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Prostate Cancer

Circulating Tumor DNA Abundance and Potential Utility in De Novo Metastatic Prostate Cancer

Gillian Vandekerkhove[a,†], *Werner J. Struss*[a,†], *Matti Annala*[b,†], *Heini M.L. Kallio*[b], *Daniel Khalaf*[c], *Evan W. Warner*[a], *Cameron Herberts*[a], *Elie Ritch*[a], *Kevin Beja*[a], *Yulia Loktionova*[a], *Antonio Hurtado-Coll*[a], *Ladan Fazli*[a], *Alan So*[a], *Peter C. Black*[a], *Matti Nykter*[b], *Teuvo Tammela*[b], *Kim N. Chi*[a,c], *Martin E. Gleave*[a], *Alexander W. Wyatt*[a,*]

[a] *Department of Urologic Sciences, Vancouver Prostate Centre, University of British Columbia, Vancouver, Canada;* [b] *Prostate Cancer Research Center, Faculty of Medicine and Life Sciences and BioMediTech Institute, University of Tampere, Tampere, Finland;* [c] *Department of Medical Oncology, British Columbia Cancer Agency, Vancouver, Canada*

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Abstract

Background: Several systemic therapeutic options exist for metastatic castrate-sensitive prostate cancer (mCSPC). Circulating tumor DNA (ctDNA) can molecularly profile metastatic castration-resistant prostate cancer and can influence decision-making, but remains untested in mCSPC.
Objective: To determine ctDNA abundance at de novo mCSPC diagnosis and whether ctDNA provides complementary clinically relevant information to a prostate biopsy.
Design, setting, and participants: We collected plasma cell-free DNA (cfDNA) from 53 patients newly diagnosed with mCSPC and, where possible, during treatment. Targeted sequencing was performed on cfDNA and DNA from diagnostic prostate tissue.
Results and limitations: The median ctDNA fraction was 11% (range 0–84%) among untreated patients but was lower (1.0%, range 0-51%) among patients after short-term (median 22 d) androgen deprivation therapy (ADT). *TP53* mutations and DNA repair defects were identified in 47% and 21% of the cohort, respectively. The concordance for mutation detection in matched samples was 80%. Combined ctDNA and tissue analysis identified potential driver alterations in 94% of patients, whereas ctDNA or prostate biopsy alone was insufficient in 19 cases (36%). Limitations include the use of a narrow gene panel and undersampling of primary disease by prostate biopsy.
Conclusions: ctDNA provides additional information to a prostate biopsy in men with de novo mCSPC, but ADT rapidly reduces ctDNA availability. Primary tissue and ctDNA share relevant somatic alterations, suggesting that either is suitable for molecular subtyping in de novo mCSPC. The optimal approach for biomarker development should utilize both a tissue and liquid biopsy at diagnosis, as neither captures clinically relevant somatic alterations in all patients.
Patient summary: In men with advanced prostate cancer, tumor DNA shed into the bloodstream can be measured via a blood test. The information from this test provides complementary information to a prostate needle biopsy and could be used to guide management strategies.
Sequencing data were deposited in the European Genome-phenome Archive (EGA) under study identifier EGAS00001003351.

† These authors contributed equally to this work.
* Corresponding author. Vancouver Prostate Centre, Department of Urologic Sciences, University of British Columbia, 2660 Oak Street, Vancouver, BC, V6H 3Z6, Canada. Tel. +1 604 875 4818; Fax: +1 604 875 5654.

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1. Introduction

De novo metastatic disease represents 5–10% of prostate cancer (PC) diagnoses but contributes to almost 50% of PC-related deaths [1,2]. The incidence of de novo metastatic diagnoses is rising, potentially related to better imaging modalities and a reduction in prostate-specific antigen (PSA) screening [3,4]. Historically, affected men were managed with systemic androgen deprivation therapy (ADT) alone, but recent phase III data supports treatment combination with taxane chemotherapy or androgen receptor (AR) targeted therapy in high burden disease [5–8]. Other targeted therapies such as PARP inhibitors are also being tested in metastatic castration-sensitive prostate cancer (mCSPC). Thus, there is increasing interest in the potential of tumor molecular features to help in guiding therapy choice.

The majority of patients with de novo mCSPC will not undergo surgery for management of their primary tumor, and metastatic biopsy is not routine. The only source of tissue is typically the diagnostic prostate biopsy. In some cases, diagnosis is based solely on clinical parameters such as exceptionally elevated PSA and concurrent radiographic bone lesions. Although next-generation sequencing of formalin-fixed, paraffin-embedded (FFPE) tissue-derived DNA is now routine, it is unknown whether tumor cells obtained from prostate biopsy are representative of synchronous metastatic deposits.

Plasma circulating tumor DNA (ctDNA) is a promising minimally invasive biomarker in progressing metastatic castration-resistant PC (mCRPC) [9–11]. The fraction of ctDNA as a proportion of total cell-free DNA (cfDNA) can approach 90% in mCRPC [10,12]. High ctDNA levels reflect proliferative disease and poor prognosis, and ctDNA-based mutational and copy number profiles are consistent with matched metastatic tissue [10,12,13]. However, plasma ctDNA is largely unexplored in mCSPC; it remains unclear whether acute ADT impacts ctDNA levels, which is a relevant question because patients with de novo mCSPC may start ADT before a decision on treatment intensification (eg, with chemotherapy). In this study, our objective was to determine ctDNA abundance at de novo mCSPC diagnosis and establish the degree to which molecular subtyping profiles obtained from prostate biopsy tissue and ctDNA are complementary.

2. Patients and methods

2.1. Clinical cohort

We prospectively enrolled 53 men diagnosed with de novo mCSPC at Vancouver General Hospital/University of British Columbia (UBC) Department of Urologic Sciences and the British Columbia Cancer Agency from June 2014 to March 2018. A confirmatory transrectal ultrasound (TRUS)-guided prostate biopsy was performed in 50 patients. Diagnoses were established via histology, PSA levels, and radiographic imaging (computed tomography and/or bone scan). All patients underwent blood collection for ctDNA analysis within 50 d of diagnosis. Where possible, blood was obtained at follow-up appointments. Three additional men with de novo mCSPC were enrolled at Tampere University Hospital from October 2017 to June 2018. Study approval was granted by the UBC Clinical Research Ethics Board (certificates H18-00944, H16-00934 and H09-01628) and the Regional Ethics Committee of Tampere University Hospital (certificate R03203). Written informed consent was obtained from all participants before enrolment.

Table 1 – Clinical characteristics at diagnosis with de novo metastatic castration-sensitive prostate cancer

Parameter	Result
Median age at diagnosis, yr (IQR)	68 (60–76)
Median prostate-specific antigen at diagnosis, ng/ml (IQR)	110 (32–280)
Gleason grade group, *n* (%)	
1	1 (2)
2	0 (0)
3	3 (6)
4	6 (11)
5	38 (72)
Unknown	5 (9)
Metastatic extent of disease at diagnosis, *n* (%)	
Lymph node only	5 (9)
Regional	2
Non-regional	3
Bone	40 (75)
Lung	6 (11)
Liver	2 (4)
Initial therapy regimen after diagnosis, *n* (%)	
ADT only	14 (26)
ADT + docetaxel (without AR-targeted therapy)	18 (34)
ADT + AR targeted therapy[a]	9 (17)
ADT + docetaxel + AR-targeted therapy[a]	8 (15)
Unknown[b]	4 (8)
Patients with cfDNA collected before ADT initiation (%)	67
Patients with cfDNA collected after ADT initiation, % (range in d)	33 (1–49)

IQR = interquartile range; ADT = androgen deprivation therapy; AR = androgen receptor; cfDNA = cell-free DNA.
[a] Abiraterone acetate, enzalutamide, or apalutamide.
[b] Patients enrolled in a blinded study.

2.2. Sample processing, DNA sequencing, and bioinformatics

Blood and tissue processing was performed as previously described (Supplementary material) [10,14,15]. We used an established targeted sequencing strategy capturing the exons of 73 PC driver genes in cfDNA and tissue samples [10], modified by the inclusion of 4-bp molecular barcodes to the index sequence for cfDNA libraries. Sequence data analysis, including identification of somatic mutations and copy number alterations, was performed according to published protocols [10]. The ctDNA fraction was estimated on the basis of somatic mutation allele fractions and leveraged matched tissue sample mutations in cases with low ctDNA fractions (Supplementary material). Deidentified sequencing data were deposited in the European Genome-phenome Archive (EGA) under study identifier EGAS00001003351.

2.3. Outcome measures

Castration resistance was defined according to Prostate Cancer Clinical Trials Working Group 3 guidelines [16]. Time to progression and follow-up were calculated from the start of ADT. Survival fractions were estimated using the Kaplan-Meier method and differences between groups were identified using the log-rank test. All hypothesis tests were two-tailed and used a 5% significance threshold. Hazard ratios (HRs)

were calculated using Cox proportional hazards regression with binary covariates (dichotomized at cohort median), using *survival* v.2.41.3 in R v.3.5.0 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

Patient characteristics are provided in Table 1 and Supplementary Table 1. Plasma cfDNA sequencing was successful in 52/53 patients (median depth 927×; Supplementary Table 2). Of the 53 patients, 48 had diagnostic tissue available. Of the five patients without tissue, three had no local biopsy performed (clinical diagnoses only), while two had no remaining tumor after pathology slides were prepared. Tissue sequencing was successful in all 48 patients (median depth 189×).

3.1. ADT rapidly reduces ctDNA abundance

For 35/53 patients, plasma cfDNA was collected before ADT initiation; 74% (26/35) of these had detectable ctDNA (fraction range 2.0–84%; Fig. 1A; Supplementary Table 3), similar to the proportion of mCRPC patients who have detectable ctDNA using our approach [10,12]. Eighteen patients received 1–49 d of ADT (degarelix or goserelin plus bicalutamide) prior to cfDNA collection (median 22 d; Fig. 1A); only ten of 17 (59%) with successfully sequenced cfDNA had detectable ctDNA, and ctDNA fractions were significantly lower than among treatment-naïve patients (mean 6.7% vs 23%; median 1.0% vs 11%; $p = 0.02$, rank-sum test). The reduction in ctDNA fraction was more pronounced after 1 wk of ADT.

For six patients with detectable ctDNA at diagnosis, we obtained follow-up plasma samples within 4 mo of ADT initiation. In five of these patients, ctDNA was undetectable at follow-up (Fig. 1B). In one patient, the ctDNA fraction increased from 50% to 70% between days 4 and 40 on ADT, despite a PSA decline. This patient subsequently began chemotherapy and ctDNA was undetectable in the third collection (102 d after ADT initiation). To confirm the overall trend, we examined serial samples from three patients

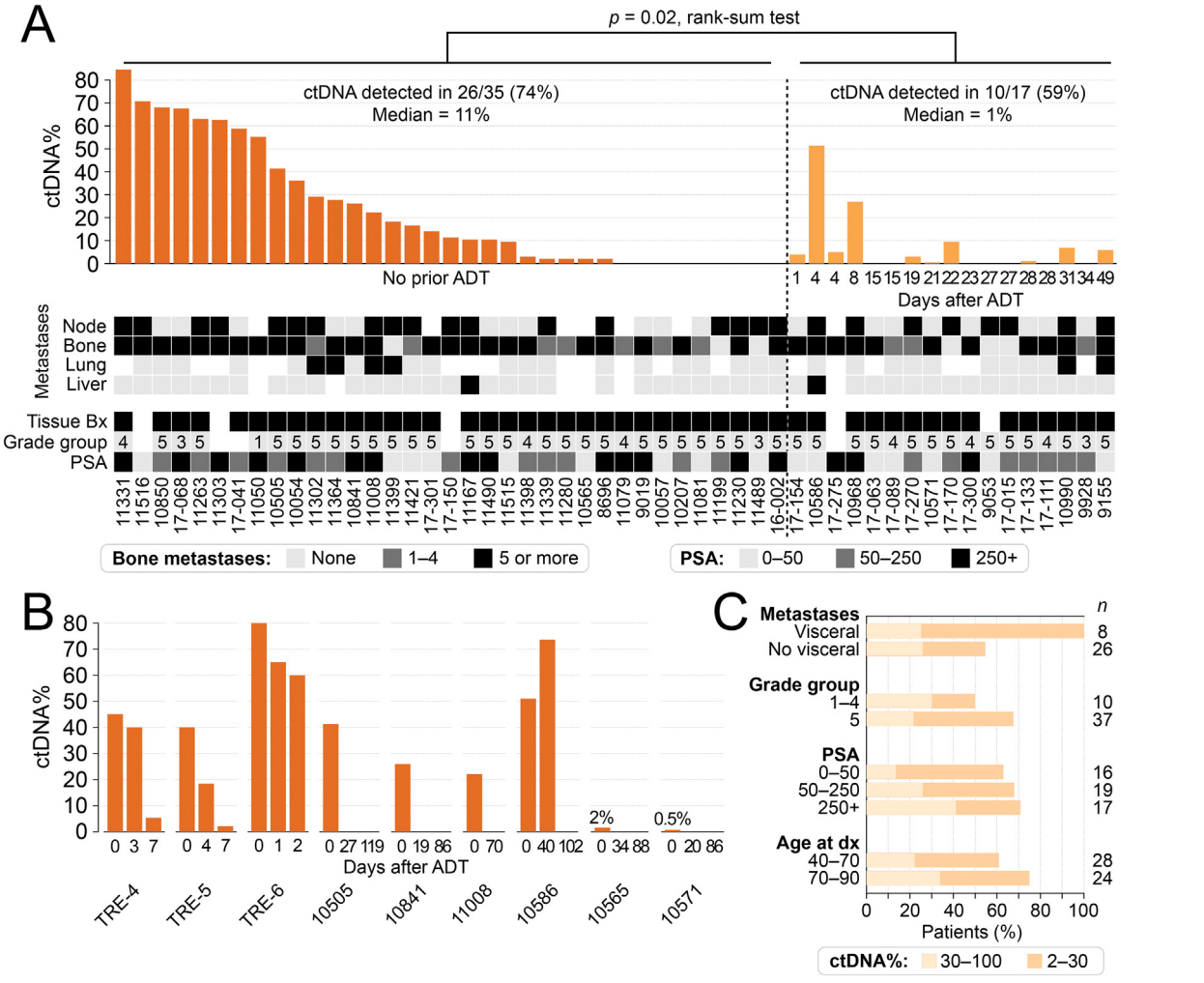

Fig. 1 – Circulating tumor DNA (ctDNA) abundance and impact of androgen deprivation therapy (ADT). (A) Bar plot illustrating the percentage of cell-free DNA that is tumor-derived (ctDNA %) for each patient. Dark orange bars (left) reflect patients who were entirely treatment-naïve at the time of blood collection; light orange bars (right) indicate those exposed to short-term androgen deprivation therapy (ADT). Key clinical variables at diagnosis are indicated in the matrix below the bar plot. (B) Bar plot illustrating ctDNA fraction decline in serial blood collections after commencement of ADT (see also Supplementary Fig. 1). (C) Proportion of patients with ctDNA detected by clinical variable category. PSA = prostate specific antigen; Bx = biopsy; dx = diagnosis.

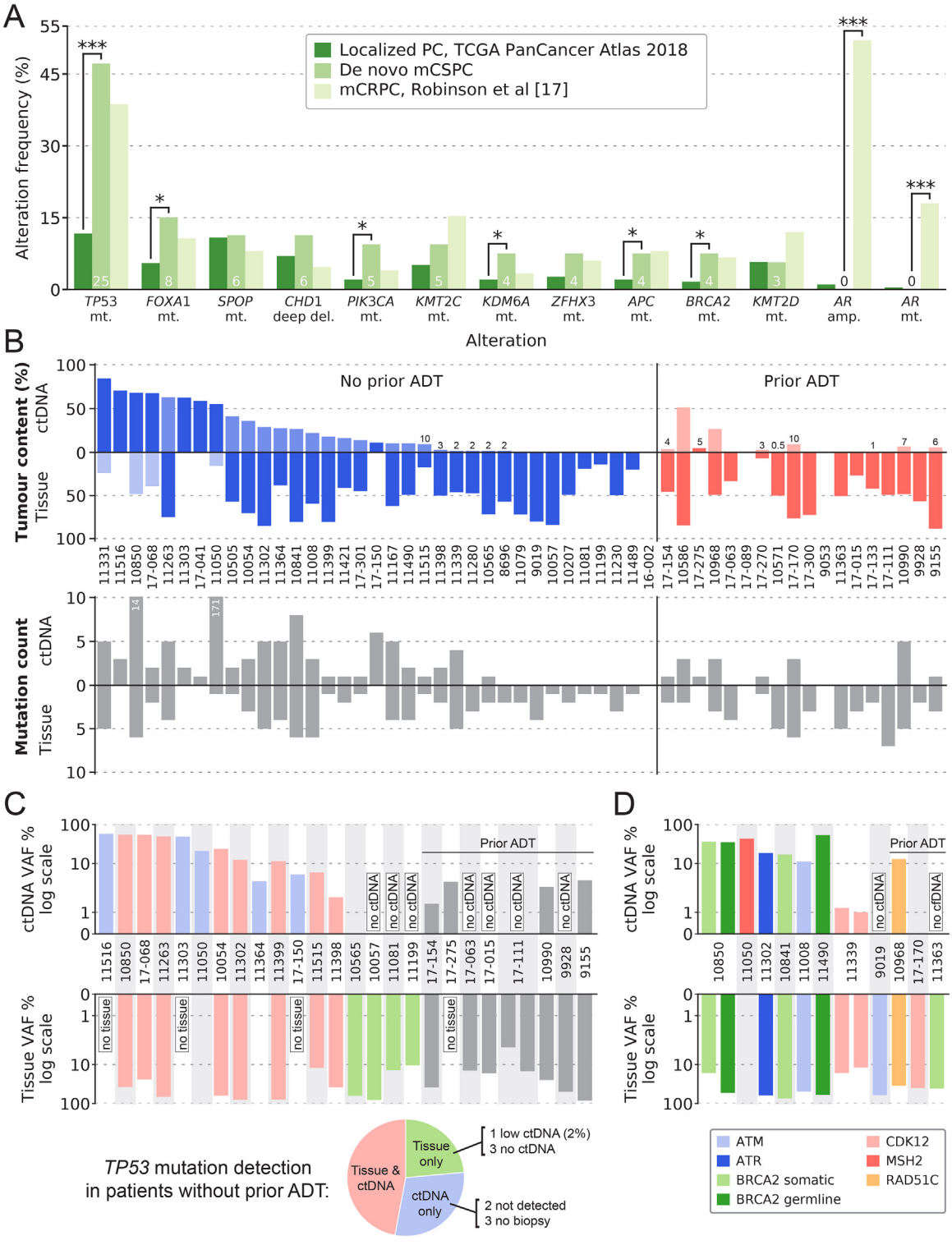

Fig. 2 – Combined analysis of circulating tumor DNA (ctDNA) and primary tissue reveals aggressive genomic features. (A) Frequency of recurrent somatic alterations in de novo metastatic castration-sensitive prostate cancer (mCSPC) as compared to localized prostate cancer (PC) and metastatic castration-resistant PC (mCRPC). Note that sequencing platforms and bioinformatics approaches differ between each study, limiting the conclusions that can be drawn by study-to-study comparison. TGCA = The Cancer Genome Atlas. (B) Bar plots demonstrating tumor content in tissue compared to matched ctDNA (upper panel), and somatic mutation count as derived from these samples (lower panel); stratified by exposure to androgen deprivation therapy (ADT). (C) Concordance of *TP53* mutation detection between matched samples. The pie chart indicates the proportion of *TP53* mutations detected by each assay in androgen deprivation therapy (ADT)-naïve patients. (D) Concordance of DNA damage repair gene calls. mt. = mutation; deep del. = deep deletion; amp. = amplification.

collected within 1 wk of commencing ADT. A clear reduction in ctDNA fraction was observed 1 d after ADT initiation. By day 7, ctDNA fractions were reduced to near zero (Fig. 1B; Supplementary Fig. 1).

Comprehensive diagnostic imaging data was available for 32 patients. All eight patients with liver or lung lesions (including 3 patients exposed to ADT) had detectable ctDNA that was at significantly higher levels than for the remainder of the cohort with confirmed lymph node and/or bone metastases only (14/26; $p = 0.03$, Fisher's exact test; Fig. 1C). Three of the eight patients with visceral metastases had intraductal features in their prostate biopsy. We observed no relationship between ctDNA fraction and PSA, Gleason grade, or age (Fig. 1C).

3.2. Aggressive genomic features with frequent TP53 mutations and DNA repair defects

Combining somatic information from ctDNA and tumor tissue revealed a landscape similar to mCRPC [17], albeit without *AR* gene alterations (Fig. 2A; Supplementary Fig. 2; Supplementary Tables 4 and 5). *TP53* mutations were identified in almost half of the cohort (triple the frequency in localized disease [18]) while a further 11 patients without *TP53* mutations harbored gene deletions. Eleven patients (21%) exhibited truncating mutations in DNA damage repair (DDR) genes, including four patients with *BRCA2* mutations (2 germline). We identified two patients with *CDK12* mutations and copy number profiles with multiple amplifications (eg, *CDK6*, *CCND1*; Supplementary Fig. 3) consistent with the *CDK12*-associated tandem duplication genotype [19,20]. We also identified truncating mutations in *RAD51C* and *ATR*, but in neither case was deletion or mutation of the second allele evident (unlike all deleterious *BRCA2* and *ATM* mutations; Supplementary Fig. 4). We identified one case with an *MSH2* frameshift mutation (and deletion of the second allele) and a high tumor mutation burden consistent with mismatch repair (MMR) deficiency.

3.3. ctDNA and tissue biopsy provide complementary insight to driver gene status

Neither tumor tissue nor plasma cfDNA sequencing in isolation was sufficient to capture somatic information from all patients. We restricted analyses to the 35 patients with no prior ADT, thereby avoiding any confounding influence on ctDNA abundance. This subset included five patients (14%) for whom either a tissue biopsy was not performed or the biopsy core lacked somatic alterations (Fig. 2B). Importantly, in four of these patients somatic alterations were detected in ctDNA. There were also four ADT-naïve patients for whom, despite informative tumor tissue, the ctDNA fraction proved higher than the tumor tissue cellularity (as assessed via the same bioinformatics approach). Conversely, ten ADT-naïve samples had detectable ctDNA but at levels between 2% and 15%, for which low-level gene copy number changes are challenging to resolve. The majority of these patients had tumor tissue cellularity sufficient for copy number analysis (Fig. 2B). Finally, nine ADT-naïve samples had no ctDNA detected via our approach; tissue biopsy profiling better serves these patients. Across the entire cohort, no somatic information was obtained from either approach in only three cases (6%). Two of the three patients had received prior ADT at the time of cfDNA collection, compromising ctDNA abundance.

TP53 alterations are linked to poor prognosis and may represent an important variable to capture at initial diagnosis [10,21]. For ADT-naïve patients, more than half (9/17) of the nonsilent *TP53* mutations were missed by either tissue biopsy profiling or cfDNA sequencing, primarily because of failure of one approach to capture any somatic information, as described above (Fig. 2C). For DDR gene mutations, 9/13 were identified in both tissue and ctDNA (Fig. 2D). However, the *MSH2* truncating mutation and accompanying hypermutation was only identified in the ctDNA of patient 11050; there was no evidence for this clone in matched tumor tissue, and the Gleason grade group 1 suggests that the prostate biopsy undersampled disease. Three DDR gene mutations present in tumor tissue were not identified by cfDNA profiling; two alterations were in patients exposed to prior ADT at sample collection, confounding ctDNA detection. One patient with an ATM truncating mutation and monoallelic deletion in tissue had no detectable ctDNA despite being ADT-naïve and carrying a high plasma cfDNA concentration (16.7× the cohort median). He had marrow infiltration and pancytopenia at the time of blood collection, suggesting that the ctDNA signal may have been diluted by elevated nonmalignant cfDNA.

It is unclear whether a primary tissue sample is representative of metastatic lesions in patients with de novo mCSPC. Here, mutational profiles for de novo mCSPC primary tissue and ctDNA were similar in cases in which both approaches yielded sufficient tumor content for comparison. Among the 26 cases with somatic mutations detected in both tissue and ctDNA (excluding the MMR deficient case), 51/64 mutations (80%) were identified in both compartments (Supplementary Table 4; Supplementary Figs. 2, 5 and 6). Of the 13 mutations detected in only one sample, seven were unique to ctDNA, while six were found only in tissue.

3.4. The majority of alterations in CSPC are shared at CRPC progression

The follow-up for the cohort was 11 mo. At the time of writing, 18 patients had progressed to CRPC (including 2 with neuroendocrine PC); this included seven of the 11 patients (63%) harboring DDR gene mutations, for whom the median time to progression was 7.3 mo (95% confidence interval [CI] 3.2–18.7) versus not reached (95% CI 10.6–not reached) for the remainder of the cohort ($p = 0.01$, log-rank test; Fig. 3A and B). Note that the time to CRPC should be interpreted in the context of variable treatment regimens (Table 1). DDR gene status did not remain significant in multivariate analysis (HR 2.21, 95% CI 0.77–6.37; $p = 0.1$; Supplementary Table 6) because PSA levels were higher in

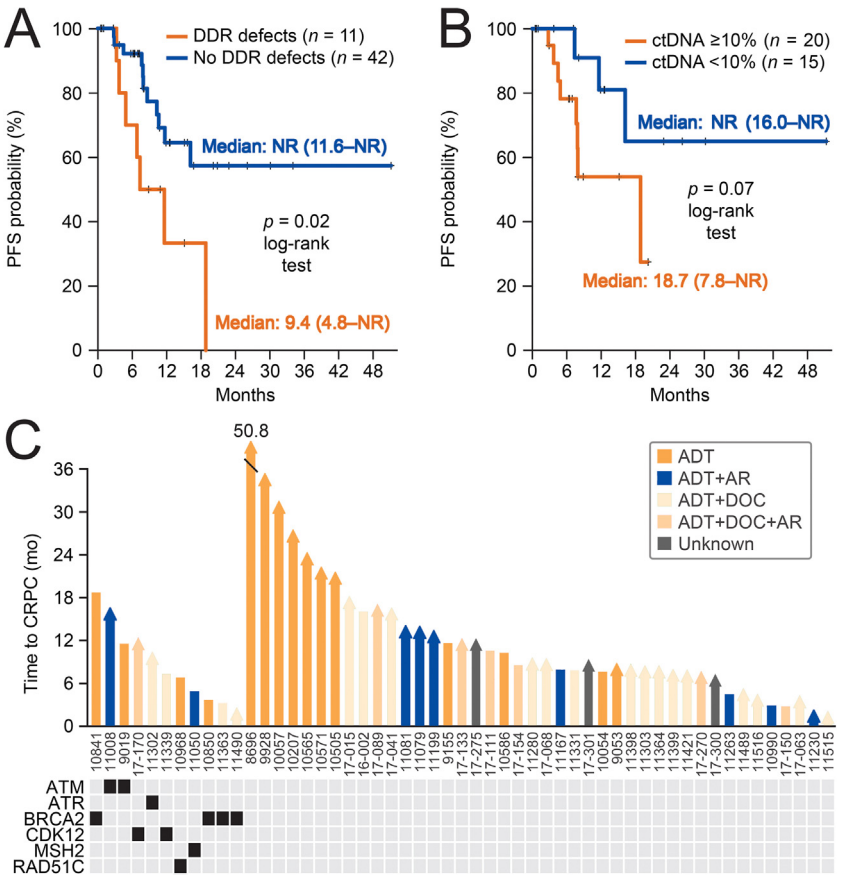

Fig. 3 – DNA damage repair (DDR) gene defects are associated with earlier progression to castration-resistant prostate cancer (CRPC). (A) Kaplan-Meier plot of time to CRPC from ADT initiation in patients with and without deleterious DDR gene alterations. PFS = progression-free survival. (B) Kaplan-Meier plot showing the association of circulating tumor DNA (ctDNA) fraction and time to CRPC from ADT initiation. (C) Swimmers plot of time to CRPC progression from diagnosis, stratified by DDR gene status. ADT = androgen deprivation therapy; AR = androgen receptor-targeted therapy; DOC = docetaxel chemotherapy.

patients with DDR defects (median 290 vs 77 ng/ml; $p = 0.005$, rank-sum test).

For 11 patients, plasma cfDNA was collected after CRPC progression. Seven of the nine patients with detectable ctDNA after progression developed an *AR* amplification or mutation (Fig. 4; Supplementary Table 4 and Supplementary Fig. 7). In general, few changes were detected outside of the *AR*, although one patient (who did not develop an *AR* alteration) exhibited a hotspot *CTNNB1* missense mutation at the time of CRPC progression that was not identified in his diagnostic tissue sample (Fig. 4). Only one patient (17-111) demonstrated marked genomic differences between his diagnostic and CRPC specimens; however, a shared *PTEN* stopgain mutation confirmed shared clonal ancestry.

4. Discussion

Plasma ctDNA is abundant in most patients with treatment-naïve de novo mCSPC, providing additional insight into metastatic disease beyond that available from prostate biopsy. However, ADT exposure before blood collection significantly reduced ctDNA abundance, thereby impairing detection of clinically relevant somatic alterations. Since ctDNA originates from apoptosis of cancer cells [22,23], a transient spike in ctDNA fractions a few hours after therapy initiation remains possible. Furthermore, ADT type (eg, degarelix vs goserelin) differentially impacts the rate at which a castrate testosterone level is achieved, and may be related to the rate of ctDNA decline. We also did not assess whether the biopsy procedure impacts ctDNA or nonmalignant cfDNA release. Nevertheless, our data suggests that for ctDNA to guide treatment intensification in mCSPC, the timing of blood collection (relative to ADT initiation) warrants careful consideration.

Particularly high ctDNA levels were observed in patients with visceral metastases, consistent with mCRPC, for which ctDNA fractions correlate with clinical prognostic markers [9–11]. Therefore, clinical metrics of proliferative tumor volume may help in guiding implementation of ctDNA assays in mCSPC. Also similar to mCRPC [12], somatic mutations identified in ctDNA were highly concordant with matched tissue biopsies. However, while there were cases for which ctDNA proved more informative than tissue biopsy (for detection of driver gene alterations), the opposite was also true, as some patients had low ctDNA levels. Technological advances continue to improve detection sensitivity for ultrarare mutations in cfDNA [24], but common PC copy number alterations such as *PTEN* or *CHD1*

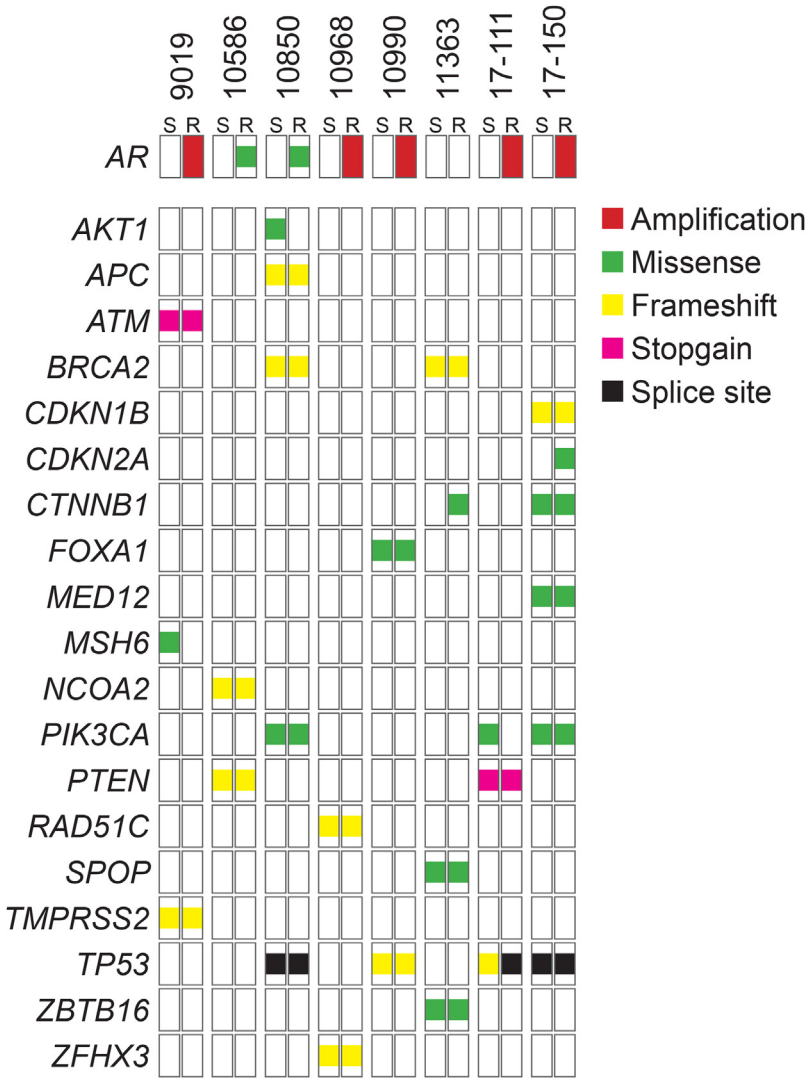

Fig. 4 – Genomic changes at progression to castration-resistant prostate cancer. Oncoprint illustrating similarities between matched castrate-sensitive (S) and castrate-resistant (R) collections, with the exception of the *AR* gene. Copy number alterations only included for the *AR* gene.

deletion remain undetectable when ctDNA constitutes a few percent of total cfDNA. Many of the alterations identified by either cfDNA or tissue sequencing alone have clinical relevance, from DDR gene defects and potential sensitivity to PARP inhibition or immunotherapy [25,26], to *TP53* and *SPOP* mutations that infer poor and favorable prognosis, respectively [10,27,28]. Therefore, the optimal approach for correlative studies or biomarker development in the de novo mCSPC setting should incorporate both tissue and plasma analyses, or risk undersampling disease.

De novo mCSPC is poorly characterized since sequencing efforts have focused on either localized disease or mCRPC. In our study, the similarity between primary tissue and ctDNA may suggest that de novo mCSPC is a highly clonal disease at diagnosis, although follow-up studies are required to confirm this hypothesis. In localized PC, intratumor heterogeneity is common, and truly independent tumor foci can arise within the same prostate [29,30]. It is possible that de novo mCSPC represents later-stage disease after the most aggressive tumor clone expands and predominates. Alternatively, it might represent a different disease trajectory, characterized by the emergence of a singularly aggressive clone that rapidly proliferates. Regardless, de novo mCSPC is characterized by aggressive genomics including frequent *TP53* and DDR gene mutations; this appears to be distinct from localized disease, but different sequencing and analysis approaches between studies prevent definitive conclusions. Among patients who progressed to CRPC, ctDNA at progression yielded highly similar profiles to their CSPC counterpart, suggesting that de novo mCSPC is primed for therapy resistance. Future studies assessing greater patient numbers and a broader range of somatic alterations are required.

To maintain cost efficiency, we captured a small fraction of the genome and did not perform ultradeep sequencing (ie, ~10,000×). Some samples with apparent low tumor content might harbor somatic alterations at high variant frequency outside the panel, or conversely might harbor

alterations below our detection sensitivity. The unavoidable sampling bias associated with TRUS-guided needle biopsy could account for mutations detected only in ctDNA. Future studies could instead assess saturation template biopsies. Finally, given the level of noise associated with FFPE tissue-derived copy number profiles, comparisons with ctDNA-derived copy number alterations were limited.

5. Conclusions

Plasma ctDNA fractions are elevated in de novo mCSPC, especially in patients with visceral metastases. However, exposure to ADT compromises the potential utility of ctDNA. When measurable, ctDNA defines the driver alterations in de novo mCSPC, but combined use of ctDNA and primary tissue is optimal for assessing molecular subtype and could aid targeted therapy implementation in a precision oncology framework.

Author contributions: Alexander W. Wyatt had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Chi, Gleave, Wyatt.
Acquisition of data: Vandekerkhove, Struss, Kallio, Khalaf, Beja, Loktionova, Hurtado-Coll, Fazli, So, Black, Tammela, Chi, Gleave.
Analysis and interpretation of data: Vandekerkhove, Struss, Annala, Warner, Herberts, Ritch, Gleave, Chi, Wyatt.
Drafting of the manuscript: Vandekerkhove, Annala, Warner, Herberts, Wyatt.
Critical revision of the manuscript for important intellectual content: Vandekerkhove, Annala, Gleave, Chi, Wyatt.
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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.eururo.2018.12.042.

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