Circulating Tumor Cell Chromosomal Instability and Neuroendocrine Phenotype by Immunomorphology and Poor Outcomes in Men with mCRPC Treated with Abiraterone or Enzalutamide

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ABSTRACT

Purpose: While the detection of AR-V7 in circulating tumor cells (CTC) is associated with resistance to abiraterone or enzalutamide in men with metastatic castration-resistant prostate cancer (mCRPC), it only accounts for a minority of this resistance. Neuroendocrine (NE) differentiation or chromosomal instability (CIN) may be additional mechanisms that mediate resistance.

Experimental Design: PROPHECY was a multicenter prospective study of men with high-risk mCRPC starting abiraterone or enzalutamide. A secondary objective was to assess Epic CTC CIN and NE phenotypes before abiraterone or enzalutamide and at progression. The proportional hazards (PH) model was used to investigate the prognostic importance of CIN and NE in predicting progression-free survival and overall survival (OS) adjusting for CTC number (CellSearch), AR-V7, prior therapy, and clinical risk score. The PH model was utilized to validate this association of NE

with OS in an external dataset of patients treated similarly at Memorial Sloan Kettering Cancer Center (MSKCC; New York, NY).

Results: We enrolled 118 men with mCRPC starting on abiraterone or enzalutamide; 107 were evaluable on the Epic platform. Of these, 36.4% and 8.4% were CIN positive and NE positive, respectively. CIN and NE were independently associated with worse OS [HR, 2.2; 95% confidence interval (CI), 1.2–4.0 and HR 3.8; 95% CI, 1.2–12.3, respectively] when treated with abiraterone/enzalutamide. The prognostic significance of NE positivity for worse OS was confirmed in the MSKCC dataset ($n = 173$; HR, 5.7; 95% CI, 2.6–12.7).

Conclusions: A high CIN and NE CTC phenotype is independently associated with worse survival in men with mCRPC treated with abiraterone/enzalutamide, warranting further prospective controlled predictive studies to inform treatment decisions.

Introduction

The treatment paradigm for men with metastatic castration-resistant prostate cancer (mCRPC) has changed dramatically with

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the approval and availability or approval and widespread use of next-generation androgen receptor (AR) antagonists such as enzalutamide or the androgen synthesis inhibitor abiraterone acetate (1–4). Unfortunately, approximately 10%–20% of men have tumors that resist these agents at the outset, while virtually all men progress on therapy after a median of 1–2 years. Circulating tumor cell (CTC) AR-V7 mRNA or nuclear AR-V7 protein detection has been shown to be associated independently with a poor prognosis among men with high-risk mCRPC who have progressed on abiraterone or enzalutamide, but detection only accounts for a minority of the cross-resistance to subsequent AR inhibitor therapy (5–9). Additional predictive biomarkers are needed to optimally select men at highest likelihood for a beneficial response to therapy.

Recognized now through molecular profiling efforts of mCRPC is that a proportion of patients harbor germline or somatic alterations in DNA repair genes such as BRCA1/BRCA2/ATM that leads to homologous recombination deficiency (HRD) and is detected in 20%–25% of men with mCRPC (10). Studies have suggested that men with tumors with HRD have worse outcomes when treated with AR signaling inhibitors (ARSI; refs. 11, 12) while others have shown no difference in outcome relative to those without HRD (13). A hallmark of HRD is a high number of chromosomal breaks, which can be measured by the number of large-scale transitions (LST) on single-cell whole-genome sequencing, defined as breakages that lead to chromosomal gains or losses of greater than 10 Mb termed chromosomal instability (CIN; refs. 14–16). Epic Sciences has developed and validated a CTC assay that reports CIN positivity based on a phenotypically defined CIN high

Translational Relevance

Here we present further evidence to support the clinical validation of circulating tumor cell (CTC) prognostic biomarkers: chromosomal instability (CIN) and neuroendocrine (NE) immunomorphology phenotypes. In this prospective study, we found that the CTC CIN phenotype was associated with shorter progression-free and overall survival times in univariate and multivariable analysis among men with mCRPC treated with abiraterone or enzalutamide, after adjusting for CTC AR-V7 status, CTC enumeration by CellSearch, prior therapy, and clinical risk score. CTC NE phenotype was similarly associated with worse overall survival in multivariable analysis in two independent datasets and strongly associated with CTC CIN. These data support the utility of the defined CIN and CTC NE phenotype biomarkers to identify men with mCRPC and poor outcomes—when considering androgen receptor targeting therapies—who should be considered for clinical trials investigating alternative therapeutic approaches directed toward the underlying CIN or NE biology.

score, which aims to predict the presence of nine or more LSTs (pLST) in a CTC and has been shown to be associated with worse overall survival (OS) among men with mCRPC treated with ARSIs and taxanes (17).

A second mechanism of AR therapy resistance is lineage plasticity that can result in a small cell or neuroendocrine (NE) like phenotype (18–21). These transitions can develop from treated adenocarcinoma and is likely found in up to 20% of mCRPC compared with 1%–2% at initial diagnosis (19, 22, 23). Outcomes are poor (24) despite treatment with platinum-based combinations such as carboplatin plus etoposide or cabazitaxel plus carboplatin (25, 26), considered by some to be the *de facto* standard. Epic Sciences has also developed a CTC-based assay that identifies cells that are primarily small, round, and have high nuclear to cytoplasmic ratios to detect men potentially harboring *de novo* or undergoing NE or small-cell transition (27).

Detection of CIN or NE phenotype on a blood-based assay may improve diagnostic accuracy and impact therapeutic decision making. Here we sought to explore the relationship between the CIN biomarker and progression-free survival (PFS) and confirm the association with worse OS previously shown, and separately to relate the presence of the NE biomarker with clinical outcomes in the PROPHECY trial: an independent, multicenter, prospective, and blinded validation study of men with high-risk mCRPC treated with abiraterone or enzalutamide.

Materials and Methods

Patients

The PROPHECY study was a prospective multicenter clinical trial investigating clinical outcomes among men with progressive mCRPC initiating standard-of-care enzalutamide or abiraterone. Study details have been described previously (9, 28). See Supplementary Appendix for full eligibility criteria and definitions of high-risk disease, which required ≥2 poor prognosis clinical factors (29, 30). All patients provided written informed consent. The study was approved by institutional review boards (IRB) of all participating centers within the Department of Defense Prostate Cancer Clinical Trial Consortium (31) and Duke University (Durham, NC) as lead coordinating center. All the authors vouch for the completeness and integrity of the

data and for the fidelity of the study to the clinical protocol (available online). A second cohort was used to validate findings observed in the PROPHECY trial. Independently, blood samples were collected from men with progressing mCRPC about to initiate either abiraterone or enzalutamide and treated at Memorial Sloan Kettering Cancer Center (MSKCC; New York, NY) between December 2012 and September 2016 and followed until July 2020. All patients provided informed consent for biospecimen collection as part of an IRB-approved protocol and all had histologic confirmation of prostate cancer.

Epic analysis of CTC CIN and NE phenotypes

The PROPHECY study was designed initially to validate the ability of baseline AR-V7 status in CTCs to predict clinical outcomes with abiraterone/enzalutamide. CellSearch CTC enumeration was performed at these timepoints on all men and processed in a College of American Pathologists (CAP)/Clinical Laboratory Improvement Amendments (CLIA)-approved central laboratory at MSKCC (29, 32). Peripheral blood was collected for CTC analysis from men at prespecified timepoints: baseline and at the time of clinical, radiographic, or biochemical progression. CTCs were sent to two blinded central laboratories, one of which was Epic Sciences (Epic). Epic Sciences performed the approved CTC nuclear-specific AR-V7 protein assay and CTC heterogeneity evaluations as described previously (9). As an exploratory but prospectively defined objective, CTCs were also analyzed by Epic for CIN phenotype and NE phenotype as predicted by an algorithm based on CTC immunofluorescent staining (DAPI, CK, CD45, and AR) and cell phenotypic characteristics (cytoplasmic and nuclear characteristics). The CIN phenotype algorithm was developed previously to predict CTCs harboring CIN high score defined as the prediction of the presence of nine or more LSTs (pLST) in a CTC (17) and the NE phenotype algorithm to identify men with tumors that have undergone a NE transformation (27). Laboratory investigators were blinded to the clinical information and patient outcomes.

The CIN algorithm uses digital pathology features including CTC morphologic and immunofluorescent staining to make a binary prediction of whether an individual CTC has nine or more LSTs. (**Fig. 1**). Important features in the CIN classifier include among others cell size, nuclear entropy (texture metric), CK protein expression, and AR protein expression. CIN positivity was previously defined as having ≥3 CIN positive CTC/mL, a cutoff chosen for 100% specificity for identifying CTCs with nine or more LSTs. The NE algorithm uses digital pathology features such as CTC morphologic and immunofluorescent staining to predict whether an individual CTC is NE positive (**Fig. 1**). NE positive CTCs generally have lower AR expression, higher cytoplasmic circularity and small cell features including a high nuclear to cytoplasmic ratio. NE positivity was previously defined as having ≥3 NE positive CTC/mL.

The laboratory data were sent to the study statistician (S. Halabi) and unblinded after the database was locked. The primary clinical efficacy endpoint of the PROPHECY study was PFS, defined as the time from registration to clinical or radiographic progression, clinical progression, or death, whichever occurred first, and excluded PSA progression (9). Radiographic progression was determined using Prostate Cancer Working Group 2 soft tissue and bone scan criteria (33). Clinical progression was defined by death, pain, or other symptomatic progression; initiation of new systemic therapy; or a new skeletal event. Key secondary endpoints were confirmed 50% or greater PSA declines (PSA50) on therapy from baseline, radiographic response per RECIST version 1.1 (defined as partial or complete response), and
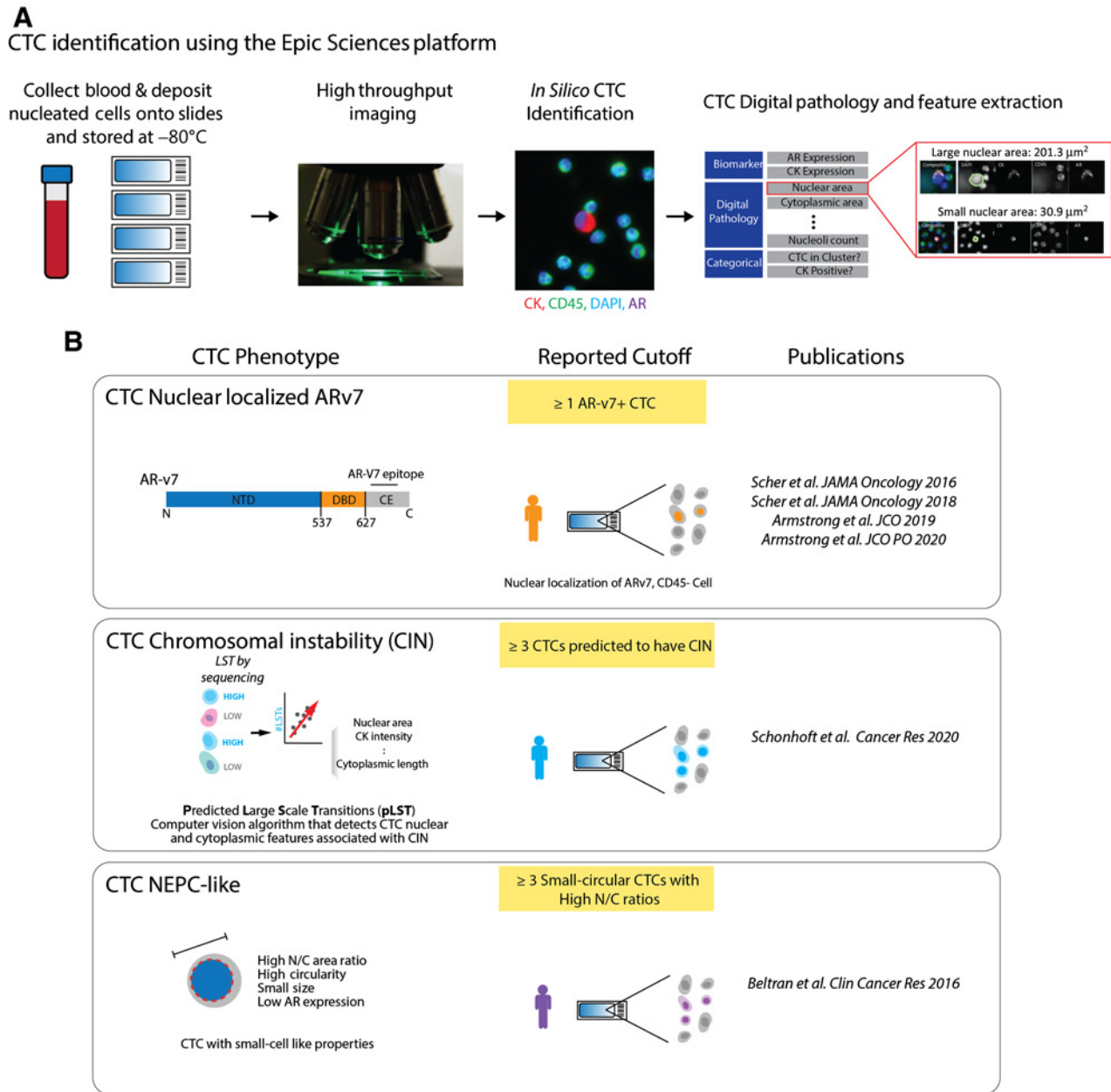
Figure 1.
CTC identification on Epic Sciences platform (**A**) and biomarker identification algorithm (**B**).

OS defined from the date of study registration to date of death or last follow-up. Confirmed 50% decline in PSA required subsequent PSA measurement at least 2 weeks later. Separately from Epic CTC processing, CTCs were enumerated using the FDA-approved Cell-Search platform (34) in a CLIA-approved laboratory at baseline and at progression on abiraterone/enzalutamide.

The MSKCC cohort was analyzed separately to validate the NE biomarker. Pretreatment CTCs from blood draws taken within 30 days of therapy initiation were analyzed and categorized as NE positive or negative (27). Overall survival was calculated as the date from initiation of therapy until date of death from any cause or date of last follow-up (July 2020).

Data analysis

A key secondary analysis of the PROPHECY study was to correlate the CTC biomarkers (CIN and NE) with clinical outcomes, and thus no specific hypothesis testing was performed. The prespecified analysis was to correlate each biomarker with PFS, OS, ≥50% PSA decline from baseline, and objective response rate. The maximum rank statistical method based on asymptotic distribution was used to find cutoff points for CIN and NE corresponding to the largest discrepancy between the lower- and higher-risk groups (35). The primary analysis was based on the optimal cutoff points. Men who had biomarker levels above these cutoff points were considered positive status for the biomarker. The proportional hazards model was then used to test

whether these biomarkers were prognostic of PFS and OS adjusting for AR-V7 status, CellSearch CTC number (≥5), prior therapy, and established prognostic factors (risk score; ref. 36). In addition, two exploratory analyses of the association of CIN/NE with clinical outcomes were performed. The first exploratory analysis analyzed the number biomarker-positive CTCs/mL for NE and CIN as a continuous variable [$\log_2$(biomarker level+1)]. The second exploratory analysis utilized the biomarker cutoffs for the CIN and NE assays from prior publications (17, 27) that defined ≥3 positive CTCs/mL as biomarker positive in contrast to the optimized cutoffs. The Kaplan–Meier product-limit approach was used to estimate the PFS and OS distributions by each biomarker status. Investigators and laboratory personnel were blinded to the biomarker status and outcome data prior to statistical analysis. The clinical database was locked on February 4, 2020.

For the MSKCC cohort of men treated with an ARSI, the Kaplan–Meier method was used to estimate the OS distribution. The proportional hazards model was utilized to assess the prognostic importance of the NE biomarker as a binary variable, using the optimal cutoffs from the PROPHECY study, as well as a continuous variable [$\log_2$(CTC NE+1)] in univariate and multivariable analyses for OS. The proportional hazards models included NE biomarker status, prior therapy, pretreatment clinical prognostic variables, and Epic CTCs ≥3/mL.

Results

Between May 2015 and January 2017, 118 men were enrolled in the PROPHECY trial from five academic medical centers. Of the 118 men enrolled, 11 were unevaluable for Epic CTC analysis leaving 107 eligible for inclusion in this analysis who were evaluable for CIN, NE, and AR-V7 by Epic Nuclear AR-V7 protein assay at baseline (Fig. 1). See Supplementary Fig. S1 for CONSORT diagram and Supplementary Fig. S2 for example images of biomarker-positive and biomarker-

negative CTCs. Table 1 summarizes patient characteristics from the PROPHECY study for the overall cohort and by CTC biomarker–defined cohorts. Analysis of the association between CIN and NE with clinical outcomes was performed and determined that the optimal threshold for biomarker positivity was ≥1 CTC/mL for CIN and ≥2 CTC/mL for NE given CTC counts are whole integers. CIN or NE positivity, unless otherwise specified, is defined using these optimized thresholds.

CTC biomarkers at baseline

At baseline, 39 men (36.4%) were positive for the CTC CIN phenotype, 9 men (8.4%) for the CTC NE phenotype, and 11 men (10%) had nuclear AR-V7–positive CTCs by Epic (≥ nuclear localized AR-V7–positive CTC). Of the 39 CIN-positive men, 9 were also positive for NE phenotype and 9 were positive by AR-V7 (Fig. 2A; Supplementary Table S1). All 9 NE positive men were CIN positive, and 3 of 9 (33.3%) were AR-V7 positive. The number of men who were negative for CIN, NE, and AR-V7 was 66 of 107 (61.7%). Both CIN-positive and NE-positive men had baseline characteristics suggestive of a higher burden of disease such as higher rates of anemia; higher baseline lactate dehydrogenase (LDH), alkaline phosphatase, and PSA; higher CellSearch CTC burden; visceral metastases; and a greater number of bone metastasis (Table 1). AR N-terminal protein expression was common and overlapped with CTC CIN and NE phenotype detection. Biomarker-positive CTC numbers are shown for each patient along with Epic CTC and CellSearch CTC quantification in Fig. 2C. Men who were CIN biomarker positive had a median of 4.7 CIN positive CTCs/mL (range, 1.1–880.7 CTCs/mL) while NE-positive men had a median of 7.2 NE-positive CTCs/mL (range, 2.6–85.1; Supplementary Table S2). On the single CTC level, most NE-positive CTCs were also CIN positive (83%, 186/223) while a minority of CIN-positive CTCs were also NE positive (8.3%, 186/2,235). AR-V7 is performed on a separate set of slides from CIN or NE and thus cannot be correlated on a single CTC level.

Table 1. Baseline characteristics of evaluable men from the PROPHECY study.

| Baseline characteristics	All Men (n = 107)	CIN(+) Men (≥1 CTCs/mL) (n = 39)	CIN(−) Men (<1 CTC/mL) (n = 68)	NE(+) Men (≥2 CTCs/mL) (n = 9)	NE(−) Men (<2 CTC/mL) (n = 98)
Age, median years (range)	73 (44–92)	74 (48–92)	72 (44–87)	77 (58–87)	72 (44–92)
Race: White/Black/other (%)	81/12/7	77/13/10	84/12/4	67/22/11	83/11/6
Gleason sum 8-10 (%)	60	56	62	33	62
Karnofsky score ≥90 (%)	73	56	82	33	77
High-risk features					
Hemoglobin <12 g/dL (%)	39	49	34	89	35
Elevated alkaline phosphatase (%)	44	64	32	89	40
Elevated serum LDH (%)	36	74	13	100	30
Prior abiraterone or enzalutamide (%)	37	36	38	33	38
Presence of liver or lung metastasis (%)	29	31	28	33	29
Presence of clinically significant pain requiring opiates (%)	28	26	29	33	28
CellSearch CTC ≥ 5 cells per 7.5 mL (%)	48	82	28	100	43
Radiographic progression at entry (%)	77	85	72	67	78
PSA doubling time <3 months (%)	64	67	62	78	62
Prior docetaxel for mHSPC (%)	19	21	18	11	19
M1 stage at diagnosis (%)	32	49	22	33	32
>20 bone metastases (%)	35	54	24	67	32
Median baseline PSA ng/mL (range)	22 (0.08–4,195)	42 (0.32–4,195)	14 (0.08–482)	297 (0.54–4,195)	18 (0.08–1,105)
Epic nuclear AR-V7 positive (%)	10.3	23.1	2.9	33.3	8.2
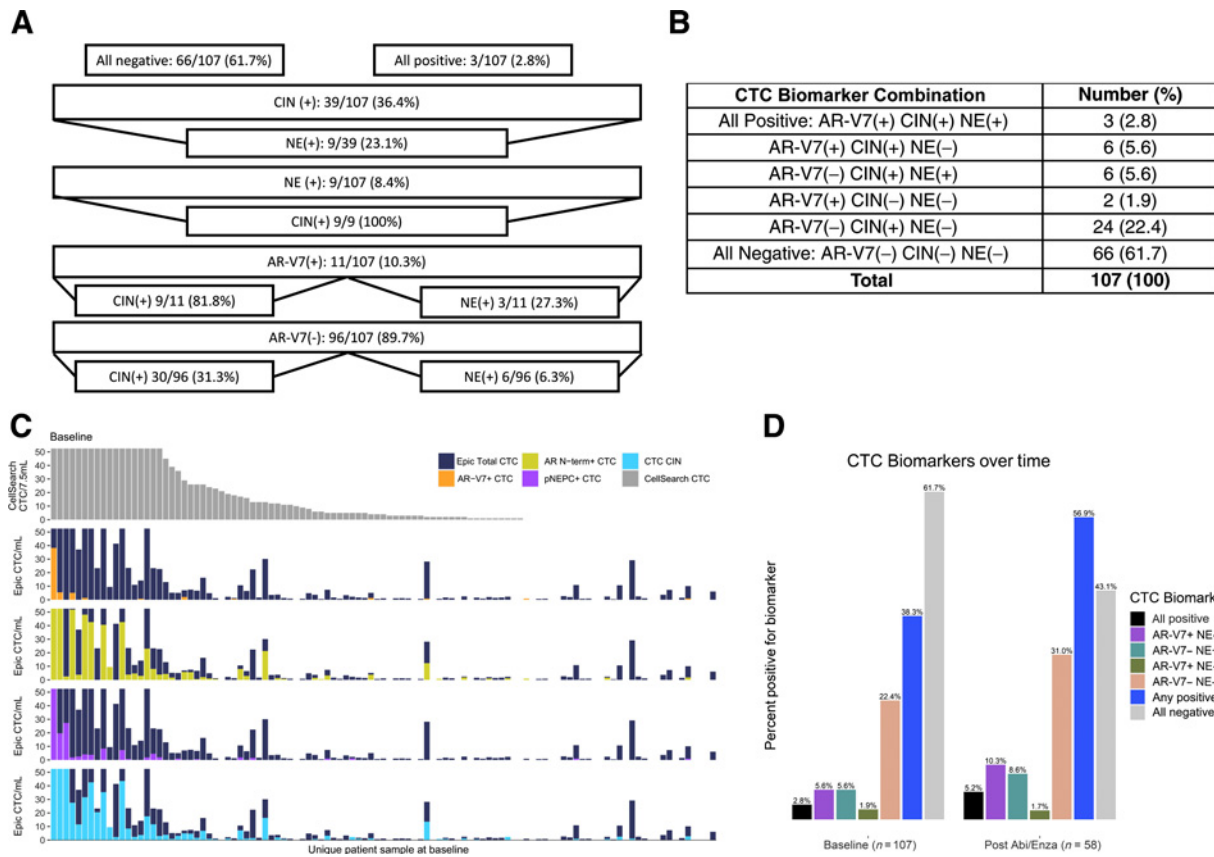
Figure 2.
A, CTC biomarker overlap. **B,** CTC biomarker group incidence. **C,** CTC enumeration by patient at baseline including CellSearch CTC/7.5 mL (gray), AR-V7 (orange), AR N-terminal positive (yellow), NE (purple), and CIN (cyan). Dark blue bars show total number of Epic CTC/mL. Note that CellSearch CTC enumeration is provided per 7.5 mL whole blood, while Epic CTC enumeration is reported per mL whole blood, so enumeration results are not directly comparable. **D,** CTC biomarker groups at baseline and progression. Positive CIN defined as ≥1 CTCs/mL, negative CIN <1 CTC/mL; positive NE defined as ≥2 CTCs/mL, negative NE <2 CTC/mL.

AR N-terminal overexpression, which is part of the digital pathology algorithm, was positive in 39% of NE-positive CTCs and 48% of CIN-positive CTCs.

CTC CIN and NE phenotypes and clinical outcomes

The median follow-up among surviving men was 31 months (range, 3.4–42.3), 104 of 107 men have progressed on abiraterone or enzalutamide and 83 of 107 men have died. The Kaplan–Meier curves and analysis for PFS and OS for each biomarker are shown in **Fig. 3A–D** and **Table 2**. CIN-positive men experienced shorter median PFS compared to CIN-negative men, 3.7 versus 7.5 months, [univariate HR = 2.3; 95% confidence interval (CI), 1.5–3.6], and OS of 11.5 versus 25.0 months (univariate HR, 3.2; 95% CI, 2.0–5.1) for positive versus negative cases, respectively. NE positivity was similarly associated with shorter PFS, 1.6 versus 6.0 months (univariate HR, 3.4; 95% CI, 1.7–6.8) and OS of 5.7 versus 20.5 months (univariate HR, 5.5; 95% CI, 2.7–11.2) for positive and negative cases.

The prognostic significance of CIN positivity was retained in multivariable analysis of PFS adjusting for AR-V7 status, CellSearch CTC number (≥5), prior therapy, and clinical prognostic factors for a shorter PFS (adjusted HR, 1.8; 95% CI, 1.0–3.0) while NE was not (adjusted HR, 2.1; 95% CI, 0.6–7.5), shown in **Table 2**. In contrast, the significance of both biomarkers was retained in the multivariable model of OS (adjusted HR, 2.2; 95% CI, 1.2–4.0; adjusted HR, 3.8;

95% CI, 1.2–12.3, respectively). Similar results were found when CIN and NE were included in the same model with CellSearch CTC number as a continuous covariate [log$_2$(CTC+1); **Table 2**]. Furthermore, in univariate analysis where the number of CIN- or NE-positive CTCs were modeled as continuous variables [log$_2$(biomarker+1)], CIN and NE were associated with worse OS and PFS. However, in multivariable analysis, CIN as a continuous variable was associated with OS but not PFS. Conversely, NE as a continuous variable was associated with PFS but not OS (Supplementary Table S3). Using the threshold of positivity of ≥3 CTC/mL for CIN and NE positivity from prior publications, similar results were observed where both CIN positivity and NE positivity were associated with worse OS, but not worse PFS (Supplementary Table S4; Supplementary Figs. S3 and S4A–S4F).

Confirmed ≥50% PSA declines and radiographic responses were also assessed as key secondary endpoints and the waterfall plots by biomarker status are shown in **Fig. 4A**. A lower proportion of CIN-positive men experienced a confirmed ≥50% PSA decline, 18% (95% CI, 10–41) compared with 28% (95% CI, 25–54) for CIN-negative men (**Table 2**). Similarly, a smaller proportion of NE-positive men experienced a confirmed PSA decline compared with NE-negative men, 12% (95% CI, 0.4–58) versus 26% (95% CI, 24–46), respectively. Objective responses were similar between CIN-positive and CIN-negative as well as NE-positive and NE-negative men (**Table 2**; Supplementary Table S5). A swimlane plot is shown in **Fig. 4B** with
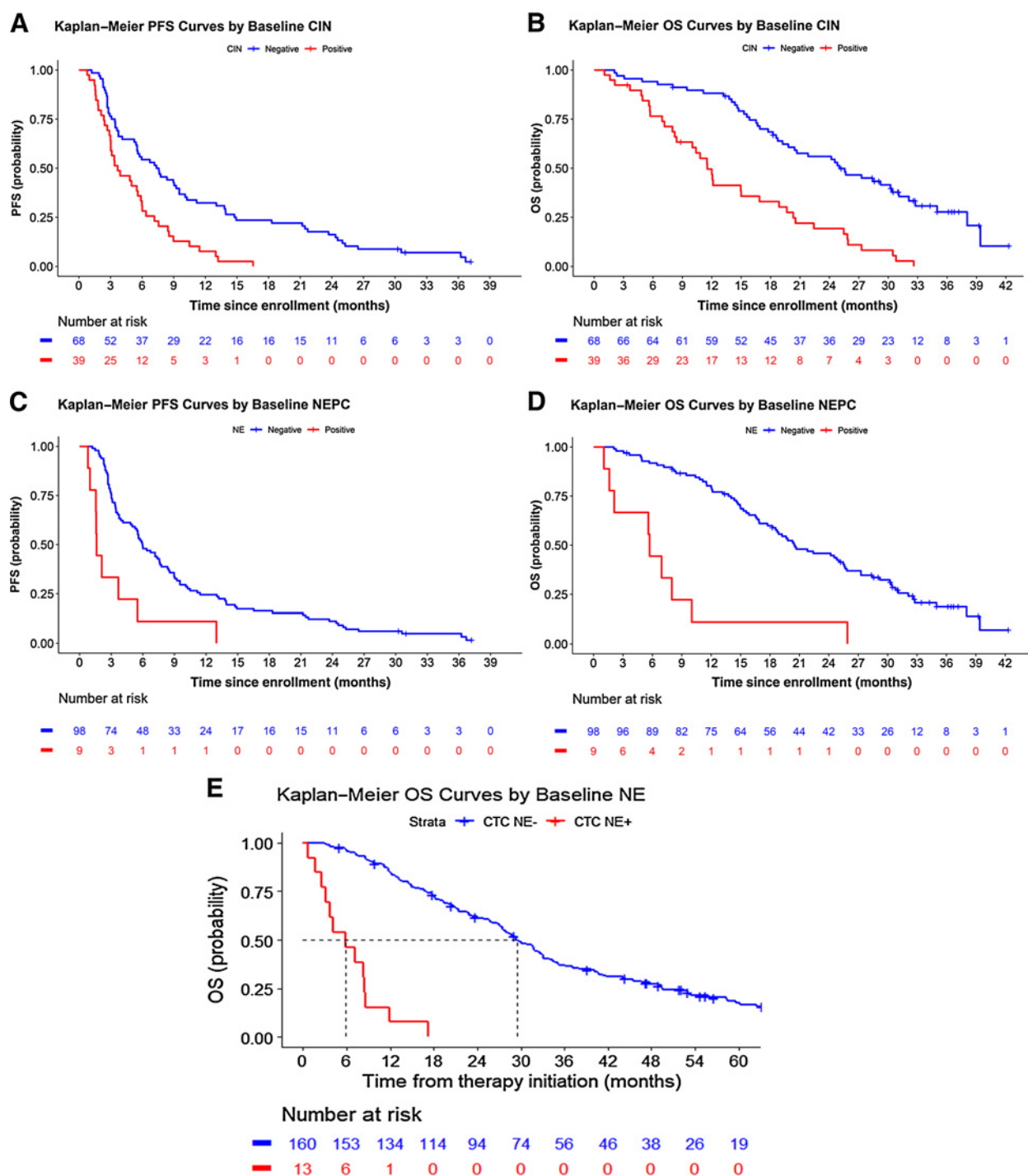
Figures 3.

A–D, PFS and OS curves for CIN and NE biomarkers. Positive CIN defined as ≥1 CTCs/mL, negative CIN <1 CTC/mL, positive NE ≥2 CTCs/mL, and negative NE <2 CTC/mL. **E,** OS curves for MSKCC cohort treated with ARSI for external validation of NE biomarker.

patients grouped by biomarker category using the three CTC bio-markers (AR-V7, CIN, and NE), demonstrating the shorter PFS and OS times in biomarker-positive men.

In exploratory analyses, the CTC biomarkers were categorized into three groups: any positive biomarker (AR-V7, NE, or CIN; $n = 41$),

triple biomarker negative and CellSearch CTC >0 ($n = 38$), and triple biomarker negative and CTC count of 0 ($n = 23$). The Kaplan–Meier PFS and OS curves for this analysis are shown in Supplementary Fig. S5A and S5B. These analyses showed that men positive for any of the three biomarkers experienced worse outcomes

Table 2. Association of CIN and NE biomarkers with clinical outcomes from the PROPHECY study.

Biomarker status	CIN phenotype $n = 107$		NE phenotype $n = 107$	
	Positive (≥1 CTCs/mL)	Negative (<1 CTC/mL)	Positive (≥2 CTCs/mL)	Negative (<2 CTC/mL)
Number (%)	39 (36.4)	68 (63.6)	9 (8.4)	98 (91.6)
Median PFS (months; 95% CI)	3.7 (3.0–6.0)	7.5 (5.5–10.1)	1.6 (1.5–NR)	6.0 (5.3–7.8)
HR[a] (95% CI)	2.3 (1.5–3.6)		3.4 (1.7–6.8)	
HR[b] (95% CI)	1.8 (1.0–3.0)		2.1 (0.6–7.5)	
HR[c] (95% CI)	1.5 (0.8–2.7)		2.2 (0.7–7.2)	
Median OS (months; 95% CI)	11.5 (8.4–18.9)	25.0 (19.8–32.1)	5.7 (2.1–NR)	20.5 (18.2–25.7)
HR[a] (95% CI)	3.2 (2.0–5.1)		5.5 (2.7–11.2)	
HR[b] (95% CI)	2.2 (1.2–4.0)		3.8 (1.2–12.3)	
HR[c] (95% CI)	2.0 (1.1–3.8)		3.0 (1.0–9.4)	
% of men ≥ 50% confirmed PSA decline (95% CI)[a]	18 (10–41)	28 (25–54)	12 (0.4–58)	26 (24–46)
% of men with best response (CR+PR, 95% CI)[a]	7.7 (1.8–22.5)	4.4 (1.0–12.9)	11.1 (0.3–52.7)	5.1 (1.8–12.1)

Abbreviations: CR, complete response; NR, not reached; PR, partial response.
[a]Univariate.
[b]Adjusted for CellSearch CTCs (≥5), AR-V7 status, prior therapy, and clinical risk score.
[c]Model with CIN and NE and adjusted for CellSearch CTCs [continuous (log2(CTC+1)], AR-V7 status, prior therapy, and clinical risk score.

with a median PFS of 3.5 months (95% CI, 3.0–5.9) and OS of 11.5 (95% CI, 8.4–18.9) months. Men who were negative for all three biomarkers with CTC >0 by CellSearch, had a median PFS and OS of 5.5 months (95% CI, 3.5–9.5) and 19.2 months (95% CI, 16.7–30.2), respectively, while men who had no CTCs by Epic or CellSearch and thus negative for all three CTC biomarkers had the best outcomes with a median PFS of 9.2 months (95% CI, 6.7–24.3) and OS of 30.4 months (95% CI, 24.5—not reached).

CTC NE biomarker external validation

An independent cohort of 173 men contributed pretreatment CTC samples collected before starting therapy with an ARSI at MSKCC and were used for validation of the association between NE and OS. This cohort was utilized previously as part of the clinical validation of the CIN biomarker showing relationship between CIN and OS (17). Demographics for the MSKCC cohort are shown in Supplementary Table S6. As a group, the median PSA was 20.6 ng/mL (0.5–2,010) and 57.8%, 27.7%, and 14.5% of samples were from patients starting an ARSI in the first, second, or third line or greater setting, respectively (17). When defining NE and CIN by the biomarker cutoffs established from the PROPHECY study, the NE positivity (≥2 CTC/mL) rate was 7.4% (13/173), and all 13 (100%) NE-positive patients were also CIN positive (≥1 CTC/mL).
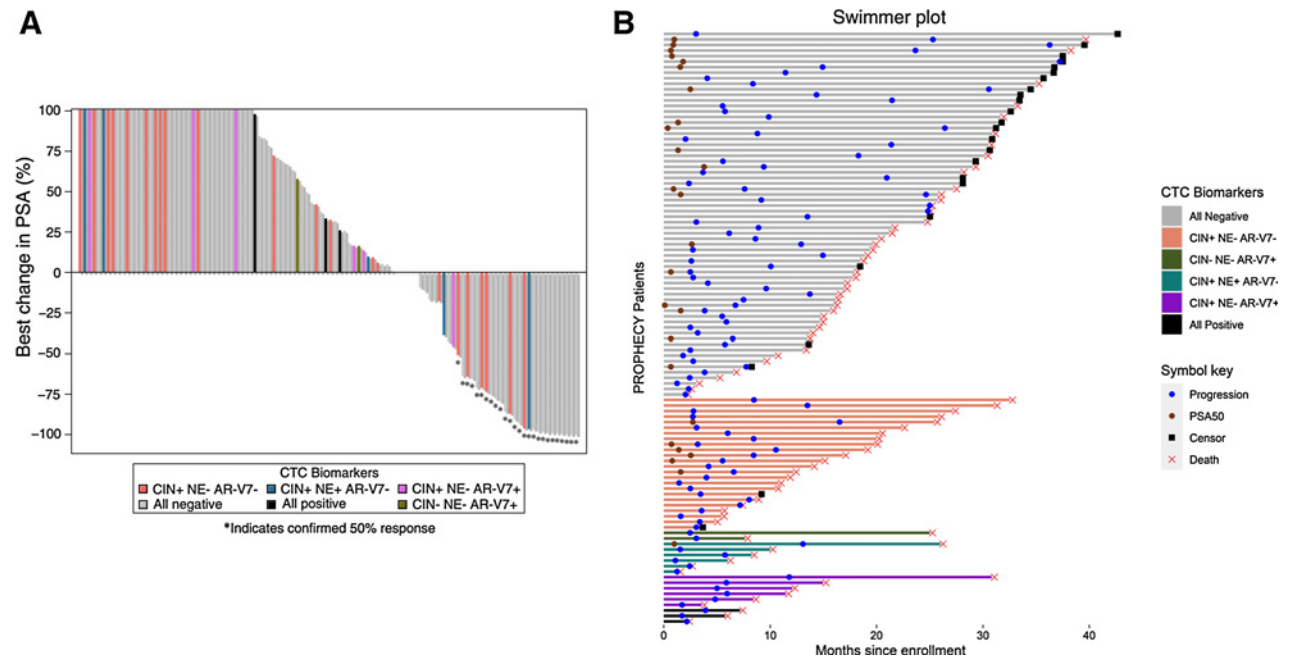

Figure 4.
A, PSA waterfall plot showing CTC biomarker categories ($n = 106$). Asterix (*) indicates confirmed 50% PSA decline. One patient is excluded because of no PSA follow-up measurements who was CIN$^+$, NE$^+$, and AR-V7$^-$. **B,** Swimmer plot showing CTC biomarker categories ($n = 107$).

NE-positive men had a worse OS when treated with an ARSI (HR, 14.5; 95% CI, 7.5–28.0) in univariate analysis (Supplementary Table S7; **Fig. 3E**), which was retained in the multivariable analysis (HR, 5.7; 95% CI, 2.6–12.7) when adjusting for treatment line (1, 2, 3+), lung or liver metastasis, PSA >20 ng/mg, LDH >250 U/L, hemoglobin >12 g/dL, alkaline phosphatase >140 U/L, and total CTC (Epic defined CK$^+$, CD45$^-$) ≥3/mL versus < 3/mL. This association with OS was similarly retained when the NE biomarker was considered as a continuous variable [log$_2$ (CTC NE+1)] in univariate (HR, 1.7; 95% CI, 1.4–2.0) and in the above multivariable model (HR, 1.5; 95% CI, 1.3–1.9) for OS (Supplementary Table S7).

CTC biomarkers at progression

In the PROPHECY trial, CTCs were again collected at progression on abiraterone or enzalutamide and analyzed for multiple biomarkers; 68 men had CTCs measured at progression for analysis, of whom 10 (15%) were missing AR-V7 status. At baseline and first progression, 74% (79/107) and 79% (46/58) men, respectively, had detectable CTCs on the Epic platform. Biomarker-positive CTC numbers are shown for each patient at progression along with Epic CTC and CellSearch CTC quantification in Supplementary Fig. S6. The incidence of CIN, NE, and AR-V7 biomarker positivity at progression was 55% (32/58), 14% (8/58), and 17% (10/58; 17.2%), respectively, all of which increased from their incidence at baseline. The percentage of men with baseline and progression samples who were positive for any of the three CTC biomarkers increased from 38% at baseline to 57% at progression (**Fig. 2D**). Of the 39 men positive for CIN at baseline, 15 remained CIN positive, 5 became CIN negative, and 19 were not assessed at progression, whereas of the 9 men positive for NE at baseline, 2 converted to NE negative and 7 were not assessed at progression. Of the 32 men positive for CIN at progression, 15 men were previously CIN positive while 17 men were previously CIN negative. All 8 NE-positive men at progression were previously NE negative at baseline prior to treatment with abiraterone or enzalutamide. NE-positive patients at baseline were more likely to not have biomarkers assessed at progression: 7 of 9 NE-positive men compared with 41 of 98 NE-negative men, possibly due to more rapid clinical progression. The prevalence of CIN, NE, and AR-V7 positivity among men with three or more CTCs/mL (CTCs defined by Epic) was found to be 34/38 (89.5%), 9/38 (23.7%), and 8/38 (21.1%) at baseline, respectively, and 24/27 (88.9%), 8/27 (29.6%), 7/27 (25.9%) at first progression, respectively.

Association of CIN- and NE-positive CTCs with CTC genomic alterations

To associate CTC genotype with CTC phenotype, we performed copy-number variation analysis on pooled CTCs by array comparative genomic hybridization and whole-exome sequencing on a cohort of men from PROPHECY (n = 12) with sufficient evaluable CTCs and CTC DNA (see Supplementary Materials and Methods). These results are very limited by the low sample size and the bias toward genomic data being available on patients with sufficient CTCs and CTC DNA and are presented only as hypothesis generating. For individual gene alteration analysis, we focused on genes found to be associated with aggressive prostate cancer (MYCN, PTEN, RB1, TP53) and DNA repair genes (BRCA1, BRCA2, FANCA, ATM, PALB2). See Supplementary Fig. S7A–S7E for mutation and copy-number alteration frequency for each gene stratified by biomarker status as well as the total copy-number burden for patients stratified by biomarker status. Both CIN- and NE-positive men had a higher average of whole-

genome copy-number alterations, 3,988 versus 2,430 for CIN and 6,589 versus 2,845 for NE (Supplementary Fig. S8).

CIN-positive men (n = 8) had a higher rate of pathogenic mutation or copy-number loss compared with CIN-negative men (n = 4) in TP53 (4/8 vs. 0/4), BRCA1 (6/8 vs. 0/4), and BRCA2 (4/8 vs. 1/4). The rates of copy-number loss or mutation for CIN-positive versus CIN-negative men was similar for PTEN (5/8 vs. 2/4), RB1 (2/8 vs. 1/4), FANCA (2/8 vs. 2/4), ATM (1/8 vs. 0/4), and PALB2 (0/8 vs. 1/4). Notably MYCN gain was seen in 2 of 8 CIN-positive men compared with 3 of 4 CIN-negative men. NE-positive men (n = 2) had a higher rate of pathogenic mutation of copy-number loss compared with NE negative men (n = 10) in TP53 (2/2 vs. 2/10) and RB1 (1/2 vs. 2/10), but not PTEN (1/2 vs. 6/10). MYCN gain was found in 1 of 2 NE-positive men and 4 of 10 NE-negative men. Higher rates of BRCA1 (2/2 vs. 4/10) and FANCA (2/2 vs. 2/10) mutation or copy-number loss were also observed in NE positive compared with negative. Other genes assessed include BRCA2 (1/2 vs. 4/10), ATM (1/2 vs. 0/8), and PALB2 (0/2 vs. 1/10). Finally, patients with available CTC whole genomic data were categorized by the previously described aggressive variant prostate cancer (AVPC) molecular criteria proposed by Aparicio and colleagues (21) which defines the molecular AVPC subtype as harboring two or more alterations in PTEN, RB1, and TP53. AVPC molecular criteria were met for 4 of 8 CIN-positive men and 1 of 4 CIN-negative men as compared with 2 of 2 NE-positive men and 3 of 10 NE-negative men.

Discussion

The current study intended to associate two CTC biomarkers, CIN and NE phenotype, with OS and PFS in men with mCRPC and identify optimal thresholds for outcome discrimination for future predictive studies. These results from the PROPHECY study serve as an optimization cohort for the previously described CTC CIN biomarker (17). For the CTC NE biomarker, we present both the initial biomarker analysis and optimization from the PROPHECY study as well as separate external clinical validation cohort at MSKCC. We observed that both biomarkers are independently associated with worse OS among men with high-risk mCRPC in the PROPHECY study treated with abiraterone or enzalutamide.

After adjusting for AR-V7 status, CTC enumeration by CellSearch, prior therapy, and clinical risk score, CIN positivity (defined as ≥1 CIN positive CTC/mL) was associated with worse PFS following treatment with abiraterone or enzalutamide, though NE positivity (defined as ≥2 NE positive CTC/mL) was not. This could be due to the small NE-positive sample and the high rates of early progression in this high-risk cohort of men treated with abiraterone or enzalutamide. Pretreatment detection of CTC CIN or NE was not associated clearly with differential PSA declines or objective responses, and thus our data refutes our initial hypothesis that CIN and NE may be mediators of primary resistance to novel hormonal therapies. Rather, these biomarkers may identify men at high-risk of rapid progression through hormonal as well as subsequent nonhormonal treatments (37, 38). PSA declines were uncommon for each biomarker-positive category (18% for CIN and 12% for NE), though this is higher than the 0% confirmed PSA declines seen in men with Epic AR-V7–positive mCRPC treated with AR inhibitors. Together these data suggest a poor prognosis for both CIN- and NE-positive men but does not directly suggest that novel hormonal agents should be withheld in these cases. Rather, these men represent an identifiable subgroup with poor survival outcomes that could benefit from novel approaches such as PARP inhibitors, immunotherapy, or platinum-based chemotherapy combinations (25, 26).

Cross-resistance between AR-targeted therapies is an unmet medical of clinical significance given the earlier use of AR therapies in men with metastatic hormone-sensitive prostate cancer (mHSPC; refs. 39–43) and nonmetastatic CRPC (44, 45). However, while AR-V7 has been shown to have prognostic value in mCRPC for poor outcomes and predictive of a lack of sensitivity to AR-targeted therapies in mCRPC, its clinical predictive utility is limited by the low prevalence of AR-V7 detection and that AR-V7 detection only accounts for a minority of this cross-resistance. Thus, additional actionable predictive biomarkers are needed to guide treatment choice. CIN attempts to predict men with CTCs harboring a high number of chromosomal breaks, which has been hypothesized to be associated with HRD such as mutations or deletions of BRCA1/2, ATM, or other DNA repair enzymes. Our preliminary and limited results showing BRCA1 and BRCA2 alterations in 6 of 8 and 4 of 8 CIN-positive patients versus 0 of 4 and 1 of 4 CIN-negative patients, respectively, need further validation. Whether CIN or other markers of a functional HRD phenotype in prostate cancer may complement genomic testing for selecting men for PARP inhibitor therapy will require prospective testing, given the enrichment for TP53 alterations in CIN-positive patients as well. Additional studies are also warranted to investigate whether platinum agents alone or platinum agents in addition to a taxane such as cabazitaxel could also be a therapeutic strategy for these men (26).

The emergence of NE phenotype (NEPC) in tissue biopsies or clinically has been known to convey a poor prognosis and detecting this aggressive form of transformed prostate cancer on a blood-based assay is appealing (20, 25). Our study had a lower than expected overall number of NE-positive men at 8.4%, though this is consistent with the NE positivity rate from the separate MSKCC validation cohort at 7.4%. Among men enrolled in PROPHECY with sufficient evaluable CTCs, the prevalence of NE positivity was 23.7%, which is similar to contemporary biopsy prevalence studies of men with mCRPC (20). The PROPHECY trial specifically excluded men with known small cell/NEPC and included only men with prostate adenocarcinoma suitable for treatment with abiraterone or enzalutamide for mCRPC, reducing the overall number of men who would be expected to test NE positive. Systematic metastatic biopsies were not performed, and thus tissue correlation is not available. We have previously published one case of documented NEPC transformation at progression on enzalutamide from the PROPHECY study, and subsequently found this patient to be NE positive at baseline (46). In addition, two thirds of men had not previously received an AR antagonist or abiraterone and thus have not been exposed to the therapeutic pressure that may drive NEPC development. Finally, sufficient CTCs were required to detect NEPC, thus potentially leading to false negatives for those with NEPC and low number of CTCs. We did note an increase in biomarker rates at progression for both NE (8.4% increased to 13.8% at progression) and CIN (36.4% increased to 55.2%) at progression, using the newly defined cutoffs suggesting that these biomarkers may emerge in the setting of hormonal therapies. Of the men who were biomarker positive at progression, many were previously biomarker negative (8/8 NE-positive men at progression and 17/32 CIN-positive men at progression). A small group of men converted from biomarker positive at baseline to negative at progression (2/9 NE-positive men at baseline and 5/39 CIN-positive men at baseline). Collectively, these results suggest biomarker positivity with either CIN or NE increases over time.

One interesting observation that we made was that all NE-positive men at baseline were also CIN positive. This may either reflect an overlap of the digital pathology algorithm or suggest that NE men have a high number of chromosomal breaks and DNA repair machinery defects, suggesting a connection to platinum sensitivity. Malihi and colleagues recently performed a single CTC genome analysis that noted an association between a higher number of LSTs and aggressive variant prostate cancer both defined by clinical variables as well as an aggressive variant molecular signature (loss of at least two of PTEN, RB1, and TP53; ref. 47), which may explain the overlap of CIN and NE in this study as many men with aggressive variant prostate cancer may harbor neuroendocrine features. Our limited genomic analysis of 12 patients do show that this aggressive variant molecular signature may be enriched among NE- and CIN-positive men (2/2 and 4/8, respectively) but this molecular signature was also observed in some NE- and CIN-negative men (3/10 and 1/4, respectively). Of the 9 men who were NE positive, 3 were noted to be AR-V7 positive and AR N-terminal positivity was common in NE-positive cases (Fig. 2C). While NEPC is not considered AR dependent, it has been shown that increased AR expression but low AR activity is common in transformed NEPC/small cell prostate cancer (20) and also that AR-V7 can be detected by IHC in prostate cancer tissue among men after neoadjuvant hormonal therapy (48). We are unable to correlate AR-V7 with NE or CIN positivity on a single CTC level because AR-V7 is run on a separate set of slides from NE and CIN; however, we did find that 39% of NE-positive CTCs and 48% of CIN-positive CTCs were also positive for AR N-terminal overexpression on the single-cell level. These results concur that while NEPC may be AR independent, AR expression can still be detected. Importantly, we show enrichment of both CTC CIN and NE immunophenotypes at progression on AR-targeted therapies, suggesting the importance of these genetic phenotypic transitions or clonal selections in drug resistance. Only through specific AR variant and NE/CIN-specific therapeutic clinical trials, can the question of whether these are passenger or driver alterations be directly addressed.

Men with de novo or transformed small cell/NEPC are frequently treated with platinum-based chemotherapy (either etoposide/platinum or taxane/platinum), though making this diagnosis can be challenging as it frequently requires a biopsy. In addition, given the known heterogeneity within individual men with mCRPC, the utility of a single site biopsy that is negative for NEPC is not known. A theoretical benefit of a blood-based assay is to aggregate this heterogeneity. Future clinical trials should assess whether men with mCRPC and are NE positive by a peripheral blood assay have improved outcomes with platinum-based chemotherapy. A limitation of this assay is the lack of tissue validation of small cell transformation for some of the NE-positive patients. The response to abiraterone/enzalutamide among the NE-positive patients suggests that these patients may have harbored tumor heterogeneity, wherein the AR-positive clones responded to AR inhibition, which the AR-independent NE clones eventually progressed. Future studies of CTC genomic and phenotypic evolution over time with tissue correlates are needed. Other promising methods of detecting transformation to NEPC include detection of ctDNA (49). In addition, given the higher number of mutations found in NEPC and the improvements in survival with immunotherapy seen in small cell lung cancer, clinical trials of chemotherapy plus immune checkpoint inhibitor therapy should be considered (50, 51). Future refinement of this assay may include incorporating staining for NE markers such as synaptophysin, chromogranin, or CD56, higher magnification/resolution to better assess nuclear texture (i.e., salt-and-pepper chromatin; ref. 52), as well as incorporation of CTC or cell-free genomic analysis.

The strengths of our study include blinded application of previously defined CTC biomarker algorithms to a prospectively enrolled multicenter cohort of men with mCRPC treated with

abiraterone or enzalutamide with a long follow-up. Laboratory personnel and investigators were blinded to the biomarker status and clinical outcomes of men prior to data analysis. An additional strength is that we further optimized the cutoffs for both the CIN and NE assay and found that a threshold of ≥1 CTC/mL for CIN and ≥2 CTC/mL for NE improved prognostic ability of each assay, though these new cutoffs will require future validation. Weaknesses include a relatively small sample size of biomarker-positive men due to the low prevalence of CIN and NE and that these analyses were secondary analyses of the overall PROPHECY trial. In addition, treatments were not assigned prospectively based on testing so no commentary can be made on whether these assays are predictive. An additional weakness is our relative lack of genomic and mutation information on all men to correlate with these CTC biomarkers. However, our subset phenotype–genotype analysis suggests enrichment of CIN/NE phenotypes with TP53, BRCA1, and BRCA2 alterations. Future prospective and larger trials are clearly needed to define the clinical utility of a CTC phenotypic and genotypic classifier of primary and acquired AR therapy resistance, including AR-V7 and other AR variants, chromosomal instability, lineage plasticity and neuroendocrine transformation, and other mechanisms.

In conclusion, CIN and NE phenotypes on a CTC-based assay in high-risk mCRPC are independently associated with worse OS among men treated with abiraterone or enzalutamide. Further study of these two biomarkers for prognostic as well as predictive utility is warranted and ongoing.

Authors' Disclosures

Authors' Contributions

L.C. Brown: Formal analysis, investigation, methodology, writing–original draft, writing–review and editing. S. Halabi: Conceptualization, data curation, formal analysis, supervision, validation, investigation, writing–review and editing. J.D. Schonhoft: Formal analysis, investigation, writing–review and editing. Q. Yang: Formal analysis, writing–review and editing. J. Luo: Conceptualization, investigation, writing–review and editing. D.M. Nanus: Conceptualization, investigation, writing–review and editing. P. Giannakakou: Conceptualization, investigation, writing–review and editing. R.Z. Szmulewitz: Conceptualization, investigation, writing–review and editing. D.C. Danila: Conceptualization, investigation, writing–review and editing. E.S. Barnett: Data curation, formal analysis. E.A. Carbone: Data curation, formal analysis. J.L. Zhao: Data curation, formal analysis. P. Healy: Formal analysis. M. Anand: Data curation, supervision. A. Gill: Formal analysis. A. Jendrisak: Formal analysis. W.R. Berry: Conceptualization, investigation. S. Gupta: Visualization. S.G. Gregory: Conceptualization, formal analysis. R. Wenstrup: Formal analysis, supervision. E.S. Antonarakis: Conceptualization, supervision, investigation, writing–review and editing. D.J. George: Conceptualization, supervision, investigation, writing–review and editing. H.I. Scher: Conceptualization, investigation, writing–review and editing. A.J. Armstrong: Conceptualization, supervision, funding acquisition, validation, investigation, methodology, writing–original draft, writing–review and editing.

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References

1. Ryan CJ, Smith MR, de Bono JS, Molina A, Logothetis CJ, de Souza P, et al. Abiraterone in metastatic prostate cancer without previous chemotherapy. N Engl J Med 2013;368:138–48.
2. de Bono JS, Logothetis CJ, Molina A, Fizazi K, North S, Chu L, et al. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med 2011;364:1995–2005.
3. Beer TM, Armstrong AJ, Rathkopf DE, Loriot Y, Sternberg CN, Higano CS, et al. Enzalutamide in metastatic prostate cancer before chemotherapy. N Engl J Med 2014;371:424–33.
4. Scher HI, Fizazi K, Saad F, Taplin ME, Sternberg CN, Miller K, et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med 2012;367:1187–97.
5. 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;371:1028–38.
6. Antonarakis ES, Lu C, Luber B, Wang H, Chen Y, Zhu Y, et al. Clinical significance of androgen receptor splice variant-7 mRNA detection in circulating tumor cells of men with metastatic castration-resistant prostate cancer treated with first- and second-line abiraterone and enzalutamide. J Clin Oncol 2017;35:2149–56.
7. Scher HI, Graf RP, Schreiber NA, Jayaram A, Winquist E, McLaughlin B, et al. Assessment of the validity of nuclear-localized androgen receptor splice variant 7 in circulating tumor cells as a predictive biomarker for castration-resistant prostate cancer. JAMA Oncol 2018;4:1179–86.
8. 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;2:1441–9.
9. 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;37:1120–9.
10. Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, Mosquera JM, et al. Integrative clinical genomics of advanced prostate cancer. Cell 2015;162:454.
11. Annala M, Vandekerkhove G, Khalaf D, Taavitsainen S, Beja K, Warner EW, et al. Circulating tumor DNA genomics correlate with resistance to abiraterone and enzalutamide in prostate cancer. Cancer Discov 2018;8:444–57.
12. Annala M, Struss WJ, Warner EW, Beja K, Vandekerkhove G, Wong A, et al. Treatment outcomes and tumor loss of heterozygosity in germline DNA repair-deficient prostate cancer. Eur Urol 2017;72:34–42.
13. Antonarakis ES, Lu C, Luber B, Liang C, Wang H, Chen Y, et al. Germline DNA-repair gene mutations and outcomes in men with metastatic castration-resistant prostate cancer receiving first-line abiraterone and enzalutamide. Eur Urol 2018;74:218–25.
14. Jonsson P, Bandlamudi C, Cheng ML, Srinivasan P, Chavan SS, Friedman ND, et al. Tumour lineage shapes BRCA-mediated phenotypes. Nature 2019;571:576–9.
15. Marquard AM, Eklund AC, Joshi T, Krzystanek M, Favero F, Wang ZC, et al. Pan-cancer analysis of genomic scar signatures associated with homologous recombination deficiency suggests novel indications for existing cancer drugs. Biomark Res 2015;3:9.
16. Popova T, Manie E, Rieunier G, Caux-Moncoutier V, Tirapo C, Dubois T, et al. Ploidy and large-scale genomic instability consistently identify basal-like breast carcinomas with BRCA1/2 inactivation. Cancer Res 2012;72:5454–62.
17. Schonhoft JD, Zhao JL, Jendrisak A, Carbone EA, Barnett ES, Hullings MA, et al. Morphology-predicted large-scale transition number in circulating tumor cells identifies a chromosomal instability biomarker associated with poor outcome in castration-resistant prostate cancer. Cancer Res 2020;80:4892–903.
18. Hu J, Han B, Huang J. Morphologic spectrum of neuroendocrine tumors of the prostate: an updated review. Arch Pathol Lab Med 2020;144:320–5.
19. Aggarwal R, Zhang T, Small EJ, Armstrong AJ. Neuroendocrine prostate cancer: subtypes, biology, and clinical outcomes. J Natl Compr Canc Netw 2014;12:719–26.
20. Aggarwal R, Huang J, Alumkal JJ, Zhang L, Feng FY, Thomas GV, et al. Clinical and genomic characterization of treatment-emergent small-cell neuroendocrine prostate cancer: a multi-institutional prospective study. J Clin Oncol 2018;36:2492–503.
21. Aparicio AM, Shen L, Tapia EL, Lu JF, Chen HC, Zhang J, et al. Combined tumor suppressor defects characterize clinically defined aggressive variant prostate cancers. Clin Cancer Res 2016;22:1520–30.
22. Zhang X, Coleman IM, Brown LG, True LD, Kollath L, Lucas JM, et al. SRRM4 expression and the loss of REST activity may promote the emergence of the neuroendocrine phenotype in castration-resistant prostate cancer. Clin Cancer Res 2015;21:4698–708.
23. Bluemn EG, Coleman IM, Lucas JM, Coleman RT, Hernandez-Lopez S, Tharakan R, et al. Androgen receptor pathway-independent prostate cancer is sustained through FGF signaling. Cancer Cell 2017;32:474–89.e6.
24. Tsaur I, Heidegger I, Kretschmer A, Borgmann H, Gandaglia G, Briganti A, et al. Aggressive variants of prostate cancer - are we ready to apply specific treatment right now? Cancer Treat Rev 2019;75:20–6.
25. Aparicio AM, Harzstark AL, Corn PG, Wen S, Araujo JC, Tu SM, et al. Platinum-based chemotherapy for variant castrate-resistant prostate cancer. Clin Cancer Res 2013;19:3621–30.
26. Corn PG, Heath EI, Zurita A, Ramesh N, Xiao L, Sei E, et al. Cabazitaxel plus carboplatin for the treatment of men with metastatic castration-resistant prostate cancers: a randomised, open-label, phase 1–2 trial. Lancet Oncol 2019;20:1432–43.
27. Beltran H, Jendrisak A, Landers M, Mosquera JM, Kossai M, Louw J, et al. The initial detection and partial characterization of circulating tumor cells in neuroendocrine prostate cancer. Clin Cancer Res 2016;22:1510–9.
28. Armstrong AJ, Luo J, Nanus DM, Giannakakou P, Szmulewitz RZ, Danila DC, et al. Prospective multicenter study of circulating tumor cell AR-V7 and taxane versus hormonal treatment outcomes in metastatic castration-resistant prostate cancer. JCO Precis Oncol 2020;4:PO.20.00200.
29. 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;14:6302–9.
30. Halabi S, Lin CY, Kelly WK, Fizazi KS, Moul JW, Kaplan EB, et al. Updated prognostic model for predicting overall survival in first-line chemotherapy for patients with metastatic castration-resistant prostate cancer. J Clin Oncol 2014;32:671–7.
31. Morris MJ, Basch EM, Wilding G, Hussain M, Carducci MA, Higano C, et al. Department of Defense prostate cancer clinical trials consortium: a new instrument for prostate cancer clinical research. Clin Genitourin Cancer 2009;7:51–7.
32. Scher HI, Heller G, Molina A, Attard G, Danila DC, Jia X, et al. Circulating tumor cell biomarker panel as an individual-level surrogate for survival in metastatic castration-resistant prostate cancer. J Clin Oncol 2015;33:1348–55.
33. 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;26:1148–59.
34. Heller G, McCormack R, Kheoh T, Molina A, Smith MR, Dreicer R, et al. Circulating tumor cell number as a response measure of prolonged survival for metastatic castration-resistant prostate cancer: a comparison with prostate-specific antigen across five randomized phase III clinical trials. J Clin Oncol 2018;36:572–80.
35. Hothorn T, Lausen B. On the exact distribution of maximally selected rank statistics. Comput Stat Data Anal 2003;43:121–37.
36. Halabi S, Lin CY, Small EJ, Armstrong AJ, Kaplan EB, Petrylak D, et al. Prognostic model predicting metastatic castration-resistant prostate cancer survival in men treated with second-line chemotherapy. J Natl Cancer Inst 2013;105:1729–37.
37. Kleppe A, Albregtsen F, Vlatkovic L, Pradhan M, Nielsen B, Hveem TS, et al. Chromatin organisation and cancer prognosis: a pan-cancer study. Lancet Oncol 2018;19:356–69.
38. Swanton C, Nicke B, Schuett M, Eklund AC, Ng C, Li Q, et al. Chromosomal instability determines taxane response. Proc Natl Acad Sci U S A 2009;106:8671–6.
39. Fizazi K, Tran N, Fein L, Matsubara N, Rodriguez-Antolin A, Alekseev BY, et al. Abiraterone plus prednisone in metastatic, castration-sensitive prostate cancer. N Engl J Med 2017;377:352–60.
40. Davis ID, Martin AJ, Stockler MR, Begbie S, Chi KN, Chowdhury S, et al. Enzalutamide with standard first-line therapy in metastatic prostate cancer. N Engl J Med 2019;381:121–31.
41. Chi KN, Agarwal N, Bjartell A, Chung BH, Pereira de Santana Gomes AJ, Given R, et al. Apalutamide for metastatic, castration-sensitive prostate cancer. N Engl J Med 2019;381:13–24.
42. James ND, de Bono JS, Spears MR, Clarke NW, Mason MD, Dearnaley DP, et al. Abiraterone for prostate cancer not previously treated with hormone therapy. N Engl J Med 2017;377:338–51.

43. Armstrong AJ, Szmulewitz RZ, Petrylak DP, Holzbeierlein J, Villers A, Azad A, et al. ARCHES: a randomized, phase III study of androgen deprivation therapy with enzalutamide or placebo in men with metastatic hormone-sensitive prostate cancer. J Clin Oncol 2019;37:2974–86.

44. Hussain M, Fizazi K, Saad F, Rathenborg P, Shore N, Ferreira U, et al. Enzalutamide in men with nonmetastatic, castration-resistant prostate cancer. N Engl J Med 2018;378:2465–74.

45. Fizazi K, Shore N, Tammela TL, Ulys A, Vjaters E, Polyakov S, et al. Darolutamide in nonmetastatic, castration-resistant prostate cancer. N Engl J Med 2019;380:1235–46.

46. 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;59:225–39.

47. Malihi PD, Graf RP, Rodriguez A, Ramesh N, Lee J, Sutton R, et al. Single-cell circulating tumor cell analysis reveals genomic instability as a distinctive feature of aggressive prostate cancer. Clin Cancer Res 2020;26:4143–53.

48. Zhao P, Zhu Y, Cheng L, Luo J. Detection of androgen receptor (AR) and AR-V7 in small cell prostate carcinoma: diagnostic and therapeutic implications. Asian J Urol 2019;6:109–13.

49. Beltran H, Romanel A, Conteduca V, Casiraghi N, Sigouros M, Franceschini GM, et al. Circulating tumor DNA profile recognizes transformation to castration-resistant neuroendocrine prostate cancer. J Clin Invest 2020;130:1653–68.

50. Beltran H, Prandi D, Mosquera JM, Benelli M, Puca L, Cyrta J, et al. Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. Nat Med 2016;22:298–305.

51. Horn L, Mansfield AS, Szczesna A, Havel L, Krzakowski M, Hochmair MJ, et al. First-line atezolizumab plus chemotherapy in extensive-stage small-cell lung cancer. N Engl J Med 2018;379:2220–9.

52. Scher HI, Jendrisak A, Gill A, Barnett E, Gopalan A, Zaidi S, et al. Circulating tumor cells (CTCs) with small-cell like pathology are prevalent in metastatic castration-resistant prostate cancer (mCRPC) and show selective pharmacodynamic reductions in patients treated with platinum but not ARSI or taxane. J Clin Oncol 38:15s, 2020 (suppl; abstr 5572).