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Circulating tumor cell genomic evolution and hormone therapy outcomes in men with metastatic castration-resistant prostate cancer (mCRPC)

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Abstract

Men with circulating tumor cell (CTC) AR-V7 positive mCRPC have worse outcomes when treated with enzalutamide/abiraterone. However, most men lack CTC AR-V7 detection, and additional predictive-biomarkers are needed. We conducted a retrospective secondary analysis of the prospective PROPHECY trial (NCT02269982) of men with mCRPC undergoing treatment with enzalutamide/abiraterone, analyzing pooled CTC and germline DNA for whole-genome copy number alterations (CNAs) in 73 samples from 48 men over time along with pooled CTC and germline whole-exome sequencing (WES) on 22-paired samples before and following progression on AR inhibitor therapy to identify somatic genomic alterations associated with acquired resistance. We observed broad inter-patient and longitudinal CTC genomic heterogeneity from

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Conflicts of interest

- SH sits on DSMB for Ferring, Bayer, and Eisai and receives funding via ASCO as a statistician on the TAPUR trial.
- GK, MA, PG, DMN and SG have no conflicts of interest.
- DJG has research support (to Duke) from Acerta, Astellas, BMS, Bayer, Calithera, Exelixis, Janssen, Myovant, Pfizer, Novartis, Sanofi Aventis. Consulting income from Vizuri health sciences, UroToday, Sanofi, Pfizer, Nektar, Myovant, Modra, Merck, Ipsen, Flatiron, Exelixis, Capio, EMD Serono, BMS, Bayer, AstraZeneca, Astellas.
- SGG is a paid consultant for Pfizer and receives research funding to the institution from Janssen Pharmaceuticals.
- AJA is a paid consultant with Pfizer, Astellas, Janssen, Bayer, AstraZeneca, and Merck and receives research funding (to his institution) from Pfizer, Astellas, Janssen, Bayer, Dendreon, Novartis, Genentech/Roche, Merck, BMS, AstraZeneca, Constellation, Beigene.

AR-V7 negative men with mCRPC, including common gains of *KDM6A*, *MYCN*, and *AR*, and loss of *ZFHX3*, *BRCA1*, and *PTEN*. Men who had progression-free survival of <=3 months despite enzalutamide/abiraterone treatment were more likely to have baseline CTC genomic loss of *CHD1*, *PTEN*, *PHLPP1*, and *ZFHX3* and gains of *BRCA2*, *KDM5D*, *MYCN*, and *SPARC*. After progression on abiraterone/enzalutamide, we observed clonal-evolution of CTCs harboring *TP53* mutations and gain of *ATM*, *KDM6A*, and *MYC*, and loss of *NCOR1*, *PTEN*, *RB1*, and *RUNX2*. CTC genomic findings were independently confirmed in a separate cohort of mCRPC men who progressed despite prior treatment with abiraterone/enzalutamide (NCT02204943).

Introduction

Men with metastatic castration-resistant prostate cancer (mCRPC) face a range of life-prolonging treatment options, including novel androgen receptor (AR) inhibitors, sipuleucel-T, taxane chemotherapy, the radiopharmaceutical radium-223, and PARP inhibitors (1–5). However, despite the improved survival with AR inhibitors such as enzalutamide or abiraterone, most men with mCRPC will ultimately progress after 1–2 years of therapy, and around 20% of men do not respond to these treatments. Primary resistance to AR inhibitors has been linked to the detection of the AR splice variant AR-V7 in CTCs in about 10–20% of patients, and we have recently validated two measures of CTC AR-V7 in a prospective multicenter trial called PROPHECY (3,6,7). However, many men with mCRPC lack CTC AR-V7 detection and alternative mechanisms of resistance, such as lineage plasticity and the neuroendocrine phenotype, *TP53* or *RB1* alterations, and alterations in *AR* itself may confer AR therapy resistance.

Comprehensive genomic studies of tumor biopsies in prostate cancer have revealed a complex mutational landscape with enormous interpatient and within-patient tumor heterogeneity (8,9). This heterogeneity leads to a wide range of clinical outcomes and prognosis as the molecular genotype frequently is associated with clinical phenotypic events related to the timing of disease progression, metastasis, drug resistance, and tumor relapse. A major goal of precision oncology and clinical cancer research is to enable treatment selection through the identification of genomic alterations that match a patient with a therapy most likely to be beneficial. Given the challenges in metastatic biopsies in men with mCRPC, non-invasive methods may be preferred, and recent studies of circulating tumor cells (CTCs) and ctDNA molecular alterations have demonstrated prognostic significance for a range of molecular alterations (8,10–12). We have recently shown that CTCs can provide a unique source of biological material derived from cancer that may be missed in cell-free plasma DNA sequencing and can represent a patient-friendly, non-invasive, cost-effective real-time liquid biopsy marker for longitudinal monitoring, excluding background noise from healthy or normal cells, and can provide clinical utility as a biomarker of treatment response and outcomes (3,12–16).

We recently demonstrated in the multicenter prospective PROPHECY clinical trial that men who have CTCs that express a ligand-binding domain truncated version of the androgen receptor (AR), called AR-V7 variant, have a very poor response and a short time to progression or death when treated with the potent AR inhibitors. Here, we determined

whether pre-or post-treatment genomic DNA alterations in CTCs from AR-V7-ve men with mCRPC could provide clinical utility in predicting clinical outcomes with these hormonal therapies.

Material and methods

Patients and eligibility

In this prospective translational study (PROPHECY, NCT02269982), institutional review board (IRB) approvals were obtained from Duke University IRB or the Weill Cornell Medical College IRB (PROPHECY), along with written informed consent in accordance with Declaration of Helsinki ethical guidelines from all mCRPC patients. All men had mCRPC with disease progression and were starting treatment with either abiraterone acetate with prednisone or enzalutamide. Eligibility and patient characteristics have been previously published (3), and disease progression at entry followed Prostate Cancer Clinical Trials Working Group (PCWG2) criteria (17). All patients had prostate adenocarcinoma, castrate levels of testosterone less than <50 ng/dl, and at least two poor risk factors for aggressive disease, including high serum LDH, high alkaline phosphatase, radiographic progression, PSA doubling (<3 months), a high number of CellSearch CTCs (>=5 CTCs/7.5 mL whole blood), or prior enzalutamide or abiraterone therapy (3). In the present analysis, we defined rapid progressors as a progression-free survival of <=3 months during treatment with abiraterone or enzalutamide, and extreme responders were defined as men who had progression-free survival of >=6 months. We focused on those men with mCRPC who tested negative for the Epic AR-V7 nuclear protein assay, as these patients have a highly variable outcome, with some patients exhibiting long-term responses and some patients having primary treatment resistance (3).

Circulating tumor cell (CTC) enrichment

CTCs prior to treatment and again at disease progression on abiraterone or enzalutamide were isolated using several methods in the present study in separate blood tubes from men with mCRPC. To enumerate CTCs, we collected 7.5 mL whole blood in CellSave tubes, and CTCs were counted using the FDA-approved CellSearch® CTC method based on EpCAM capture and characterization by lack of CD45 expression and positive pan-cytokeratin expression in a Clinical Laboratory Improvement Amendments (CLIA)-approved laboratory at Memorial Sloan Kettering Cancer Center.

CTC genomic studies were performed on Duke and Cornell patients with sufficient evaluable CTC DNA (Figure 1 CONSORT diagram). For CTC genome-wide copy number analysis, EpCAM positive and CD45 negative CTCs were isolated from 7.5ml mCRPC patient blood samples in independent tubes, as described in our previous study (14). Unspiked and spiked control EpCAM+ (T47D) cells into healthy volunteer blood were used for gating thresholds during fluorescence-activated cell sorting (FACS) analysis (14). Independently, peripheral blood mononuclear cells (PBMCs) were isolated from the same mCRPC patient's blood at the same time point in order to identify only somatic alterations in CTCs (14). Before hybridizing the CTCs DNA samples onto the array comparative genomic hybridization (aCGH) microarray chip (Agilent, 4×180K), all CTC's genomic

DNA was amplified by using GenomePlex® Single Cell Whole Genome Amplification Kits (Sigma, WGA4) to the yield required for cy3/cy5 labeling according to Agilent's aCGH manual (14). Further, AR-V7 nuclear protein was detected in CTCs by using the Epic AR-V7 nuclear protein detection assays, as previously described (3).

Comparative genomic hybridization (CGH)

For whole-genome copy number alterations (CNAs) analysis, two-color probe-based aCGH was performed using Agilent Oligonucleotide Array-based CGH for Genomic DNA Analysis (Sure Print G3 Human CGH array, 4×180K) (14). The aCGH raw data was analyzed by using Agilent Cytogenomics Software default settings (≥ 3 probes, log2 ratio for gain ≥ 0.25 and loss < −0.25), and all the copy number alterations (CNAs) were manually validated by two independent investigators (14,18) and later, cross-referenced with publicly-available prostate cancer datasets in cBioPortal (19).

DNA library preparation, HiSeq sequencing, and whole-exome sequencing analysis

For whole-exome sequencing (WES), initial CTC and WBC DNA samples quality assessment, DNA library preparation, sequencing, and bioinformatics analysis were conducted at GENEWIZ, Inc. (South Plainfield, USA). Details of the DNA Library Preparation, Quality Control, and HiSeq Sequencing are summarized in the Supplementary Method, separately. We selected 11 men with mCRPC from our high throughput genomic cohort of Duke and Cornell patients who had sufficient CTC and matched germline DNA at baseline and progression on abiraterone or enzalutamide for longitudinal WES and copy number analysis for the present analysis.

For WES analysis, Agilent software SureCall (v. 3.0.1.4) was used to analyze 150bp paired-end FASTQ files from all the CTCs and WBC DNA samples as a paired analysis (tumor vs. matched WBC) under default parameters. Sequence reads were aligned to the reference genome (GRCh37/hg19) using the SureCall BWA-mem alignment tool following post-alignment processing, included 100 bp region padding, removal of duplicates, and singletons (reads without a mate), followed by a normalization step, including GC and mappability corrections. Somatic variants were called using the SureCall SNPPET algorithm annotated with ClinSNP, ClinVar, Cosmic, gwasV1_ucsc, and NCBI. Only COSMIC validated variants were selected, and unknown variants were filtered out during WES analysis.

Statistics

This analysis is considered an exploratory subset analysis of the overall PROPHECY trial, which had as its primary objective to determine the prognostic and predictive association of CTC AR-V7 detection by two methods (JHU mRNA and Epic Sciences nuclear protein) with progression-free survival during treatment with abiraterone or enzalutamide (3). CTC genomic alterations were associated with outcomes using this case-control approach of sensitive vs. resistant to determine the relevance of specific genomic alterations with primary resistance to these hormonal therapies. Secondarily, we examined longitudinal CTC samples for changes in genomic alterations over time in order to identify potential alterations associated with clonal selection and acquired resistance to abiraterone or enzalutamide. Due

to the exploratory nature of the analysis, no p-values are presented, and results are considered hypothesis-generating.

Results

Patient characteristics

A total of 120 men with progressive mCRPC starting treatment with abiraterone acetate or enzalutamide were enrolled in the PROPHECY study, and baseline characteristics of this cohort have been previously published (3). Of these 120 men, two did not start treatment, and five men who failed CTC extraction or CTC DNA quality testing were excluded from the downstream genomic analyses, leaving 113 evaluable men with mCRPC. Of these 113, 52 men consented to additional tubes of blood collection for our high throughput sequencing cohort, where we enriched CTCs and paired PBMCs from 88 blood samples, including 52 baseline and 36 at disease progression, and performed whole genome amplification (WGA4, Sigma). Of these 88 samples, 73 samples (45 baseline and 28 progression) had a sufficient amount of amplified genomic DNA for aCGH and WES analysis (Figure 1 CONSORT diagram).

Overall, 48% (54/113) of mCRPC men at baseline had an unfavorable ≥ 5 CellSearch CTCs per 7.5mL blood (range 0–12972 CTCs, median 4), and 27% (29/113) of mCRPC men had zero CTCs. Note that the 88 patient CTC samples were analyzed collectively for genomic analyses from individual men and time points and not single-cell sequenced, and each CTC sample represents a range of CTC numbers from <5 CTCs per blood sample to over 1000 CTCs per sample, and a total of 5198 CTCs were analyzed according to CellSearch CTCs criteria.

After whole genome amplification of CTCs and paired PBMC germline DNA of these 52 men, 45 had sufficient pooled CTC DNA from single blood samples for a paired longitudinal analysis for CNAs at baseline (N=25) and progression (N=25). In addition, we classified patients into two groups defined by their outcomes to hormonal treatment, focusing on 37 men (23 sensitive and 14 resistant) who tested negative for the Epic Sciences CTC nuclear AR-V7 protein (3). Given our interest in exploring mechanisms of resistance to AR targeted therapies in AR-V7 negative men with mCRPC, we further classified patients as sensitive or resistant to abiraterone or enzalutamide based on a PFS of >=6 (n=23) vs. <=3 months (n=14), respectively. In this high throughput cohort, 25 men had paired longitudinal CTC and PBMC collections for CNAs, and 11 men had paired CTCs, and PBMCs were analyzed for WES.

Genome-wide chromosomal copy number alterations in CTC DNA

In order to associate genome-wide baseline and progression CTC CNAs with clinical outcomes during enzalutamide or abiraterone treatment, we first analyzed whole-genome CNAs using aCGH on 73 patients with pooled CTCs from the blood of 48 high-risk mCRPC men treated with enzalutamide or abiraterone acetate, including 45 baseline and 28 progression samples with matched germline reference genomic DNA (Figure 1). Further, we analyzed whole-genome CNAs data from CTCs DNA samples at baseline and progression

and compared (baseline versus progression CNAs). The top genes harboring genomic alterations in over 30% of baseline samples (N=45) included the top gain in *AR*, *IL3RA*, *KDM6A*, *MIR3690*, or *AKAP17A*, and loss in *ZFHX3*, *FGFR2*, or *BRCA1*. At progression (N=28), CTC DNA analysis demonstrated gains in *AKAP17A*, *CD99*, *MXRA5*, *GYG2*, or *ARSF*, and loss in *NCOR1*, *ZFHX3*, or *RUNX2*, are summarized in File S1. We next performed ingenuity pathway analysis among the above genomic altered genes at baseline and progression and found many common and distinct pathways associated with drug resistance and metastasis in mCRPC, respectively, which are summarized in Table S1A and B. Similarly, we also compared the genomic alteration in sensitive (N=23) and resistant baseline CTCs DNA at the genome level, and observed the copy gain in *IL3RA*, *MUM1*, *AKAP17A*, *ZBED1*, or *CSF2RA*, and copy loss in *ZFHX3*, *BRCA1*, or *GRHL2*, whereas, at resistant, demonstrated gain in *JAK3*, *CSF2RA*, *CRLF2*, *EPOR*, and *PIK3R2*, and loss in *ZFHX3*, *PHLPP1*, or *BRCA1*, all the genes are listed in File S2. These data support the concept that CTC genomic alterations recapitulate the heterogeneity of common genomic alterations present in metastatic biopsies from men with mCRPC and suggest that CTC molecular profiling in such patients is feasible and could provide useful biologic information(14,18).

Targeted gene-level assessment of known pathogenic copy number alterations in CTC DNA: Baseline

Given the broad whole genomic findings and heterogeneity between patients and over time, in the present analysis, we focused on a panel of sixty recurrently altered and validated genes that have been previously associated with mCRPC pathogenesis, including those linked to androgen signaling, epigenetics, DNA damage and repair, osteomimicry, cell differentiation, metastasis, and drug resistance in mCRPC (8,14,20). We identified heterogeneity of somatic copy number alterations between men with mCRPC in this PROPHECY cohort. We analyzed blood samples from the 45 men for pooled CTCs DNA aCGH and identified typical genomic gain detected in *AR* (44%), *KDM6A* (40%), *FOXA1* (36%), *UGT2B17* (33%), and *MYCN* (31%), and loss in *ZFHX3* (53%), *FGFR2* (38%), *BRCA1* (33%), *ERG* (31%), and *PTEN* (20%) (Figure 2A). Likewise, these commonly altered genes detected in baseline CTC DNA were also detected in 28 progression CTC DNA samples (Figure 2B). Comprehensively, the sixty-gene CNA status in CTC DNA at the baseline and progression are summarized in Figure S1A and B, respectively.

In order to validate these gains or losses identified in pooled CTC DNA, we analyzed publically available genomic data from 1154 mCRPC tissue samples from 1115 patients in the cBioPortal database (19). We validated many of these findings of recurrent losses or gains in mCRPC and the broad genomic heterogeneity in human metastatic tissue samples, providing some degree of cross-validation of our CTC genomic findings (Figure S2A and B).

Targeted gene-level assessment of known pathogenic copy number alterations in CTC DNA: Longitudinal Analysis

To first study the clonal evolution and selection of genomic alterations associated with acquired treatment resistance to abiraterone or enzalutamide in men with mCRPC, we

compared longitudinal CTC CNAs in 25 paired baseline and progression samples. We found a higher number of CNVs in the progression samples compared to baseline (Figure 3A), despite only a slightly higher number of CellSearch CTCs at progression as compared to 18 paired baseline (median 14 vs. median 9.5 CTCs per 7.5 mL whole blood) (Figure 3B). We also found that 56% (10/18) of patients had more CTCs at progression than their paired baseline cases (Figure 3C).

Next, we compared 25 baseline and paired progression CTC CNA data to define novel CNAs associated with acquired or de novo resistance to abiraterone or enzalutamide. After progression, we detected a greater prevalence of CTCs harboring copy gain of *ATM* (44% at progression vs. 12% at baseline), *FOXA1* (56% vs. 28%), *UGT2B17* (52% vs. 28%), *KDM6A* (48% vs. 24%), *CYP11B1* (44% vs. 20%), and *MYC* (24% vs. 4%) (Figure 3D). We also detected an enrichment for CTC copy loss of *NCOR1* (64% vs. 20%), *ZFHX3* (60% vs. 44%), *RUNX2* (44% vs. 32%), *PXN* (36% vs. 20%) and *ERG* (36% vs. 20%), *PTEN* (32% vs. 24%), and *RB1* (16% vs. 8%) (Figure 3E). Additionally, the differential change (%) in the prevalence of copy gain or loss between progression and baseline CTCs DNA are shown (bottom) (Figure 3D and E). The CNAs of sixty genes in all 25 baseline and paired progression CTCs DNA samples together are summarized in Figure S3 as a heatmap.

We independently validated highly prevalent copy gain/loss genes that were identified at progression on abi/enza in the PROPHECY study cohort in a separate cohort of men with mCRPC starting radium-223 where all mCRPC patients had progressed on prior therapy with enzalutamide or abiraterone (N=14 cases) (4). For example, in the radium-223 trial, we found gain in *CYP11B1* (71%), *AR* (64%), *FOXA1* (64%), and *KDM6A* (57%), and loss in *ZFHX3* (86%), *CHD1* (29%), and *PHLPP1* (29%), which were in accordance with our PROPHECY longitudinal findings (Figure S4). Our data suggest that specific genomic alterations in CTCs are associated with clonal evolution or selection of cells contributing to hormonal therapy resistance in mCRPC men over time.

CTCs DNA genomic alterations predict primary resistance in mCRPC

Given that many men with CTC AR-V7 negative mCRPC still have inadequate responses and rapid resistance to abiraterone or enzalutamide therapy, we next examined baseline CTC genomic alterations and associated these with clinical outcomes in Epic AR-V7 negative men (3). To understand the primary resistance mechanisms in CTCs, genomic alterations in 40 baseline AR-V7 negative (Epic AR-V7 nuclear protein assay) men's CTCs DNA were analyzed and compared. The mCRPC patients benefiting from enza/abi therapy with >=6 months progression-free survival (PFS) were considered as sensitive (N=23), whereas patients with PFS <=3 months were considered as resistant (N=14). Patients with a PFS between 3 and 6 months were excluded from the analysis (N=3) in order to enrich for outlier responses at either extreme of clinical benefit. We found that sensitive patients' PFS was broadly distributed with a PFS ranging from 6 to 25 months, whereas resistant patients' PFS had a tighter distribution by definition (Figure 4A).

Next, we compared the number of CNVs calls at the genome level in sensitive and resistant patients and found that the resistant group had a trend of a higher number of CNVs calls than the sensitive group (Figure 4B). However, we did not observe any difference in

CellSearch CTCs counts between the sensitive and resistant groups, indicating that CTC number, while prognostic and a validated measure of disease burden (15), may alone not be a predictive marker of the benefits of AR inhibitor therapy (Figure 4C). The median and range of CellSearch CTCs in resistant (N=22) and sensitive (N=14) groups were 5 (range 0–194) and 1 (range 0–193), respectively. We also compared the genomic copy gain and loss for sixty genes in the sensitive and resistant cases. We found that the resistant group harbored a higher number of genomic gains than the sensitive cases (median 20 vs. 14). However, we did not see any differences in the prevalence of large numbers of genomic losses between groups (Figure 4D).

To examine specific CNAs associated with treatment response or primary resistance, we compared genomic CNAs between 23 sensitive and 14 resistant baseline CTCs DNA samples. We observed that men who had a clinical benefit to enza/abi (median PFS 10 mo) were more likely to have CTCs with genomic gains of *ATM*, *NCOR2*, *HSD17B4*, or *PTEN*, or loss of *BRAF*, *ABL1*, *MYC*, or *NKX3–1*. Conversely, men who did not respond to enza/abi treatment (median PFS 2.6 mo) had CTCs enriched for copy gain of *BRCA2*, *APC*, *KDM5D*, *MYCN*, *CYP11B1*, *SPARC*, and *AR*, and loss of *CHD1*, *PHLPP1*, *PTEN*, *ERG*, *ZFHX3*, and *NCOR2*. The prevalence of genomic copy gain/loss and the percent difference (delta) between resistant and sensitive CTCs DNA is shown in Figure 4E and F as a superimposed bar chart and heat map, respectively. Specifically, we found that the DNA damage repair genes *ATM* and *BRCA2* were surprisingly differentially gained in resistant vs. sensitive CTCs DNA, with *BRCA2* gain enriched in resistant patients and *ATM* gain enriched in sensitive patients. We further validated the genomic gains of these genes in multiple independent metastatic prostate cancer datasets (N=1154 samples from 1115 patients) in cBioPortal (Figure 4G), suggesting that alterations in *BRCA2* and *ATM*-mediated signaling may be inversely associated with outcomes during treatment with abiraterone or enzalutamide.

Whole exome sequencing of CTCs over time

The preceding work focused mostly on copy number alterations, given their critical importance to mCRPC pathophysiology. However, mutations in oncogenes and tumor suppressors are also relevant genomic alterations associated with poor outcomes in mCRPC (8,21,22). To identify unique somatic variants that develop or undergo clonal selection in CTCs DNA overtime during abiraterone/enzalutamide treatment, we evaluated CTC exome mutations longitudinally in a subset of patients with paired samples. We performed whole-exome sequencing (WES) in 11 Epic AR-V7 negative men at baseline and progression on enzalutamide/abiraterone and compared mutational calls against patient-matched germline DNA from leukocytes as a reference control and to rule out clonal hematopoiesis contamination of samples. The average read depth in analyzable target regions was over 87X, and all calls were annotated and functionally validated in COSMIC datasets. The raw data from the WES of CTCs and WBC DNA were analyzed as a paired analysis (CTCs vs. leukocytes) using Agilent SureCall-NGS software.

Focusing initially on the same sixty prostate cancer-associated and validated pathophysiologic genes from our CNA analysis, we detected high COSMIC-validated non-

synonymous pathogenic mutation frequencies in progressing patients' CTC DNA. In particular, we identified the enrichment of several pathogenic exome mutations at progression on abi/enza, including *TP53*, *CDK12*, *KMT2D*, *BRAF*, *BRD4*, and *SPOP* (Figure 5A). For example, 45% (5/11) of progression cases harbored *TP53* mutations, whereas *TP53* mutations were detected in only 27% (3/11) of cases at baseline, as shown in Figure 5B. Likewise, 36% (4/11) of progression samples had mutations in the *CDK12* gene compared to 18% (2/11) of cases at baseline. Non-synonymous pathogenic mutations in *SPOP* (patient's ID# 809–05, AA: D144G, AF: 0.573) and *TP53* (patient's ID# 809–7, AA: T111K, AF: 0.994) were uniquely detected in progression CTCs DNA, but not in paired baseline or germline DNA, shown as a snapshot image in IGV browser (Figure 5B). On the contrary, some of the genes had a high prevalence of pathogenic mutations detected at baseline CTCs DNA than paired progression CTCs DNA samples, including *RB1*, *BRCA2*, and *PTEN* (Figure 5A). For all the genes, sequence read depth, position, genotype, COSMIC changes, genotype quality, and amino acid changes, etc., are summarized in File S3. In addition, to combine frequent CNAs and mutation in the same sample, we analyzed copy number and mutation data together for all 11 baseline and paired progression CTCs DNA samples and observed a higher somatic tumor mutation burden in progression CTCs samples compared to the baseline (Figure 5C and File S4).

To identify the mutations associated with enza/abi resistance, we compared WES data from five baseline sensitive and five resistant patients' CTCs DNA. Here, we found many genes with pathogenic mutations enriched in resistant samples, such as *PTEN*, *TP53*, *CDK12*, *NRAS*, or *BRAF* (Figure 5D). For example, *PTEN* mutations detected in sensitive (1/5) and resistant (2/5) patients are shown as a lollipop graph generated in cBioPortal (Figure 5E). For example, mutation detected uniquely in genes *PTEN* (patient's # 809–16, AA: D178G, non-syn, missense, AF: 0.290) and *CDK12* (patient's # 809–03, AA: E131K, non-syn, missense, AF: 0.993) were visualized with sequenced read depth coverage by IGV genome browser, shown in Figure 5E. We observed a high tumor mutation burden in resistant samples compared with sensitive cases (Figure 5F, bottom, green color). Moreover, a high prevalence of common mutation and copy number change was seen in resistant cases compared to sensitive cases shown in Figure 5F. For all the sensitive and resistant samples, the read depth, COSMIC annotation, position, genotype, genotype quality, or amino acid changes are summarized in File S5. Besides, all the mutations uniquely detected at baseline vs. progression and sensitive vs. resistant CTCs DNA (CTC vs. WBC) were visualized as a snapshot image showing reference and alternative alleles in the IGV browser are summarized in Figure S5 and S6, respectively. Our somatic pathogenic exome mutation analysis indicated that CTC DNA mutations were most likely associated with disease progression and clinical outcomes in mCRPC patients treated with enza/abi.

Discussion

In this prospective study, we analyzed whole genomic copy number alterations and whole exomic somatic mutations in CTCs in the context of mCRPC treatment resistance in order to identify potential predictive and resistance biomarkers of treatment outcomes with the potent AR inhibitors enzalutamide or abiraterone acetate. We found evidence for broad genomic heterogeneity in copy gains and losses using a liquid biopsy approach in AR-V7 negative

men from the multicenter PROPHECY study, and more importantly, that some of these genomic alterations were associated with clinical outcomes with AR inhibitor therapy (3). While our data confirm prior work suggesting poor outcomes in the setting of *PTEN* loss, *MYCN* gain, *AR* gain, and *TP53* mutations, we have identified novel associations with AR inhibitor sensitivity, including gains in *ATM*, *NCOR2*, and *HSD17B4*, and novel associations of primary resistance to AR inhibition with gains of *BRCA2*, *APC*, *KDM5D*, *MYCN*, *CYP11B1*, *SPARC*, and *AR*, and loss of *CHD1*, *PHLPP1*, *PTEN*, *ERG*, *ZFHX3*, and *NCOR2*. These findings, coupled with validation of this work in public datasets of mCRPC patient tissue samples, warrant further prospective validation and mechanistic studies.

We recently demonstrated that men with mCRPC in the PROPHECY trial who have CTCs that express AR-V7 and treated with enzalutamide or abiraterone have poor outcomes, including a lack of PSA or radiographic responses and short PFS or OS (3). Nevertheless, most men in this setting lack AR-V7 and additional genomic alterations are likely contributing to drug resistance [6]. In this study, we show that the men who had the clinical benefit (PFS >=6 months) to enzalutamide or abiraterone were more likely to have CTCs with genomic alterations in pathways implicated in DNA repair, steroid metabolism, PI3K, and WNT pathway signaling, chromatin and epigenetic gains linked to loss of *CHD1* (23) and gain of *KDM5D*, and lineage plasticity genes including *MYCN*, *FOXA1*, and *NKX3.1*. Moreover, after disease progression on AR inhibitor treatment, we observed the clonal evolution of CTCs harboring genomic gain of *ATM*, *FOXA1*, *UGT2B17*, *KDM6A*, *CYP11B1*, and *MYC*, and acquired loss of *NCOR1*, *ZFHX3*, and *ERG*. While we cannot establish causality from this correlative work, collectively, these genomic alterations in CTCs suggest critical mechanisms of primary or acquired resistance to hormonal therapies in men with mCRPC. Further, our somatic exome mutation analysis of CTCs DNA detected various COSMIC-validated non-synonymous mutations in progressed or resistant AR-V7 negative patients with enrichment of pathogenic mutations, such as *TP53*, *AKAP9*, *CDK12*, *KMT2D*, *BRD4*, and *SPOP*. Thus, these genomic alterations in androgen signaling, WNT, lineage plasticity, epigenetic regulators, and DNA damage pathways support a variety of pathways that were associated with poor clinical outcomes in AR-V7 negative mCRPC men treated with enzalutamide or abiraterone.

In this study, we observed several genomic alterations that may be associated with tumor progression, drug resistance, lineage plasticity, and lethal disease tumor heterogeneity in mCRPC (24). AR overexpression and genomic amplification are the most common occurrence in enza/abi treated mCRPC patients, associated with poor clinical outcomes, where a large proportion of patients may develop primary or secondary resistance to hormonal therapy (14,25). In this prospective study, we observed *AR* gain in 57% (8/14) of resistant and 39% (9/23) of sensitive baseline CTCs DNA samples, which might suggest the role of increases *AR* amplification/gain in developing primary resistant cancer. Remarkably, we did not observe any differences in *AR* gene alterations at the DNA level after disease progression (at progression 8/25 (32%), vs. baseline 11/25 (44%)), which reflects that therapy-resistant clones were present before hormonal therapy. Thus, the genomic alterations of CTCs could be indicative of clonal evolution to survive enza/abi treatment pressure, leading to the selection of resistant sub-clones while eliminating other CTC clones.

In the AR transcription complex, FOXA1, one of the key members for the AR transcriptional activity, is upregulated in advanced prostate cancer, associated with poor prognosis and disease progression, and correlated with PSA level and Gleason scores (26,27). An in vitro study showed that the silencing of FOXA1 in LNCaP cells reduced cell proliferation and growth of prostate cancer (28). In accordance, we also observed that *FOXA1* was one of the top genes with a high prevalence of copy gain detected in our CTCs DNA at baseline 7/25 (28%) and paired progression 14/25 (56%) in mCRPC patients, overtime. Therefore, the increased prevalence of *FOXA1* gain in mCRPC CTCs might be associated with acquired resistance, and AR reprogramming (27) requires further independent confirmations.

In our previous CTCs vs.cfDNA study (18), we established that *MYCN* is uniquely copy gained in CTCs DNA, but not in paired cfDNA in the same patient reflected different CTCs biologics, associated with poor clinical outcomes, neuroendocrine-like prostate cancer phenotype, and resistance to androgen deprivation therapy in mCRPC patient treated with enza/abi (18,29,30). We again observed *MYCN* gain in 43% (6/14) of resistant versus 22% (5/23) of sensitive baseline CTCs DNA. In contrast, we did not see any changes in copy number between the 25 baseline and paired progression CTCs DNA samples (28% vs. 28%). Here, our *MYCN* CNAs findings suggest that *MYCN* might be associated with the primary drug resistance and could be used as a prognostic biomarker for NEPC-like CTCs during hormonal therapy, which requires further clinical validations and functional study (31). In addition, altered genes such as AURKA might also be associated with the primary resistance in the mCRPC patients treated with abi/enza. However, on the other hand, *AURKA* gain may not be associated commonly with acquired resistance given its lack of enrichment in progression samples. Determination of AURKA's role in AR therapy resistance and neuroendocrine transformation will require further mechanistic and targeted therapy studies in selected patients (32).

In addition to genomic copy gain, there were many other genes with high prevalence in genomic copy loss, such as *ZFHX3*, *BRCA1*, *PTEN*, *ERG*, and *FGFR2*, which play an essential role in disease progression and primary resistance in mCRPC (8,14,33). For example, we found that ZFHX3, which acts as a tumor suppressor gene associated with Erk1–2/AKT signaling, development of prostatic intraepithelial neoplasia, and cell proliferation in prostate cancer (34), had a high prevalence of genomic loss in our 15/25 progressed (60%) CTCs DNA in comparison to the paired baseline 11/25 (44%) CTCs DNA. When we compared *ZFHX3* loss between resistant and sensitive baseline CTCs DNA, we found a high prevalence of *ZFHX3* genomic loss in resistant CTCs DNA (9/14, 64%), whereas 48% (11/23) was detected in sensitive baseline cases. The genomic loss of *ZFHX3* in CTCs DNA could be associated with both primary and acquired resistance in mCRPC patients treated with enza/abi, which needs further functional and clinical validations. Finally, these genomic alterations findings support that CTCs DNA genomic alterations may be the driving force in mCRPC progression and hormonal therapy failure.

In a prospective study, Zhang et al. showed that genomic loss of the *CHD1* gene in metastatic prostate cancer promotes resistance to AR-targeted hormonal therapy through chromatin modifications, and associated with worst clinical outcomes in patients treated

with enzalutamide may lead to the emergence of antiandrogen drug resistance in mCRPC (23). Here, we also observed *CHD1* gene loss in 4/14 (29%) of the resistant versus 2/23 (9%) sensitive mCRPC patients treated with enza/abi, which might be associated with hormonal therapy failure, requires further study.

It has previously been shown that lethal clones with clonal heterogeneity were distinguished from the primary tumor to metastases during disease progression in prostate cancer, longitudinally, where they observed many genetic alterations including pathogenic mutations in many vital genes such as *APC*, *SPOP*, *PTEN*, and *TP53* in metastatic tissues, but not in primary tissues, (35). Similarly, we also observed a high prevalence in many genes, for example, *SPOP*, *TP53*, and *BRD4*, where somatic exonic non-synonymous pathogenic COSMIC validated mutations were detected in progressed CTCs DNA, but not in baseline CTCs DNA. For example, we observed a range of genomic alterations at progression, including new *TP53* mutations in 3 cases, each of which had a PFS >6 months, indicating that this new mutation may be acquired during resistance. We observed 2 cases of persistent *TP53* mutations at each time point, each of whom had PFS times less than three months, indicating primary resistance. The presence of these common genomic alterations and exomic mutations may indicate common clones associated with drug resistance and metastases in mCRPC patients treated with enza/abi. Therefore, our findings suggest the emergence of progressive subclone with *PTEN*, *TP53*, *SPOP*, or *BRD4* mutations within progression CTCs requires further validations in larger cohorts of mCRPC patients (36).

The limitations of our present analysis first include the small size of our cohort that underwent whole genomic or exome sequencing within the overall broader PROPHECY study, limiting our ability to perform statistical testing between the individual alterations with clinical outcomes. The ability to detect CTC genomic alterations is directly related to the number of CTCs evaluable at that time, and thus, genomic findings may be biased by disease burden and differences in sensitivity of whole genomic assays at different time points due to changes in CTC numbers in response to therapy and at progression. Our work should be considered hypothesis-generating and exploratory, necessitating prospective validation in larger cohorts. Second, it is possible that some of the identified genomic alterations may represent low-level passenger changes that, while common, may not be driving treatment outcomes. We focused the present study on those genomic alterations previously validated pathophysiologically and clinically in mCRPC or preclinical models and those alterations that were more common across patient samples, thus reducing the potential for bias related to misclassification during genomic amplification of CTC DNA, sequencing, and variant calling. We also excluded germline alterations using paired PBMCs to avoid contamination with genomic alterations associated with clonal hematopoiesis.

In conclusion, our prospective analysis showed the practical feasibility of longitudinally profiling whole-genome CNAs and exome mutation analysis of CTCs DNA. We identified an increased prevalence of common CNAs inclusive of genomic regions associated with critical pathways related to disease progression and treatment resistance in patients with mCRPC. These genomic alterations were associated with androgen signaling, tumor suppressors, WNT, NEPC, epigenetic regulators, and DNA damage and repair relevant to mCRPC biology (Figure 6). The alterations we identified may provide insights into

metastatic biology and provide future clinical applications for biomarker discovery in men with mCRPC in context to enza/abi treatment. The study of genomic alterations in CTCs provides putative clinically actionable information and tracking clonal evolution over-time that could influence treatment options in mCRPC cases. Moreover, if validated, our results may suggest that CTC DNA biomarkers can identify and predict sensitivity and resistance to enzalutamide or abiraterone acetate treatment in men with mCRPC irrespective of AR-V7 status.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. de Bono J, Mateo J, Fizazi K, Saad F, Shore N, Sandhu S, et al. Olaparib for Metastatic Castration-Resistant Prostate Cancer. N Engl J Med 2020;382(22):2091–102 doi 10.1056/NEJMoa1911440. [PubMed: 32343890]
2. de Wit R, de Bono J, Sternberg CN, Fizazi K, Tombal B, Wulfing C, et al. Cabazitaxel versus Abiraterone or Enzalutamide in Metastatic Prostate Cancer. N Engl J Med 2019;381(26):2506–18 doi 10.1056/NEJMoa1911206. [PubMed: 31566937]
3. Armstrong AJ, Halabi S, Luo J, Nanus DM, Giannakakou P, Szmulewitz RZ, et al. Prospective Multicenter Validation of Androgen Receptor Splice Variant 7 and Hormone Therapy Resistance in High-Risk Castration-Resistant Prostate Cancer: The PROPHECY Study. J Clin Oncol 2019:JCO1801731 doi 10.1200/JCO.18.01731.
4. Armstrong AJ, Gupta S, Healy P, Kemeny G, Leith B, Zalutsky MR, et al. Pharmacodynamic study of radium-223 in men with bone metastatic castration resistant prostate cancer. PLoS One 2019;14(5):e0216934 doi 10.1371/journal.pone.0216934. [PubMed: 31136607]
5. Scher HI, Graf RP, Schreiber NA, McLaughlin B, Jendrisak A, Wang Y, et al. Phenotypic Heterogeneity of Circulating Tumor Cells Informs Clinical Decisions between AR Signaling Inhibitors and Taxanes in Metastatic Prostate Cancer. Cancer Res 2017;77(20):5687–98 doi 10.1158/0008-5472.CAN-17-1353. [PubMed: 28819021]
6. Antonarakis ES, Lu C, Wang H, Luber B, Nakazawa M, Roeser JC, et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. N Engl J Med 2014;371(11):1028–38 doi 10.1056/NEJMoa1315815. [PubMed: 25184630]
7. Scher HI, Lu D, Schreiber NA, Louw J, Graf RP, Vargas HA, et al. Association of AR-V7 on Circulating Tumor Cells as a Treatment-Specific Biomarker With Outcomes and Survival in Castration-Resistant Prostate Cancer. JAMA Oncol 2016 doi 10.1001/jamaoncol.2016.1828.
8. Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, Mosquera JM, et al. Integrative clinical genomics of advanced prostate cancer. Cell 2015;161(5):1215–28 doi 10.1016/j.cell.2015.05.001. [PubMed: 26000489]

9. Gundem G, Van Loo P, Kremeyer B, Alexandrov LB, Tubio JM, Papaemmanuil E, et al. The evolutionary history of lethal metastatic prostate cancer. Nature 2015;520(7547):353–7 doi 10.1038/nature14347. [PubMed: 25830880]
10. McGranahan N, Swanton C. Clonal Heterogeneity and Tumor Evolution: Past, Present, and the Future. Cell 2017;168(4):613–28 doi 10.1016/j.cell.2017.01.018. [PubMed: 28187284]
11. Gerlinger M, Rowan AJ, Horswell S, Math M, Larkin J, Endesfelder D, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med 2012;366(10):883–92 doi 10.1056/NEJMoa1113205. [PubMed: 22397650]
12. Heitzer E, Haque IS, Roberts CES, Speicher MR. Current and future perspectives of liquid biopsies in genomics-driven oncology. Nat Rev Genet 2019;20(2):71–88 doi 10.1038/s41576-018-0071-5. [PubMed: 30410101]
13. Wyatt AW, Azad AA, Volik SV, Annala M, Beja K, McConeghy B, et al. Genomic Alterations in Cell-Free DNA and Enzalutamide Resistance in Castration-Resistant Prostate Cancer. JAMA Oncol 2016;2(12):1598–606 doi 10.1001/jamaoncol.2016.0494. [PubMed: 27148695]
14. Gupta S, Li J, Kemeny G, Bitting RL, Beaver J, Somarelli JA, et al. Whole Genomic Copy Number Alterations in Circulating Tumor Cells from Men with Abiraterone or Enzalutamide-Resistant Metastatic Castration-Resistant Prostate Cancer. Clin Cancer Res 2017;23(5):1346–57 doi 10.1158/1078-0432.CCR-16-1211. [PubMed: 27601596]
15. de Bono JS, Scher HI, Montgomery RB, Parker C, Miller MC, Tissing H, et al. Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. Clin Cancer Res 2008;14(19):6302–9 doi 10.1158/1078-0432.CCR-08-0872. [PubMed: 18829513]
16. Moreno JG, Miller MC, Gross S, Allard WJ, Gomella LG, Terstappen LW. Circulating tumor cells predict survival in patients with metastatic prostate cancer. Urology 2005;65(4):713–8 doi 10.1016/j.urology.2004.11.006. [PubMed: 15833514]
17. Scher HI, Halabi S, Tannock I, Morris M, Sternberg CN, Carducci MA, et al. Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group. J Clin Oncol 2008;26(7):1148–59 doi 10.1200/JCO.2007.12.4487. [PubMed: 18309951]
18. Gupta S, Hovelson DH, Kemeny G, Halabi S, Foo WC, Anand M, et al. Discordant and heterogeneous clinically relevant genomic alterations in circulating tumor cells vs plasma DNA from men with metastatic castration resistant prostate cancer. Genes Chromosomes Cancer 2020;59(4):225–39 doi 10.1002/gcc.22824. [PubMed: 31705765]
19. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013;6(269):pl1 doi 10.1126/scisignal.2004088. [PubMed: 23550210]
20. Cancer Genome Atlas Research N. The Molecular Taxonomy of Primary Prostate Cancer. Cell 2015;163(4):1011–25 doi 10.1016/j.cell.2015.10.025. [PubMed: 26544944]
21. Abida W, Cyrta J, Heller G, Prandi D, Armenia J, Coleman I, et al. Genomic correlates of clinical outcome in advanced prostate cancer. Proceedings of the National Academy of Sciences of the United States of America 2019;116(23):11428–36 doi 10.1073/pnas.1902651116. [PubMed: 31061129]
22. De Laere B, Oeyen S, Mayrhofer M, Whitington T, van Dam PJ, Van Oyen P, et al. TP53 Outperforms Other Androgen Receptor Biomarkers to Predict Abiraterone or Enzalutamide Outcome in Metastatic Castration-Resistant Prostate Cancer. Clin Cancer Res 2019;25(6):1766–73 doi 10.1158/1078-0432.CCR-18-1943. [PubMed: 30209161]
23. Zhang Z, Zhou C, Li X, Barnes SD, Deng S, Hoover E, et al. Loss of CHD1 Promotes Heterogeneous Mechanisms of Resistance to AR-Targeted Therapy via Chromatin Dysregulation. Cancer Cell 2020;37(4):584–98 e11 doi 10.1016/j.ccell.2020.03.001. [PubMed: 32220301]
24. Hong MK, Macintyre G, Wedge DC, Van Loo P, Patel K, Lunke S, et al. Tracking the origins and drivers of subclonal metastatic expansion in prostate cancer. Nat Commun 2015;6:6605 doi 10.1038/ncomms7605. [PubMed: 25827447]
25. Taplin ME, Bubley GJ, Shuster TD, Frantz ME, Spooner AE, Ogata GK, et al. Mutation of the androgen-receptor gene in metastatic androgen-independent prostate cancer. N Engl J Med 1995;332(21):1393–8 doi 10.1056/NEJM199505253322101. [PubMed: 7723794]

26. Sahu B, Laakso M, Ovaska K, Mirtti T, Lundin J, Rannikko A, et al. Dual role of FoxA1 in androgen receptor binding to chromatin, androgen signalling and prostate cancer. EMBO J 2011;30(19):3962–76 doi 10.1038/emboj.2011.328. [PubMed: 21915096]
27. Robinson JL, Hickey TE, Warren AY, Vowler SL, Carroll T, Lamb AD, et al. Elevated levels of FOXA1 facilitate androgen receptor chromatin binding resulting in a CRPC-like phenotype. Oncogene 2014;33(50):5666–74 doi 10.1038/onc.2013.508. [PubMed: 24292680]
28. Imamura Y, Sakamoto S, Endo T, Utsumi T, Fuse M, Suyama T, et al. FOXA1 promotes tumor progression in prostate cancer via the insulin-like growth factor binding protein 3 pathway. PLoS One 2012;7(8):e42456 doi 10.1371/journal.pone.0042456. [PubMed: 22879989]
29. Beltran H, Rickman DS, Park K, Chae SS, Sboner A, MacDonald TY, et al. Molecular characterization of neuroendocrine prostate cancer and identification of new drug targets. Cancer Discov 2011;1(6):487–95 doi 10.1158/2159-8290.CD-11-0130. [PubMed: 22389870]
30. Mosquera JM, Beltran H, Park K, MacDonald TY, Robinson BD, Tagawa ST, et al. Concurrent AURKA and MYCN gene amplifications are harbingers of lethal treatment-related neuroendocrine prostate cancer. Neoplasia 2013;15(1):1–10. [PubMed: 23358695]
31. Beltran H, Prandi D, Mosquera JM, Benelli M, Puca L, Cyrta J, et al. Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. Nature medicine 2016;22(3):298–305 doi 10.1038/nm.4045.
32. Beltran H, Oromendia C, Danila DC, Montgomery B, Hoimes C, Szmulewitz RZ, et al. A Phase II Trial of the Aurora Kinase A Inhibitor Alisertib for Patients with Castration-resistant and Neuroendocrine Prostate Cancer: Efficacy and Biomarkers. Clin Cancer Res 2019;25(1):43–51 doi 10.1158/1078-0432.CCR-18-1912. [PubMed: 30232224]
33. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, et al. Integrative genomic profiling of human prostate cancer. Cancer Cell 2010;18(1):11–22 doi 10.1016/j.ccr.2010.05.026. [PubMed: 20579941]
34. Sun X, Fu X, Li J, Xing C, Frierson HF, Wu H, et al. Deletion of atbf1/zfhx3 in mouse prostate causes neoplastic lesions, likely by attenuation of membrane and secretory proteins and multiple signaling pathways. Neoplasia 2014;16(5):377–89 doi 10.1016/j.neo.2014.05.001. [PubMed: 24934715]
35. Haffner MC, Mosbruger T, Esopi DM, Fedor H, Heaphy CM, Walker DA, et al. Tracking the clonal origin of lethal prostate cancer. J Clin Invest 2013;123(11):4918–22 doi 10.1172/JCI70354. [PubMed: 24135135]
36. Carver BS, Chapinski C, Wongvipat J, Hieronymus H, Chen Y, Chandarlapaty S, et al. Reciprocal feedback regulation of PI3K and androgen receptor signaling in PTEN-deficient prostate cancer. Cancer Cell 2011;19(5):575–86 doi 10.1016/j.ccr.2011.04.008. [PubMed: 21575859]

Implications:

We identified common and reproducible genomic alterations in CTCs from AR-V7 negative mCRPC men associated with poor outcomes during enzalutamide/abiraterone treatment, including CNAs in genes linked to lineage plasticity and epigenetic signaling, DNA repair, AR, TP53/RB1, PTEN, and WNT pathways.

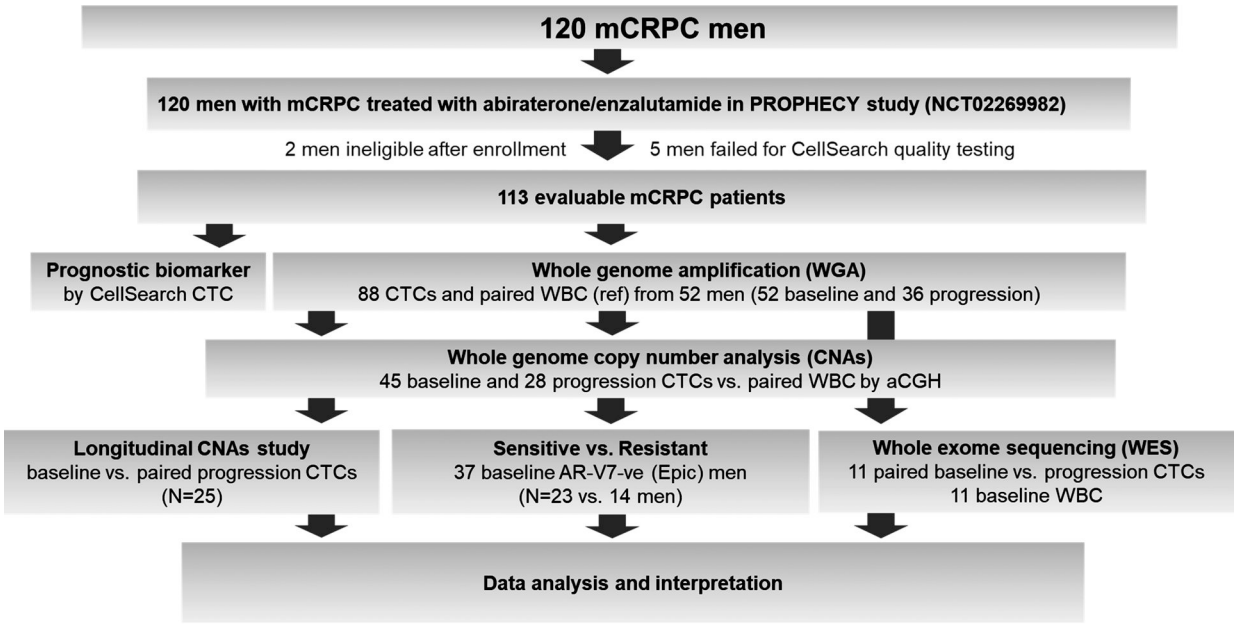

Figure 1: Summary of clinical specimens for genome-wide copy number alterations and mutations study.

Overview of the PROPHECY mCRPC patients cohorts utilized for the biomarker analyses.

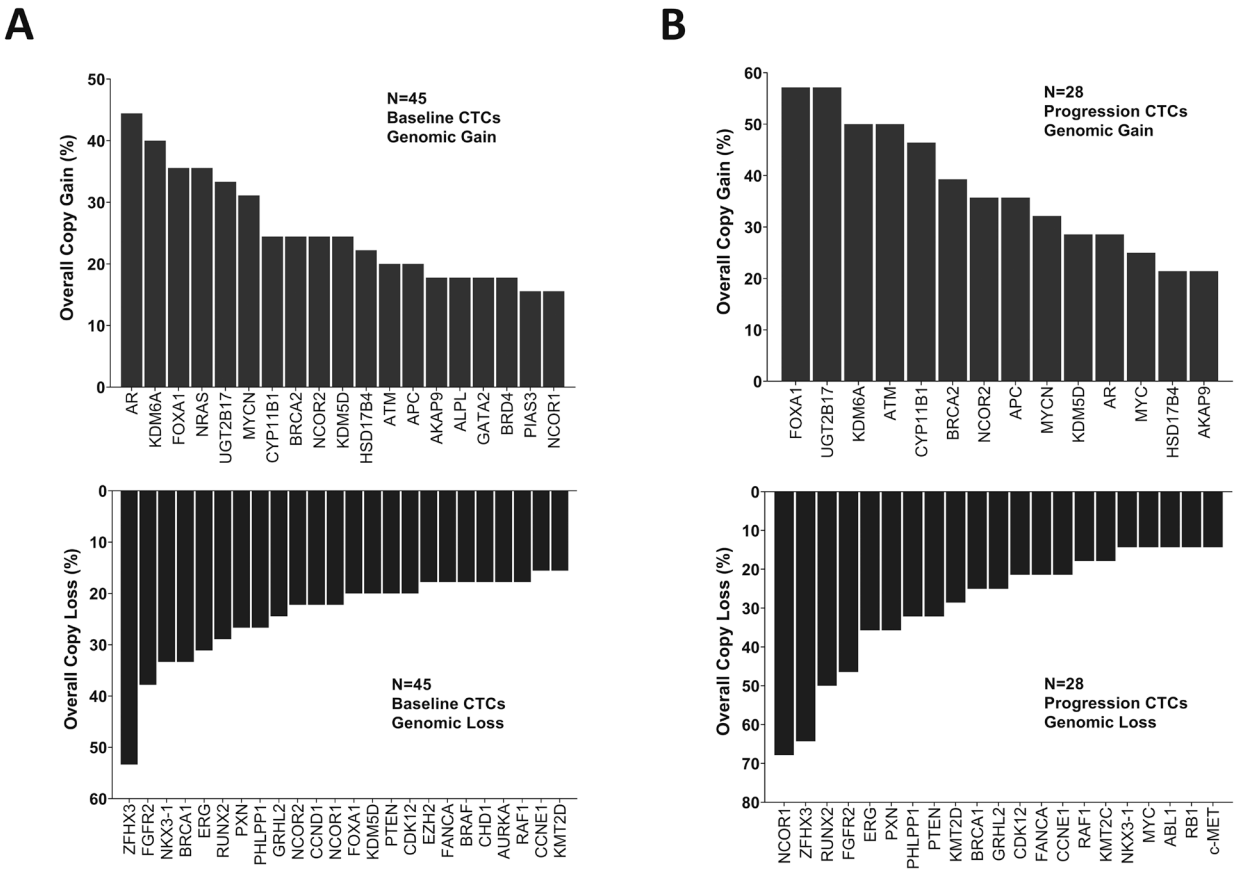

Figure 2: Overall genomic-wide copy number alterations analysis in CTCs DNA and independent clinical validations.

In this prospective study, we analyzed a panel of sixty genes identified earlier in prostate cancer progression and drug resistance across the spectrum of all baseline and progression groups. (**A**) The overall prevalence of genomic somatic alterations detected in CTCs DNA; copy gain and loss in 45 baseline CTCs DNA from mCRPC men, and ranked by their prevalence, as manifested in the bar charts. (**B**) Showing the overall prevalence of somatic alterations in 28 CTCs DNA from 28 mCRPC men. We detected widespread tumor heterogeneity between patients, and common CNAs, such as copy gain in *KDM6A*, *FOXA1*, *MYCN*, and *AR*, and loss in *BRCA1*, *PTEN*, and *ERG*.

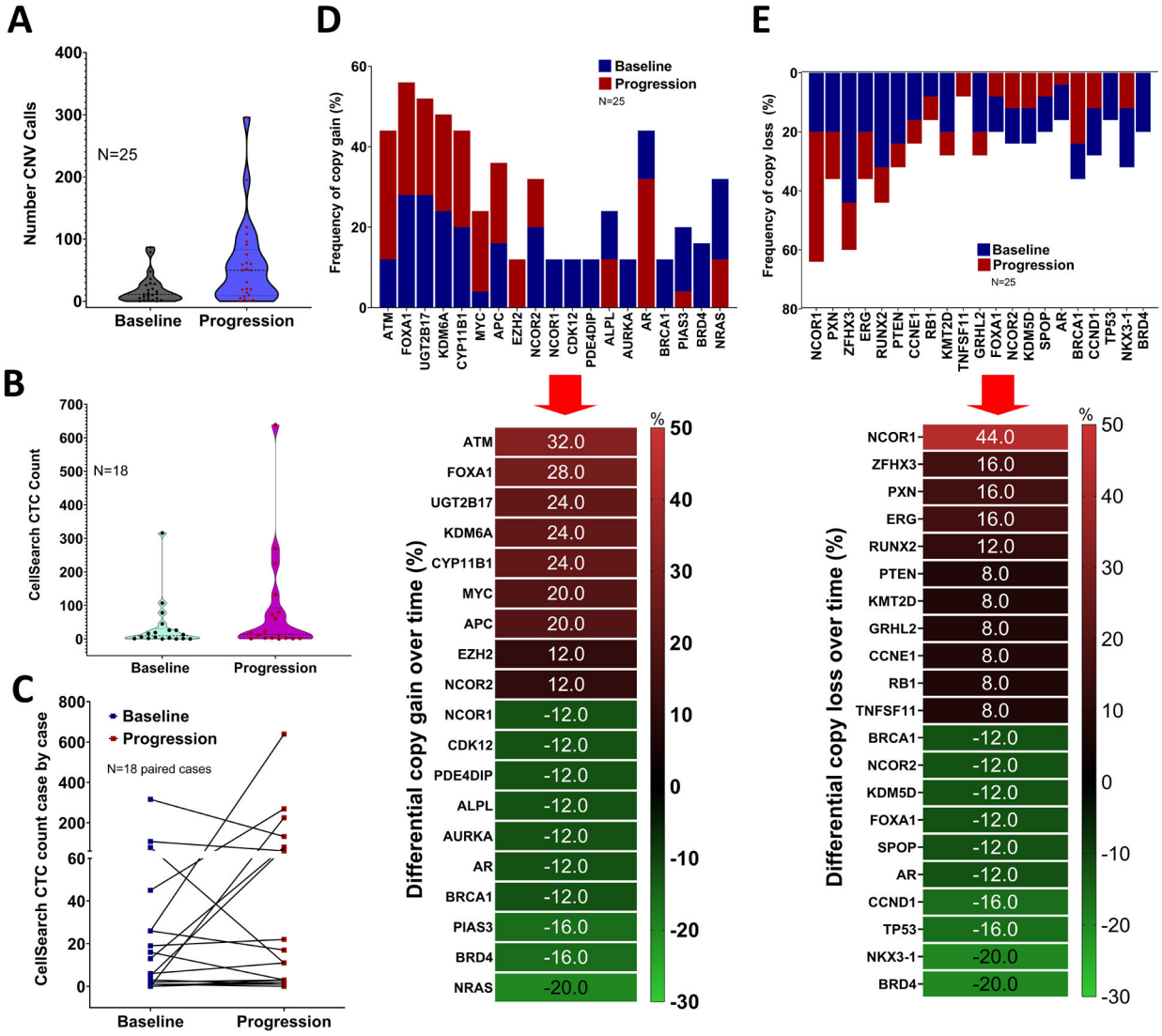

Figure 3: Genomic-wide CNAs analysis in CTCs over time.

(**A**) Violin plots showing the number of copy number variant counts were called by Cytogenomics software and compared between baseline and paired progression CTCs DNA samples (N=25 mCRPC cases). (**B**) Violin plot displaying CellsSearch CTCs enrichment between baseline and progression (N=18 cases). (**C**) CellSearch CTC number comparison case by case; baseline versus progression (N=18 cases). (**D**) and (**E**) displaying genomic alterations over time; 25 paired baseline vs. progression CTCs DNA.

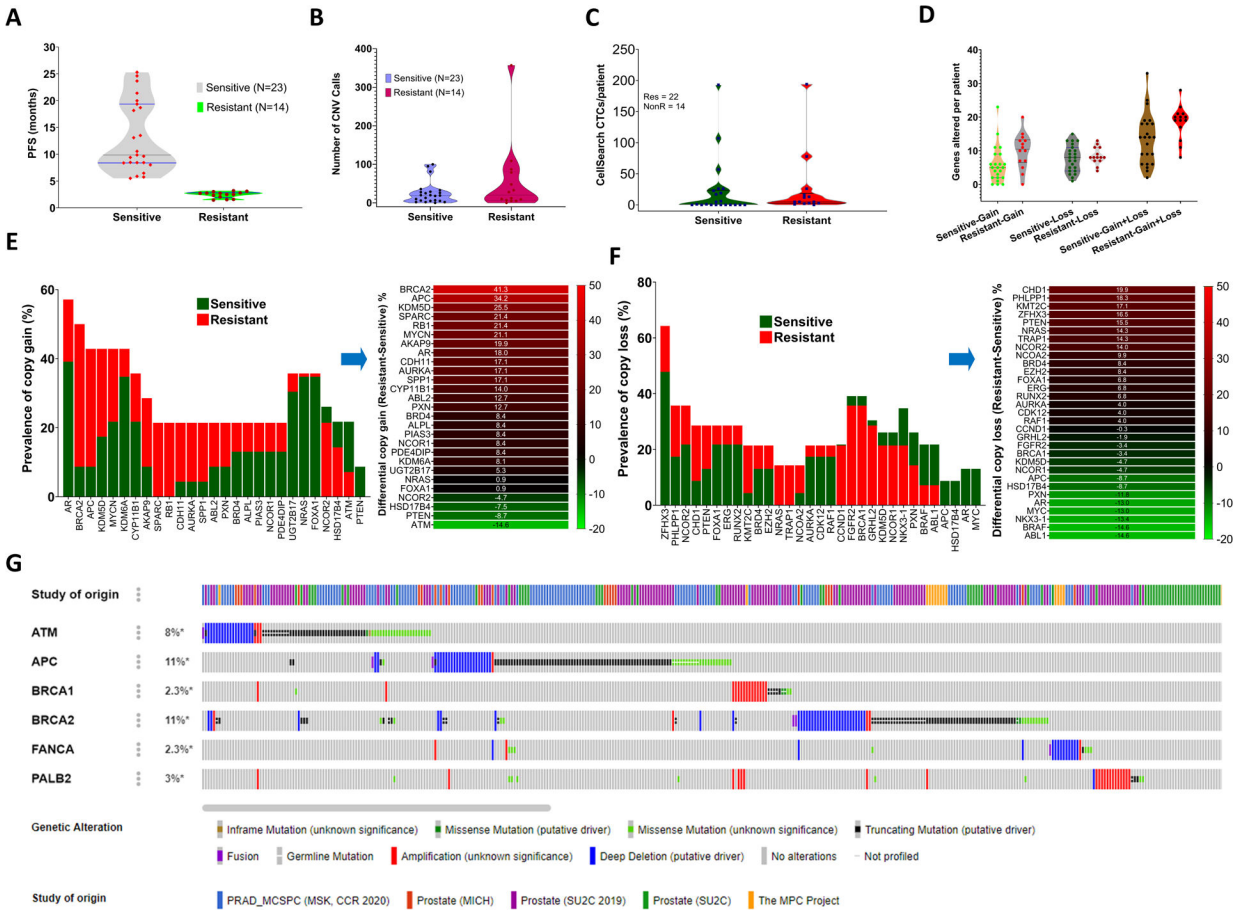

Figure 4: CTCs clonal evolution in response to enzalutamide or abiraterone therapy.

(**A**) Progression-free survival (PFS); sensitive vs. resistant (N=23 vs. 14). (**B**) Violin plot showing the number of copy number variants at genome level was called by Cytogenomics software and compared in between sensitive vs. resistant CTCs DNA samples (N=23 vs. 14 cases), and (**C**) CellSearch CTCs enrichment between sensitive vs. resistant (N=22 vs. 14 cases). (**D**) Genomic alterations for targeted sixty genes; sensitive vs. resistant. (**E**) and (**F**) displaying the top-most genomic gain and loss in sensitive vs. resistant CTC-DNA, respectively, and illustrating the difference between sensitive and resistant CTC-DNA CNAs prevalence. (**G**) Overall heatmap of DNA damage and repair genes alteration associated with DNA damage repair pathways (*ATM, BRCA2, APC*, *PALB2*, or *BRCA1*) were validated in metastatic prostate cancer datasets in cBioPortal.

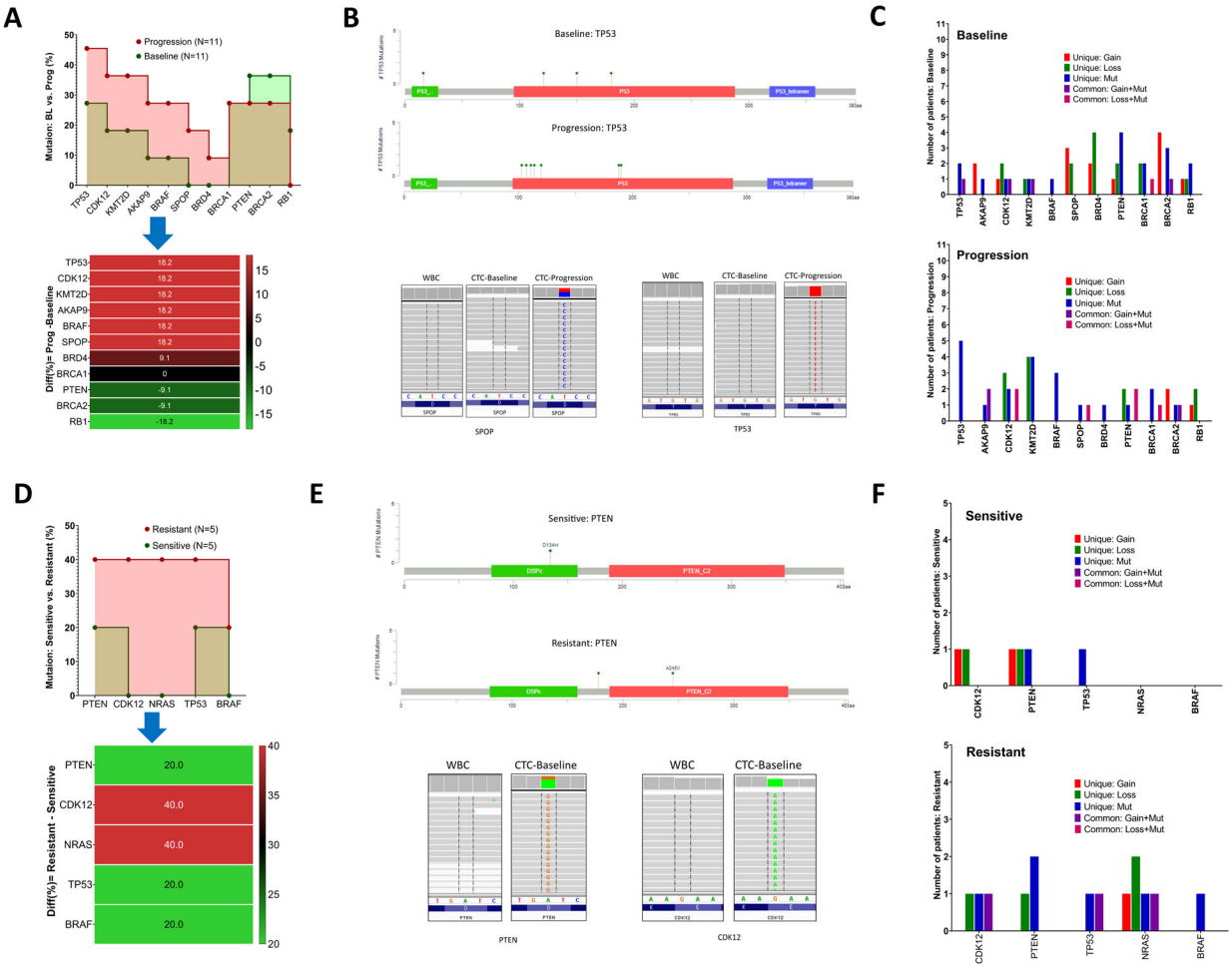

Figure 5: Comprehensive CTCs DNA analysis approach identifies mutations in sensitive and resistant mCRPC cases over time.

(**A**) The line graph illustrates top COSMIC somatic mutations detected by whole-exome sequencing analysis, compared between baseline and paired post-treatment CTC-DNA (N=11) in a set of sixty genes. (**B**) The mutation hotspot for the *TP53* gene is visualized in cBioPortal Mutation-Mapper, where read coverage is shown in the IGV browser, including matched WBC, baseline-CTC, and progression CTC DNA from the same patients, for genes *SPOP* (D144G) and *TP53* (T111K) in patients 809–5 and 809–7, respectively. (**C**) Genes with copy gain, loss, and COSMIC mutations detected in 11 common baselines and progression CTCs DNA were combined and compared to each other, are shown in stacked bars. (**D**) The line graph exemplifies the top somatic mutations validated in the COSMIC database were compared between baseline sensitive (N=5) and resistant CTC-DNA (N=5). One out of eleven patients who had a PFS in between 3 and 6 months was excluded from the analysis in order to enrich for outlier responses at either extreme of clinical benefit as determined by PFS times. (**E**) Somatic mutation in the *PTEN* gene detected in baseline sensitive and resistant are presented in cBioPortal by Mutation-Mapper software. For example, *PTEN* (D178G) and *CDK12* (E131K) mutations are visualized in the IGV browser. (**F**) Genes with copy gain, loss, and COSMIC mutations identified in five sensitive and five resistant baselines CTCs DNA were combined and compared, are shown as a bar graph.

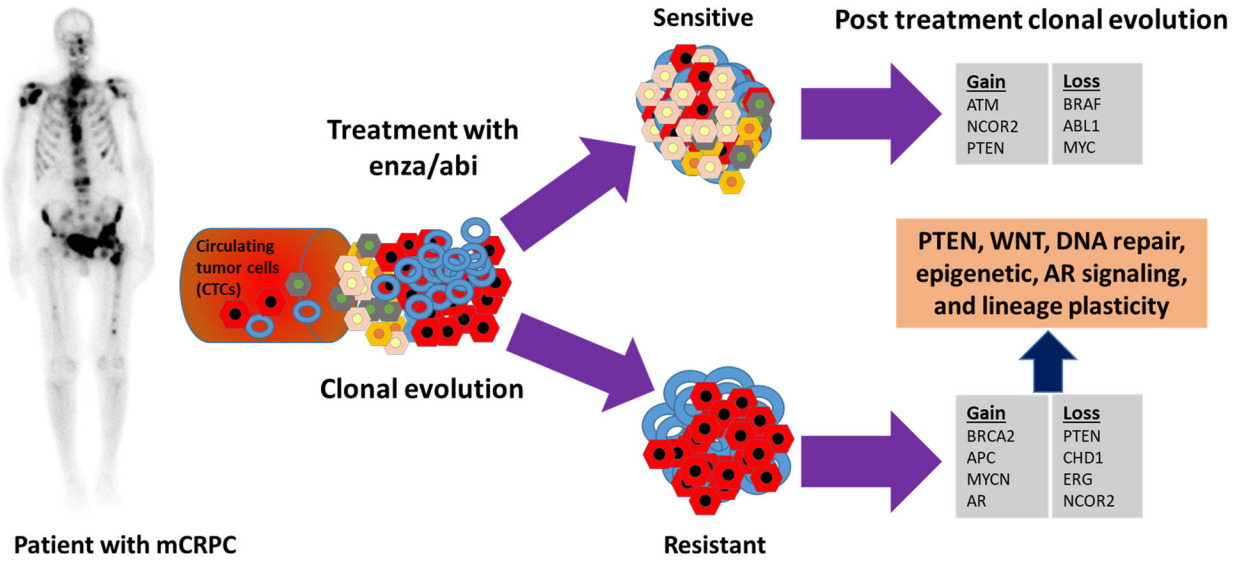

Figure 6:

Conceptual model of CTCs evolution in mCRPC: sensitive versus non-responder mCRPC patients.