Circulating tumour DNA abundance and potential utility in *de novo* metastatic prostate cancer

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Abstract

Background: Several systemic therapeutic options exist for metastatic castrate-sensitive prostate cancer (mCSPC). Circulating tumour DNA (ctDNA) can molecularly profile metastatic castration-resistant prostate cancer (mCRPC) 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 newly diagnosed patients with mCSPC and, where possible, during treatment. Targeted sequencing was performed on cfDNA and DNA from diagnostic prostate tissue.

Results and Limitations: Median ctDNA fraction was 11% (range 0-84) among untreated patients but lower (1.0%, range 0-51) in patients after short term (median 22 days) androgen deprivation therapy (ADT). *TP53* mutations and DNA repair defects were identified in 47% and 21% of the cohort, respectively. 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 are 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, tumour DNA shed into the bloodstream can be measured by a blood test. The information from this test provides complementary information to a prostate needle biopsy and could be used to guide management strategies.

Introduction

De novo metastatic disease represents 5-10% of prostate cancer (PCa) diagnoses but contributes to almost 50% of PCa related deaths [1,2]. The incidence of *de novo* metastatic diagnosis is rising, potentially related to improved imaging modalities and decreased 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 poly (ADP-ribose) polymerase inhibitors (PARPi) are also being tested in metastatic castration-sensitive prostate cancer (mCSPC). As such, there is increasing interest in the potential for tumour molecular features to help guide therapy choice.

The majority of patients with *de novo* mCSPC will not undergo surgical management of their primary tumour, 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 tumour cells obtained from prostate biopsy are representative of synchronous metastatic deposits.

Plasma circulating tumour DNA (ctDNA) is a promising minimally-invasive biomarker in progressing metastatic castration-resistant prostate cancer (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—a relevant question because *de novo* mCSPC patients may initiate ADT before the decision for treatment intensification (e.g. 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 obtained from prostate biopsy tissue and ctDNA are complementary.

Patients and Methods

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 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 by histology, PSA levels, and radiographic imaging (computed tomography and/or bone scan). All patients underwent blood collection for ctDNA analysis within 50 days 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 prior to enrollment.

Sample processing, DNA sequencing, and bioinformatics

Blood and tissue processing were performed as previously described (**Supplementary Methods)** [10,14,15]. We employed an established targeted sequencing strategy capturing the exons of 73 PCa driver genes in cfDNA and tissue samples [10], modified by the inclusion of four 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]. ctDNA fraction was estimated based on somatic mutation allele fractions and leveraged matched tissue sample mutations in cases with low ctDNA fractions (**Supplementary Methods**). De-identified sequencing data was deposited to the European Genome-phenome Archive (EGA) under study identifier (EGAS00001003351).

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 start of ADT. Survival fractions were estimated using the Kaplan-Meier method and differences between groups were identified using the logrank test. All hypothesis tests were two-tailed and used a 5% significance threshold. Hazard ratios (HR) were calculated using

Cox proportional hazards regression with binary covariates (dichotomized at cohort median), using "survival" package version 2.41.3 in R version 3.5.0.

Results

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

Androgen deprivation rapidly reduces ctDNA abundance

For 35/53 patients, plasma cfDNA was collected prior to 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 that have detectable ctDNA with our approach [10,12]. 18 patients received 1-49 days of ADT (degarelix or goserelin plus bicalutamide) prior to cfDNA collection (median 22 days) (**Fig. 1A**); only 10 of 17 (59%) with successfully sequenced cfDNA had detectable ctDNA, and ctDNA fractions were significantly lower than in treatment-naïve patients (mean 6.7% versus 23%, median 1.0% versus 11%; p=0.02, ranksum test). The reduction in ctDNA fractions was more pronounced after one week of ADT.

For six patients with detectable ctDNA at diagnosis, we obtained follow-up plasma samples within four months of ADT initiation. In 5/6 patients, ctDNA was undetectable at follow-up (**Fig. 1B**). In one patient, 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 days after ADT initiation). To confirm the overall trend, we examined serial samples from three patients collected within one week of commencing ADT. A clear reduction in ctDNA fraction was observed one day after ADT initiation. By day seven, 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 three patients exposed to ADT) demonstrated detectable ctDNA, significantly higher than 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**). 3/8 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**).

Aggressive genomic features with frequent *TP53* mutations and DNA repair defects

Combining somatic information from ctDNA and tumour 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 the cohort (triple the frequency in localized disease [18]) while a further 11 patients without *TP53* mutations harboured gene deletions. Eleven patients (21%) exhibited

truncating mutations within DNA damage repair (DDR) genes, including four patients

with *BRCA2* mutations (two germline). We identified two patients with *CDK12* mutations

and copy number profiles with multiple amplifications (e.g. *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 tumour

mutation burden consistent with mismatch repair (MMR) deficiency.

ctDNA and tissue biopsy provide complementary insight to driver gene status

Neither tumour 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%) where 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 where, despite informative tumour tissue, the ctDNA fraction proved

higher than the tumour tissue cellularity (as assessed by the same bioinformatic

approach). Conversely, ten ADT-naïve samples had detectable ctDNA but at levels

between 2 and 15%, where low-level gene copy number changes are challenging to

resolve. The majority of these patients had tumour tissue cellularity sufficient for copy

number analysis (**Fig. 2B**). Finally, nine ADT-naïve samples had no ctDNA detected with 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%). 2/3 patients had received prior ADT at 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, over half (9/17) of the non-silent *TP53* mutations were missed by either tissue biopsy profiling or cfDNA sequencing, primarily due to 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 tumour tissue, and the Gleason grade group of 1 suggests that the prostate biopsy undersampled disease. Three DDR gene mutations present in tumour 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 (16.7x cohort median). He had marrow infiltration and pancytopenia at time of blood collection, suggesting that ctDNA signal may have been diluted by elevated non-malignant cfDNA.

It is unclear whether a primary tissue sample is representative of metastatic lesions in patients with *de novo* mCSPC. Here, mutational profiles of *de novo* mCSPC primary tissue and ctDNA were similar in cases where both approaches yielded sufficient tumour content for comparison. Among the 26 cases with somatic mutations detected in both tissue and ctDNA (excluding the MMR deficient case), 51/64 (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.

Majority of alterations in CSPC are shared at CRPC progression

Follow-up for the cohort was 11 months. At time of writing, 18 patients had progressed with CRPC (including two with neuroendocrine PCa); this included 7/11 (63%) patients harbouring DDR gene mutations (median time to progression 7.3 months (95% CI: 3.2 - 18.7) compared to not reached (95% CI: 10.6 - not reached) for the remainder of the cohort (p=0.01, logrank test; **Fig. 3A-B**). Note that 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 (0.77-6.37), p=0.1; **Supplementary Table 6**), because PSA levels were higher in patients with DDR defects (median 290 versus 77 ng/mL, p=0.005, ranksum test).

For eleven patients, plasma cfDNA was collected after CRPC progression. 7/9 patients with detectable ctDNA post-progression developed either 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 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.

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 prior to 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 (e.g. degarelix versus goserelin) differentially impacts the rate at which castrate testosterone is achieved, and may be related to the rate of ctDNA decline. We have also not assessed whether the biopsy procedure impacts ctDNA or non-malignant cfDNA release. Nevertheless, our data suggests that in order for ctDNA to guide treatment intensification in mCSPC, blood collection timing (relative to ADT initiation) warrants careful consideration.

Particularly high ctDNA levels were observed in patients with visceral metastases, consistent with mCRPC where ctDNA fractions correlate with clinical prognostic markers [9–11]. Therefore, clinical metrics of proliferative tumour volume may help guide 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 where 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 ultra-rare mutations in cfDNA [24], but common PCa copy number

alterations such as *PTEN* or *CHD1* 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 PARPi or immunotherapy [25,26], to *TP53* and *SPOP* mutations that infer poor and favourable 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 PCa, intra-tumour heterogeneity is common, and truly independent tumour foci can arise within the same prostate [29,30]. It is possible that *de novo* mCSPC represents later stage disease, after the most aggressive tumour clone expands and predominates. Alternatively, it may represent a different disease trajectory, characterized by 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 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 larger 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 ultra-deep sequencing (i.e. ~10,000x). Some samples with apparent low tumour content may harbour somatic alterations at high variant frequency outside the panel, or conversely harbour alterations below our detection sensitivity. The unavoidable sampling bias associated with TRUS-guided needle biopsy may 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.

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.

Acknowledgements

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Tables and Figures

Median age at diagnosis (IQR)	68 (60-76)
Median PSA at diagnosis (IQR)	110 (32-280)
Gleason grade group	
1	1 (2%)
2	0 (0%)
3	3 (6%)
4	6 (11%)
5	38 (72%)
Unknown	5 (9%)
Metastatic extent of disease at diagnosis	
Lymph node only	5 (9%)
Regional	2
Non-regional	3
Bone	40 (75%)
Lung	6 (11%)
Liver	2 (4%)
Initial therapy regimen post-diagnosis	
ADT only	14 (26%)
ADT + docetaxel (without AR targeted therapy)	18 (34%)
ADT + AR targeted therapy*	9 (17%)
ADT + docetaxel + AR targeted therapy*	8 (15%)
Unknown**	4 (8%)
Patients with cfDNA collected prior to ADT initiation	67%
Patients with cfDNA collected post ADT initiation (range in days)	33% (1-49)

Table 1. Clinical characteristics at diagnosis with *de novo* metastatic castration-sensitive prostate cancer (mCSPC). IQR = interquartile range; ADT = androgen deprivation therapy, AR = androgen receptor. *Patients enrolled in a blinded study.
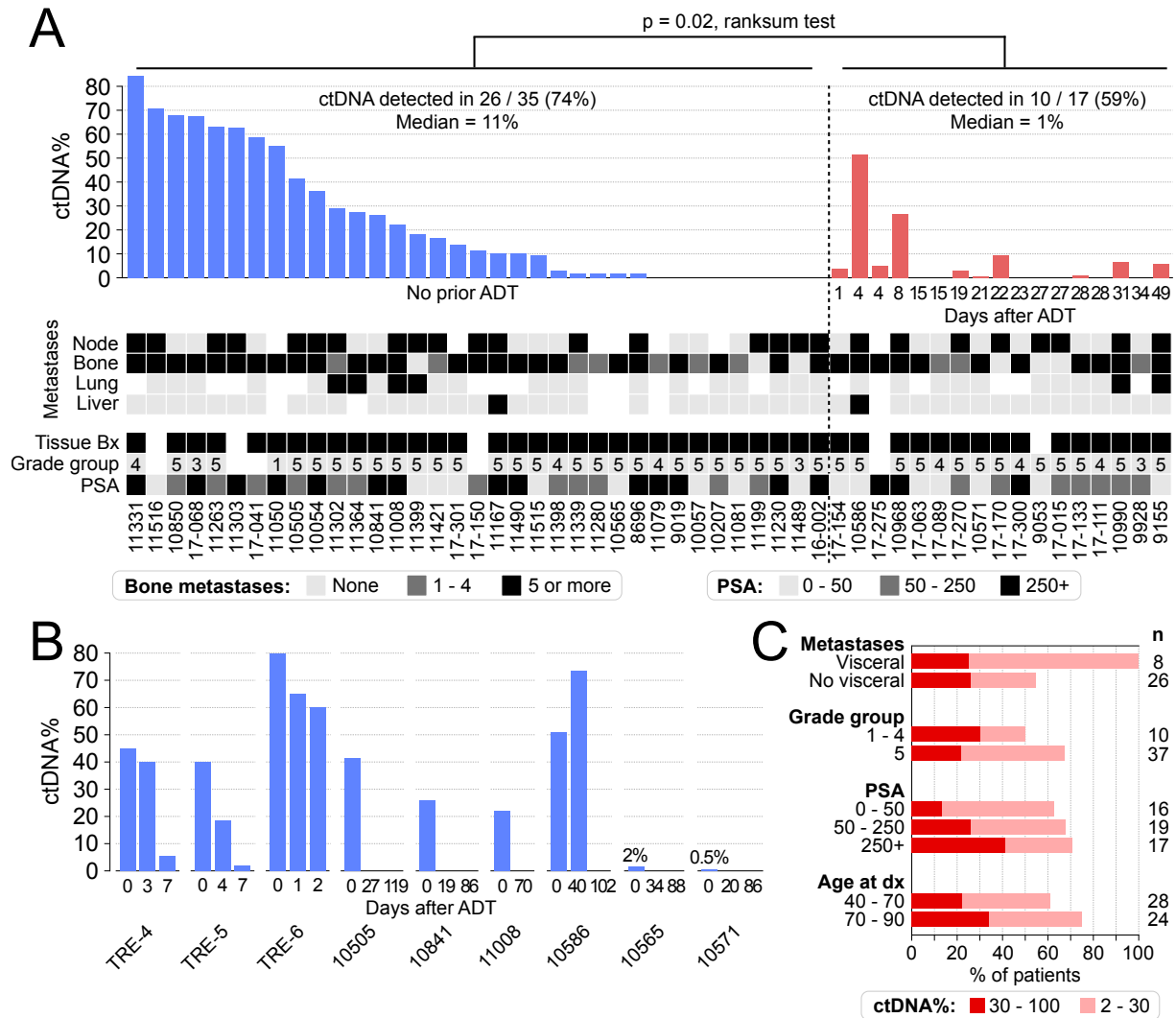
Figure 1. Circulating tumour DNA (ctDNA) abundance and impact of androgen deprivation therapy (ADT). **A)** Bar plot illustrating the percentage of cell-free DNA (cfDNA) that is tumour-derived (i.e. the ctDNA%) for each patient. Blue bars reflect patients that were entirely treatment-naïve at time of blood collection; red bars indicate those exposed to short term 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 detected ctDNA, based on clinical variables. PSA = prostate specific antigen; Bx = biopsy; dx = diagnosis.
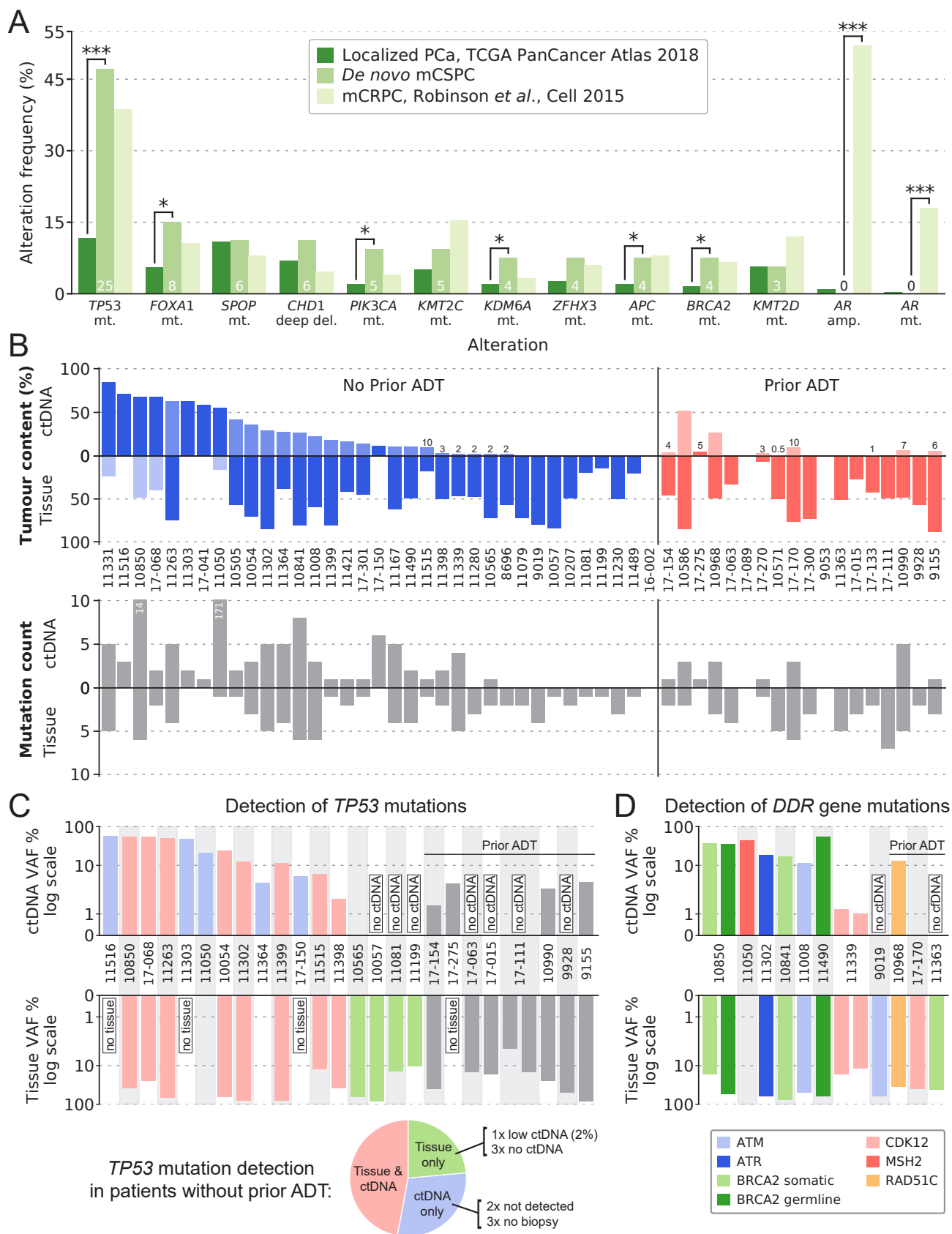
Figure 2. Combined analysis of circulating tumour DNA (ctDNA) and primary tissue reveals aggressive genomic features. **A)** Frequency of recurrent somatic alterations in *de novo* metastatic castration-sensitive prostate cancer (mCSPC) as compared to localized prostate cancer (PCa) and metastatic castration-resistant prostate cancer (mCRPC). Note that sequencing platforms and bioinformatic approaches differ between each study, limiting the conclusions that can be drawn by study-to-study comparison. **B)** Bar plots demonstrating tumour 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. Pie chart indicates proportion of *TP53* mutations detected by each assay in ADT-naïve patients. **D)** Concordance of DNA damage repair (DDR) gene calls. mt. = mutation; deep del. = deep deletion; amp. = amplification.
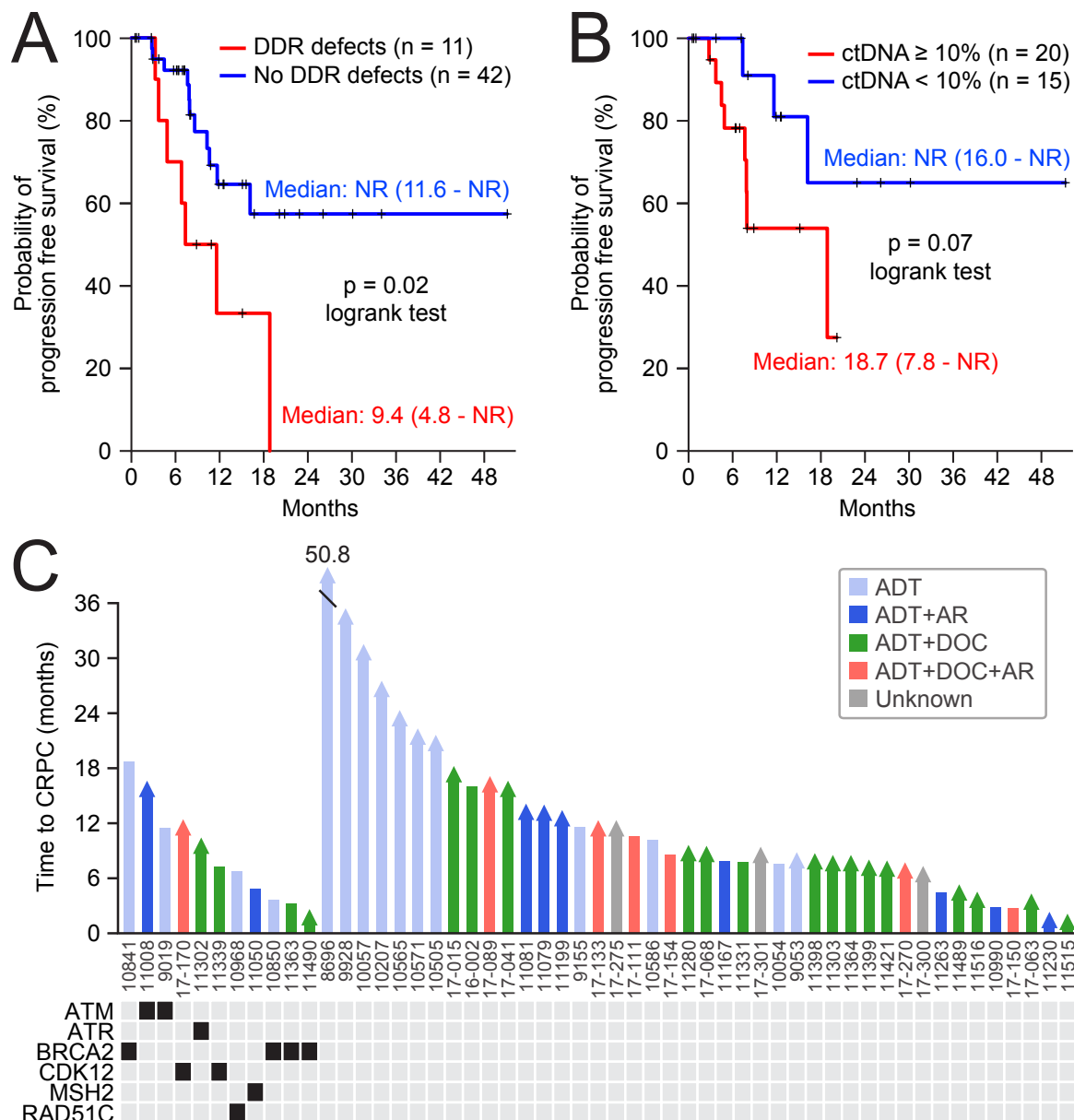
Figure 3. DNA damage repair (DDR) gene defects are associated with earlier progression to castration-resistant prostate cancer (CRPC). **A)** Kaplan-Meier plot of time to CRPC from ADT initiation in patients with and without deleterious DDR gene alterations. **B)** Kaplan-Meier plot showing the association of 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.
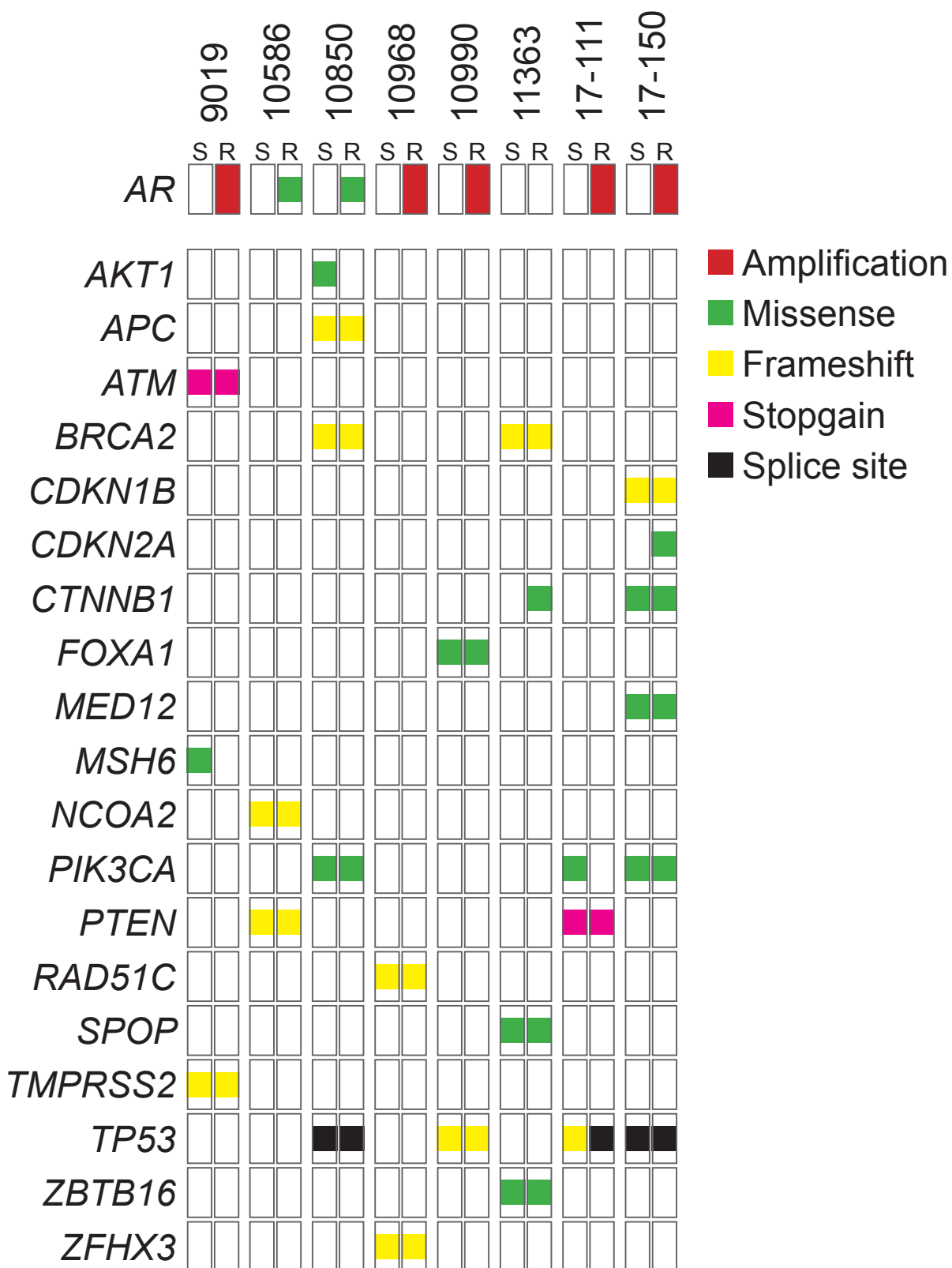
Figure 4. Genomic changes at progression to castration-resistant prostate cancer (CRPC). Oncoprint illustrating similarities between matched castrate-sensitive (S) and castrate-resistant (R) collections, with the exception of the *AR* gene. Copy number alterations only included for the *AR* gene.