0.91–2.05)
		Combined	Yes (1.55/1.15–2.09)
Longitudinal	PSA response[i]	FIRSTANA	No (C2 pcfDNA), yes (C4 pcfDNA)
C2		PROSELICA	Yes (C2 pcfDNA), yes (C4 pcfDNA)
C4		Combined	Yes (C2 pcfDNA), yes (C4 pcfDNA)
	rPFS[ii]	Combined	Yes (1.89/1.36–2.63, P<0.001) (C2 pcfDNA)
			Yes (1.88/1.32–2.68, P<0.001) (C4 pcfDNA)
	OS[ii]	Combined	Yes (1.77/1.37–2.29, P<0.001) (C2 pcfDNA)
			Yes (1.75/1.30–2.35, P<0.001) (C4 pcfDNA)

[i], determined by univariable logistic regression; [ii], modeled through multivariable Cox analysis.

and P=0.004; and OS (aHR 1.53, 95% CI, 1.18–1.97, P=0.001) in the combined population (7). Multivariable Cox model of baseline pcfDNA concentration together with other baseline characteristics including ECOG performance status, visceral metastasis, bone-only disease, Gleason score, pain, albumin, ALP, LDH, and NLR (neutrophil-to-lymphocyte ratio) revealed the baseline concentration of pcfDNA being an independent prognostic factor for rPFS and OS in the combined population (*Table 1*) (7). Both C2 and C4 pcfDNA concentrations also predicted rPFS (HR 1.89, 95% CI, 1.36–2.63, P<0.001; HR 1.88, 95% CI, 1.32–2.68, P<0.001, respectively) and OS (HR 1.77, 95% CI, 1.37–2.29, P<0.001; HR 1.75, 95% CI, 1.30–2.35, P<0.001, respectively) (*Table 1*) (7). For analysis of individual patient populations, baseline pcfDNA concentration associates with rPFS in FIRSTANA (HR 1.82, 95% CI, 1.13–2.95) but not PROSELICA (HR 1.26, 95% CI, 0.72–2.20); and OS in FIRSTANA (HR 1.80, 95% CI, 1.16–2.79) but not PROSELICA (HR 1.36, 95% CI, 0.91–2.09) (*Table 1*) (7). Furthermore, using C-index, the inclusion of baseline pcfDNA did not improve the fit of the multivariable model consisting of all aforementioned baseline characteristics in discriminating either rPFS or OS when pcfDN was not included (7). These observations suggest that pcfDNA concentration may not be a robust classifier for response to taxane chemotherapy.

Nonetheless, the study by Mehra *et al.* demonstrated an overall utility of pcfDNA quantitation in predicting mCRPC progression on taxane-based chemotherapy. In both FIRSTANA and PROSELICA groups, baseline pcfDNA concentration was significantly associated with a set of prognostic characteristics including ECOG performance status, pain at baseline, albumin, ALP, haemoglobin, LDH, PSA doubling time (<2 *vs.* ≥2 months), and NLR at baseline (7). These associations are in accordance with the reported cumulative evidence for a general association of circulating cfDNA (ccfDNA) with PC tumorigenesis and progression (10). Elevations of ccfDNA concentration were reported in PC patients compared to individuals with benign prostatic hyperplasia (BPH; patients n=142 *vs.* BPH n=19) (11) and healthy controls (patients n=133 *vs.* controls n=33) (12). High levels of baseline ccfDNA were observed to associate with biochemical recurrence (BCR) in patients with localized disease subjected to radical prostatectomy (13). Although the entry concentrations of ccfDNA did not correlate with BCR, an increase in ccfDNA following surgery was associated with a reduction in BCR free survival (12). Among the 48 mCRPC patients with PSA declines following taxane-based chemotherapy, patients (n=6) with PSA reductions less than 30% displayed a significantly higher baseline ccfDNA compared to others; however, baseline ccfDNA concentration was not associated with PSA declines ≥50% (14), which was consistent with the observations reported by Mehra *et al.* (7). Collectively, it does not appear that baseline ccfDNA quantitation has a prognostic value for PSA response (reductions ≥50%) in mCRPC patients treated with taxane-based chemotherapy.

In the combined study and FIRSTANA population of docetaxel-naïve patients with mCRPC, baseline pcfDNA concentrations were significantly associated with shortening in rPFS and OS (*Table 1*) (7). The associations did not reach statistical significance in the PROSELICA population of post-docetaxel patients judged on the low boundary of 95% CI less than 1 (*Table 1*) (7). As this result was derived from multivariable Cox analyses (7); it is possible that univariable Cox analysis might reveal baseline pcfDNA concentration being a significant risk factor of reductions in rPFS and OS in the PROSELICA population following second line cabazitaxel chemotherapy. Nonetheless, evidence suggests that the prognostic value of baseline pcfDNA concentration for rPFS and OS seems to be reduced in mCRPC patients progressed on docetaxel therapy. The inclusion of baseline pcfDNA concentration did not enhance the fit of the model composed of the clinical characteristics with demonstrated prognostic value for OS in mCRPC patients treated with chemotherapy (4,5,7). In this situation, the prognostic value of baseline pcfDNA concentration for rPFS and OS would be more appreciated should the C-index of baseline pcfDNA alone in the separation of radiological progression and fatality was reported (7).

The presence of ccfDNA in healthy individuals was reported 70 year ago (15); its levels are elevated in response to a variety of pathological conditions including benign lesions, cancer, rheumatoid arthritis, inflammation, and tissue trauma (16-18). Quantitation of ccfDNA is thus not cancer-specific, which likely contributes to the limitation of using baseline pcfDNA concentration to predict mCRPC progression on taxane-based chemotherapy (7).

Unlike quantitative alterations, qualitative changes in ccfDNA mirror tumor-associated genomic alterations. With next generation sequencing (NGS), detection of genome instability can impressively discriminate PC from non-tumor tissue with an area under curve (AUC) of 0.92 (95% CI, 0.87–0.95) (19). Methylation of CpG islands was detected in serum cfDNA, which displayed a diagnostic value on PC (20). Consistent with persistent AR signaling being a major cause in disease progression on AR-targeting therapies (21), copy number increases in the AR and CYP17A1 (a critical enzyme functioning in androgen biosynthesis) genes observed in

ccfDNA significantly associate with disease progression following abiraterone, enzalutamide, and taxane therapies (22,23). Furthermore, NGS-based deep exon sequencing of 72 mCRPC driver genes using pcfDNA detected somatic mutations in 72 genes with a high level of concordance with tumor associated mutations (R^2 =0.9) in treatment-naïve mCRPCs to second line AR-targeted therapy of abiraterone or enzalutamide (24). Among these mutations, defects in BRCA2 and ATM strongly predict poor outcome (24). In this regard, there exists great opportunities for future studies to investigate the prognostic value of AR abnormalities, genomic alterations in the 72 mCRPC driver genes, and epigenetic alterations in ccfDNA for rPFS and OS in patients with mCRPC to taxane-based chemotherapy using FIRSTANA and PROSELICA clinical trials.

Qualitative analyses of ccfDNA using comprehensive clinical materials available in phase III trials will certainly enhance its ability to predict treatment response in patients with mCRPC and improve clinical decision making regarding treatment regimen choices. This will make the non-invasive ccfDNA-based liquid biopsy even more attractive. However, ccfDNA as a biomarker is not without its limitations. Although evidence suggests apoptosis and necrosis as mechanisms response for releasing cellular DNA into circulation, there is a lack of comprehensive understanding on the process of how blood cfDNA are produced (25). Without detailed knowledge, it is difficult to envisage how the ccfDNA composition reflects tumor-associated heterogeneity. For example, prostate cancer stem cells may be quiescent and their DNA content will unlikely be released through cell damaging mechanisms including apoptosis and necrosis. A large body of evidence exists to support a critical role of prostate cancer stem cells in PC progression under ADT and second line therapies involving abiraterone, enzalutamide, and taxane (21). Furthermore, blood cfDNA is under active clearance by the liver and kidney (25), which results their presence at low levels. This clearance may also alter the composition of blood cfDNA, thereby compromising its representation of tumor-associated heterogeneity.

A number of treatment options are available to patients with mCRPC, none are curative. Good biomarkers or prognostic models facilitate decision making in treatment selection. Collective effort in this domain has indeed formulated a set of clinical characters to predict outcome in patients with mCRPC, which include bone pain, LDH, disease site, ECOG performance status, albumin, hemoglobin, ALP, PSA, and others (4,5). Further research in the field of biomarker exploration for mCRPC should include mechanism-based molecular events in addition to those of demonstrated clinical characteristics.

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Footnote

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