original report

Genetic Alterations Detected in Cell-Free DNA Are Associated With Enzalutamide and Abiraterone Resistance in Castration-Resistant Prostate Cancer

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abstract

PURPOSE Androgen receptor (*AR)* gene alterations, including ligand-binding domain mutations and copy number (CN) gain, have yet to be fully established as predictive markers of resistance to enzalutamide and abiraterone in men with metastatic castration-resistant prostate cancer (mCRPC). The goal of this study was to validate *AR* gene alterations detected in cell-free DNA (cfDNA) as markers of enzalutamide and abiraterone resistance in patients with mCRPC.

METHODS Patients with mCRPC (N = 62) were prospectively enrolled between 2014 and 2018. Blood was collected before therapies—enzalutamide (n = 25), abiraterone (n = 35), or enzalutamide and abiraterone (n = 2) —and at disease progression. We used deep next-generation sequencing to analyze cfDNA for sequence variants and CN status in *AR* and 45 additional cancer-associated genes. Primary end points were prostate-specific antigen response, progression-free survival (PFS), and overall survival (OS).

RESULTS Elevated tumor-specific cfDNA (circulating tumor DNA) was associated with a worse prostate-specific antigen response (hazard ratio [HR], 3.17; 95% CI, 1.11 to 9.05; $P = .031$), PFS (HR, 1.76; 95% CI, 1.03 to 3.01; $P = .039$), and OS (HR, 2.92; 95% CI, 1.40 to 6.11; $P = .004$). *AR* ligand-binding domain missense mutations (HR, 2.51; 95% CI, 1.15 to 5.72; $P = .020$) were associated with a shorter PFS in multivariable models. *AR* CN gain was associated with a shorter PFS; however, significance was lost in multivariable modeling. Genetic alterations in tumor protein p53 (HR, 2.70; 95% CI, 1.27 to 5.72; $P = .009$) and phosphoinositide 3-kinase pathway defects (HR, 2.62; 95% CI, 1.12 to 6.10; $P = .026$) were associated with a worse OS in multivariable models.

CONCLUSION These findings support the conclusion that high circulating tumor DNA burden is associated with worse outcomes to enzalutamide and abiraterone in men with mCRPC. Tumor protein p53 loss and phosphoinositide 3-kinase pathway defects were associated with worse OS in men with mCRPC. *AR* status associations with outcomes were not robust, and additional validation is needed.

ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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INTRODUCTION

Next-generation therapies that target the androgen–androgen receptor (AR) axis, such as abiraterone and enzalutamide, have improved survival outcomes for men with metastatic castration-resistant prostate cancer (mCRPC),[1-4] but both primary and acquired resistance to these drugs continue to be a substantial clinical challenge. Resistance mechanisms are not fully understood; however, some forms of resistance likely involve alterations to *AR*, including amplification and ligand-binding domain (LBD) missense mutations. Although rare in primary prostate cancers,[5-7] *AR* gene alterations are highly prevalent in mCRPC.[8-13] Metastatic tissue biopsies as a sole means to detect and observe changes in *AR* status is impractical, and thus cell-free DNA (cfDNA) is gaining traction as a minimally invasive and easily obtainable tumor biopsy surrogate. Previous studies using cfDNA from the blood to evaluate the association of *AR* gene aberrations with resistance to abiraterone and enzalutamide are inclusive.[14-17] *AR* copy number (CN) gain[18,19] and/or amplification[20] or detection of two or more *AR* mutations[20] have been associated with worse outcomes to such therapies as abiraterone and enzalutamide. In

contrast, a recent study demonstrated that neither *AR* CN gain, nor *AR* LBD mutations, were significantly associated with time to progression on abiraterone and enzalutamide therapies in multivariable models.[17] Thus, the role of *AR* gene aberrations in mediating resistance to androgen–AR axis therapies has not been fully determined, and additional prospective studies are needed for clinical validation.

AR gene alterations are only detected in a subset of patients who have either primary or acquired resistance to androgen–AR therapies, thereby highlighting the need to determine other mechanisms that mediate resistance. The *AR* splice variant *AR*-V7 is associated with resistance to enzalutamide and abiraterone[21-23] and is also associated with increased *AR* CN.[24] In addition to *AR*, alterations in other genes, including tumor protein p53 (*TP53*), phosphatase and tensin homolog (*PTEN*), and breast cancer gene 2 (*BRCA2*), are enriched in lethal prostate cancer.[8-11] Studies support the idea that lineage plasticity from an AR-dependent to an AR-independent state through loss of *TP53* and retinoblastoma-associated protein 1 (*RB1*) mediates resistance to AR-targeted therapies.[25-28]

Consistent with this, *TP53* defects have been shown to be associated with worse outcomes with abiraterone and enzalutamide therapies.[17] The role of *BRCA2* and other homology-directed repair (HDR) genes in mediating resistance to enzalutamide and abiraterone has not been definitively determined. Although it has been reported that truncating mutations in *BRCA2* and ataxia-telangiectasia mutated (*ATM*) gene are associated with a shorter time to progression on enzalutamide and abiraterone,[17] other studies have indicated that HDR defects may be associated with a better response to therapy.[29,30]

The primary goal of this study was to determine whether *AR* CN gain and/or LBD mutations detected in cfDNA were associated with enzalutamide and abiraterone resistance in patients with mCRPC. The secondary goal was to determine if alterations in other genes that are enriched in lethal prostate cancer, including *TP53, PTEN,* and *BRCA2*, were associated with response to enzalutamide and abiraterone. In this study, high circulating tumor DNA (ctDNA) burden was significantly associated with prostate-specific antigen (PSA) response, progression-free survival (PFS), and overall survival (OS). *AR* LBD mutations were associated with a shorter PFS, whereas *AR* CN gain was associated with both a shorter PFS and worse OS, but lost significance in multivariable analyses. *TP53* loss and defects in the phosphoinositide 3-kinase (PI3K) pathway were both associated with worse OS. Study limitations, including sample size and patient heterogeneity, necessitate larger and prospective validation of the association of plasma *AR* status with outcomes.

METHODS

Patient information, study end points, sample collection, deep next-generation sequencing (NGS), sequence alignment and analysis of variants, CN variation, estimation of ctDNA fraction, and statistical analyses are found in the Data Supplement.

RESULTS

Patient Cohort

Patient characteristics are listed in Table 1. PSA, PSA response, and PFS were not significantly different between patients on abiraterone and enzalutamide (Table 1 and Data Supplement). Approximately one quarter of patients had received prior abiraterone or enzalutamide. Prior abiraterone or enzalutamide exposure trended toward an association for worse outcomes, including PSA response (odds ratio [OR], 2.41; 95% CI, 0.74 to 7.93; $P = .146$), PFS (hazard ratio [HR], 1.17; 95% CI, 0.63 to 2.14; $P = .620$), and OS (HR, 1.51; 95% CI, 0.71 to 3.24; $P = .284$); however, these associations did not reach statistical significance (Tables 2 and 3 and Data Supplement). ClinVar-annotated pathogenic or likely pathogenic missense mutations, truncating mutations, and/or CN alterations were detected in cfDNA from 89% of patients before therapy initiation and in 92% of patients at disease progression (Figs 1A-1D and Data Supplement).

ctDNA

Total cfDNA concentration before therapy was associated with PSA ($P = .002$; Data Supplement). We used deep NGS to analyze cfDNA for CN variation and mutations in 46 cancer-associated genes (Data Supplement). Nearly all patients (61 of 62) had detectable CN variation(s) and/or mutation(s) with an allelic frequency above the 1% cutoff before therapy (Figs 1B and 1C). High ctDNA was detected in approximately 44% of patients before therapy (Fig 1C). Consistent with previous findings,[14,17,20] high ctDNA was significantly associated with a worse PSA response (OR, 3.17; 95% CI, 1.11 to 9.05; $P = .031$) by logistic regression analyses (Table 2). High ctDNA was associated with a significantly shorter median time to progression (14.0 weeks *v* 34.0 weeks; $P = .022$) and, using proportional hazards regression modeling, a shorter PFS (HR, 1.76; 95% CI, 1.03 to 3.01; $P = .039$; Table 3 and Fig 2A). High ctDNA was also significantly associated with a shorter median survival (62.7 weeks *v* 134.9 weeks; $P = .003$) and worse OS (HR, 2.92; 95% CI, 1.40 to 6.11; $P = .004$; Table 3 and Fig 3A). Other clinical variables, such as PSA, age, and visceral metastases, were not significantly associated with PSA response, PFS, or OS in univariable analyses (Tables 2 and 3 and Data Supplement).

AR

Previous studies that evaluated associations between *AR* gene alterations, including CN gain and LBD missense mutations, with therapeutic outcomes are not definitive.[14,15,17-20,31] *AR* CN gain was detected in approximately one half of patients before therapy and at disease progression (Figs 1C and 1D and Data Supplement). *AR* CN gain was not

TABLE 1. Patient Characteristics (N = 62)

Characteristic	Total Cohort (N = 62)	Abiraterone (n = 35)	Enzalutamide (n = 25)	Abiraterone Plus Enzalutamide (n = 2)
Age, years, median (range)	71.5 (41-90)	71 (51-90)	73 (41-90)	79 (70-87)
Race				
White	51 (82.3)	27 (77.1)	24 (96.0)	0 (0)
Black	7 (11.3)	6 (17.1)	0 (0)	1 (50)
Other	4 (6.5)	2 (5.7)	1 (4.0)	1 (50)
Local treatment of prostate cancer				
Radical prostatectomy	24 (38.7)	14 (40.0)	9 (36.0)	1 (50)
Radiation	9 (14.5)	3 (8.6)	5 (20.0)	1 (50)
Other	4 (6.5)	3 (8.6)	1 (4.0)	0 (0)
None	24 (38.7)	14 (40.0)	10 (40.0)	0 (0)
Not available	1 (1.6)	1 (2.9)	0 (0)	0 (0)
Gleason sum				
≤ 7	14 (22.6)	10 (28.6)	2 (8.0)	2 (100)
≥ 8	42 (67.7)	22 (62.9)	20 (80.0)	0 (0)
Not available	6 (9.7)	3 (8.6)	3 (12.0)	0 (0)
Prior treatment of metastatic prostate cancer				
Prior chemotherapy	13 (21.0)	5 (14.3)	8 (32.0)	0 (0)
Prior abiraterone	10 (16.1)	2 (5.7)	8 (32.0)	0 (0)
Prior enzalutamide	5 (8.1)	4 (11.4)	1 (4.0)	0 (0)
Median PSA, ng/mL (range)	19.3 (0.6-1966)	18.2 (0.6-1966)	18.9 (0.9-205.9)	102.6 (54.2-151.0)
Site of metastases				
Bone only	34 (54.8)	21 (60.0)	13 (52.0)	0 (0)
Visceral only	3 (4.8)	0 (0)	3 (12.0)	0 (0)
Lymph node only	4 (6.5)	1 (2.9)	3 (12.0)	0 (0)
Bone and visceral	3 (4.8)	1 (2.9)	1 (4.0)	1 (50)
Bone and lymph node	18 (29.0)	12 (34.3)	5 (20.0)	1 (50)
Study therapy				
Abiraterone plus prednisone	35 (56.5)	35 (100)	0 (0)	0 (0)
Enzalutamide	25 (40.3)	0 (0)	25 (100)	0 (0)
Abiraterone plus prednisone and enzalutamide	2 (3.2)	0 (0)	0 (0)	2 (100)
Median progression-free survival, weeks (range)	25.9 (2.3-162.7)	26.1 (3.7-162.7)	24.7 (2.3-103.4)	7.9 (4.6-11.1)
Prostate cancer–specific mortality	30 (48.4)	11 (31.4)	17 (68.0)	2 (100)
Median follow-up, weeks (range)	74.0 (4.7-182.1)	74.6 (4.7-182.1)	76.9 (21.4-144.9)	35.6 (16.4-54.7)

NOTE. Data are presented as No. (%) unless otherwise noted.

significantly associated with PSA response by logistic regression analysis ($P = .119$; Table 2 and Fig 2B), but was associated with a shorter median time to progression (16.1 weeks *v* 34.0 weeks; $P = .013$) and a shorter median survival (62.7 weeks *v* 144.9 weeks; $P = .002$; Figs 2C and 3B). Using proportional hazards regression modeling, PFS (HR, 2.07; 95% CI, 1.20 to 3.57; $P = .009$) and OS (HR, 3.26; 95% CI, 1.52 to 7.11; $P = .002$) were shorter in patients with *AR* CN gain; however, significance was lost upon inclusion of ctDNA burden in multivariable modeling (Figs 2C and 3B and Table 3).

Pathogenic *AR* LBD missense mutations were detected in cfDNA from 13% (eight of 62) of patients before therapy initiation and in an additional 15% (four of 26) of evaluable patients at disease progression (Figs 1C, 1D, and 2D and Data Supplement). Of the eight patients who had detectable *AR* LBD mutations before therapy, six did not have a PSA response, whereas two patients who harbored the

TABLE 2. Response to Therapy: Univariable and Multivariable Logistic Regression Analyses (N = 62)

		PSA Response (≥ 50% decrease in PSA from baseline)		
		OR	95% CI	*P*
Response	**Patients, No.**		**Univariable**	
Prior abiraterone or enzalutamide	15	2.41	0.74 to 7.93	.146
PSA ≥ 20 ng/mL	30	1.67	0.61 to 4.59	.323
Age ≥ 72 years	32	1.32	0.48 to 3.63	.586
Visceral metastasis	6	0.62	0.10 to 3.67	.598
ctDNA high	27	3.17	1.11 to 9.05	**.031**
AR CN gain and/or LBD mutation	34	1.80	0.65 to 5.01	.261
AR LBD mutation	8	4.71	0.87 to 25.28	.072
AR CN gain	32	2.27	0.81 to 6.34	.119
TP53 mutation and/or CN loss	23	1.32	0.47 to 3.72	.602
RB1 mutation and/or CN loss	17	2.35	0.75 to 7.35	.141
TP53 and *RB1* mutation and/or CN loss	6	7.73	0.85 to 70.65	.070
PI3K pathway defect	15	8.53	2.09 to 34.81	**.003**
WNT pathway defect	9	3.05	0.69 to 13.53	.143
BRCA1/BRCA2/ATM mutation and/or CN loss	24	1.53	0.55 to 4.30	.417
BRCA1/BRCA2/ATM truncating mutations	14	0.96	0.29 to 3.21	.953
			Multivariable	
AR LBD mutation	8	4.88	0.85 to 28.08	.076
TP53 and *RB1* mutation and/or CN loss	6	5.40	0.56 to 52.15	.145
PI3K pathway defect	15	7.09	1.40 to 35.94	**.018**

NOTE. Significant *P* values are shown in bold. Multivariable analyses controlled for ctDNA high.

Abbreviations: AR, androgen receptor; ATM, ataxia-telangiectasia mutated gene; BRCA1/2, breast cancer gene 1/2; CN, copy number; ctDNA, circulating tumor DNA; LBD, ligand-binding domain; OR, odds ratio; PI3K, phosphoinositide 3-kinase; PSA, prostate-specific antigen; RB1, retinoblastoma-associated protein 1; TP53, tumor protein 53; WNT, wingless-type MMTV integration site.

H875Y mutation had PSA responses on abiraterone (Fig 2C). Using logistic regression analyses, *AR* LBD mutations were not significantly associated with a worse PSA response rate (*P* = .072; Table 2). However, pathogenic *AR* LBD missense mutations were associated with a worse 30% or more decline in PSA (OR, 6.00; 95% CI, 1.10 to 32.76; *P* = .039) that remained significant in multivariable logistic regression analyses (Data Supplement).

Median time to progression was shorter in patients who had a detectable *AR* LBD mutation than in patients without a detectable *AR* LBD mutation (11.4 weeks *v* 28.7 weeks; *P* = .021; Fig 2F). Using proportional hazards regression modeling, *AR* LBD mutations detected before therapy were associated with a shorter time to progression (HR, 2.39; 95% CI, 1.11 to 5.14; *P* = .026), even when controlled for ctDNA burden (*P* = .020) and other variables (Table 3 and Data Supplement). However, detectable *AR* LBD mutations were not significantly associated with worse OS (*P* = .364; Table 3).

AR CN gain and LBD mutations were not mutually exclusive in cfDNA (Fig 1C). Two *AR* mutations at different allelic frequencies—T878A at 9.4% and L702H at 1.5%—were detected in one patient who experienced disease progression on abiraterone plus prednisone who also had *AR* CN gain (Data Supplement). Studies support the idea that the *AR* L702H mutation mediates an acquired response to glucocorticoids, thereby providing rationale to switch from prednisone to dexamethasone.[14,20,32] In support of this notion, replacement of prednisone with dexamethasone resulted in a greater than 80% PSA decline for this patient (Data Supplement).

TP53 and RB1

Genetic alterations in *TP53* are highly enriched in lethal prostate cancer[8-11] and have recently been shown to be associated with worse PFS and OS in patients treated with abiraterone and enzalutamide.[17] *TP53* was highly altered in patients' cfDNA (Figs 1C, 1D, and 3C); however, *TP53*

TABLE 3. Progression-free Survival and Overall Survival: Univariable and Multivariable Cox Proportional Hazards Regression Analyses (N = 62)

		Progression-Free Survival						Overall Survival					
		Univariable			Multivariable			Univariable			Multivariable		
Variable	Patients, No.	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Prior abiraterone or enzalutamide	15	1.17	0.63 to 2.14	.620	1.25	0.67 to 2.31	.480	1.51	0.71 to 3.24	.284	2.06	0.93 to 4.56	.074
PSA ≥ 20 ng/mL	30	1.43	0.83 to 2.45	.193	1.51	0.88 to 2.60	.137	1.44	0.70 to 2.95	.326	1.61	0.77 to 3.34	.204
Age ≥ 72 years	32	1.04	0.60 to 1.78	.895	1.07	0.62 to 1.84	.805	1.04	0.50 to 2.12	.925	1.11	0.54 to 2.28	.782
Visceral metastasis	6	1.29	0.51 to 3.26	.595	1.44	0.56 to 3.69	.450	2.50	0.95 to 6.63	.064	3.24	1.19 to 8.81	**.021**
ctDNA high	27	1.76	1.03 to 3.01	**.039**	—	—	—	2.92	1.40 to 6.11	**.004**	—	—	—
AR CN gain and/or LBD mutation	34	2.00	1.15 to 3.47	**.014**	1.74	0.95 to 3.19	.072	2.95	1.36 to 6.36	**.006**	2.07	0.87 to 4.89	.099
AR LBD mutation	8	2.39	1.11 to 5.14	**.026**	2.51	1.15 to 5.45	**.020**	1.64	0.56 to 4.82	.364	1.69	0.57 to 4.97	.345
AR CN gain	32	2.07	1.20 to 3.57	**.009**	1.82	0.98 to 3.40	.060	3.26	1.52 to 7.00	**.002**	2.33	0.96 to 5.62	.060
TP53 mutation and/or CN loss	23	1.33	0.77 to 2.30	.314	1.26	0.73 to 2.19	.406	3.19	1.53 to 6.64	**.002**	2.70	1.27 to 5.72	**.009**
RB1 mutation and/or CN loss	17	0.94	0.52 to 1.70	.849	0.69	0.36 to 1.32	.265	1.47	0.68 to 3.18	.321	0.99	0.43 to 2.24	.974
TP53 and *RB1* mutation and/or CN loss	6	1.66	0.71 to 3.91	.246	1.58	0.67 to 3.73	.299	4.50	1.79 to 11.28	**.001**	4.56	1.78 to 11.71	**.002**
PI3K pathway defect	15	1.77	0.97 to 3.22	.064	1.40	0.71 to 2.77	.327	3.64	1.69 to 7.86	**.001**	2.62	1.12 to 6.10	**.026**
WNT pathway defect	9	1.32	0.64 to 2.73	.450	1.13	0.54 to 2.39	.746	2.92	1.28 to 6.68	**.011**	2.36	0.995 to 5.60	.051
BRCA1/BRCA2/ATM mutation and/or CN loss	24	0.95	0.55 to 1.64	.855	0.89	0.52 to 1.54	.683	1.45	0.69 to 3.06	.326	1.32	0.62 to 2.85	.472
BRCA1/BRCA2/ATM truncating mutations	14	0.89	0.47 to 1.68	.715	0.86	0.45 to 1.63	.641	1.03	0.41 to 2.57	.948	0.97	0.39 to 2.44	.952

NOTE. Significant *P* values are shown in bold. Multivariable analyses controlled for ctDNA high.

Abbreviations: AR, androgen receptor; ATM, ataxia-telangiectasia mutated gene; BRCA1/2, breast cancer gene 1/2; CN, copy number; ctDNA, circulating tumor DNA; HR, hazard ratio; LBD, ligand binding domain; PI3K, phosphoinositide 3-kinase; PSA, prostate-specific antigen; RB1, retinoblastoma-associated protein 1; TP53, tumor protein 53; WNT, wingless-type MMTV integration site.

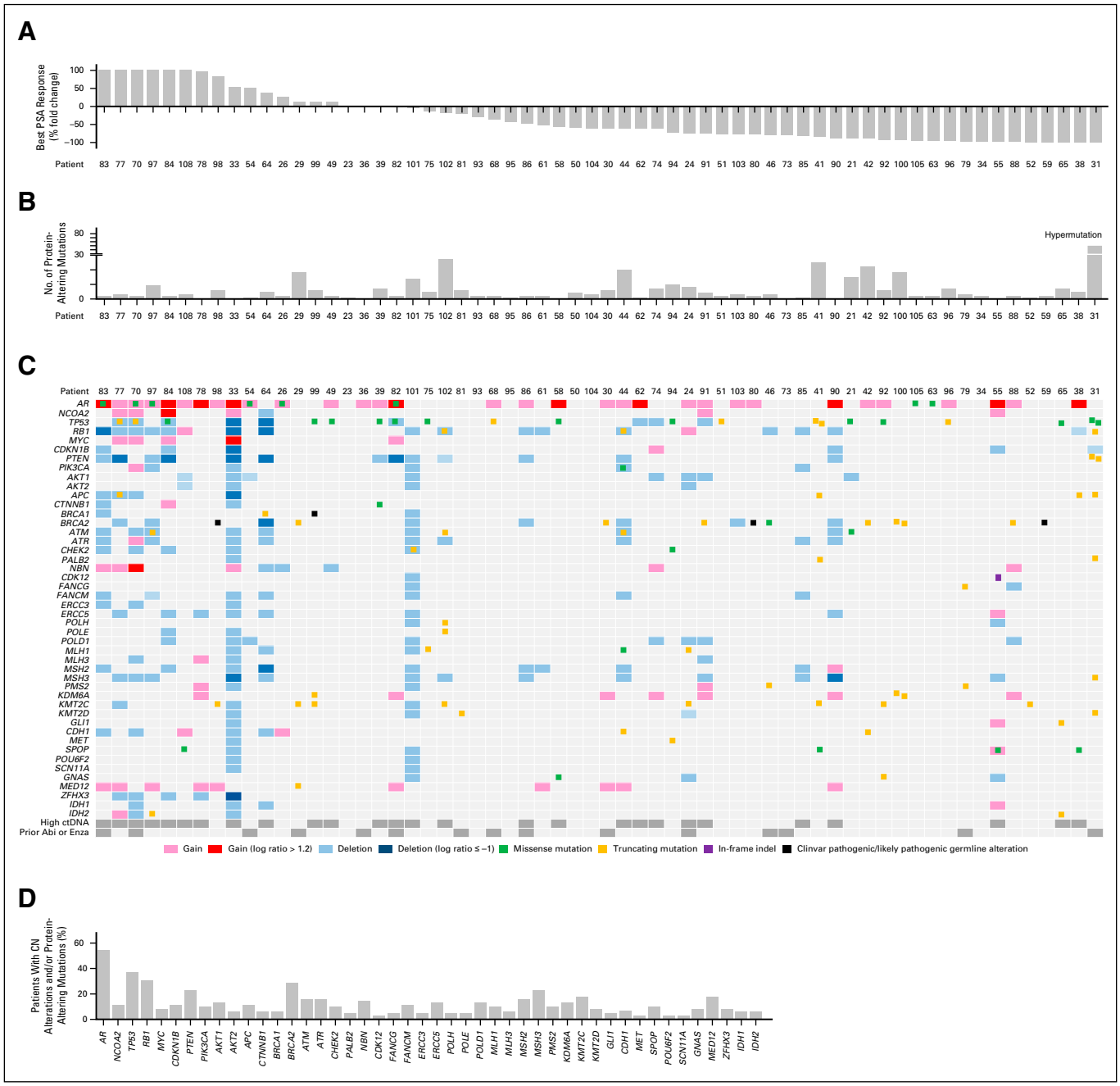


FIG 1. Genetic alterations detected in cell-free DNA (cfDNA) before therapy and best prostate-specific antigen (PSA) response. (A) Waterfall plot of best PSA response for all patients (N = 62) after therapy as determined by best percentage fold change in PSA. (B) Total number of protein-altering genetic changes in 46 genes detected by next-generation sequencing (NGS) of cfDNA from 62 patients before abiraterone (Abi) and enzalutamide (Enza) therapy. (C) Genetic alterations—copy number (CN) status, ClinVar pathogenic/likely pathogenic missense and germline mutations, and truncating mutations—in 46 genes detected by NGS of cfDNA from 62 patients before abiraterone and enzalutamide therapy in order of best PSA response. (D) Total number of genetic alterations—CN, ClinVar pathogenic/likely pathogenic missense and germline mutations, and truncating mutations—in 46 genes detected by NGS of cfDNA from 62 patients before abiraterone and enzalutamide therapy. AR, androgen receptor; ctDNA, circulating tumor DNA.

defects—pathogenic mutations and/or CN loss—were not associated with PSA response ($P = .602$) or PFS ($P = .314$; Tables 2 and 3). Conversely, median OS was shorter in patients with a *TP53* defect compared with patients without a detectable *TP53* defect (68.1 weeks *v* 134.9 weeks; $P = .001$; Fig 3D). Using proportional hazards regression modeling, *TP53* defects were associated with worse OS (HR, 3.19; 95% CI, 1.53 to 6.64; $P = .002$) that remained significant after adjusting for clinical variables (Table 3 and Data Supplement). Patients with both *TP53* and *RB1* defects had shorter median OS compared with patients with a *TP53* defect alone or with patients with intact *TP53*

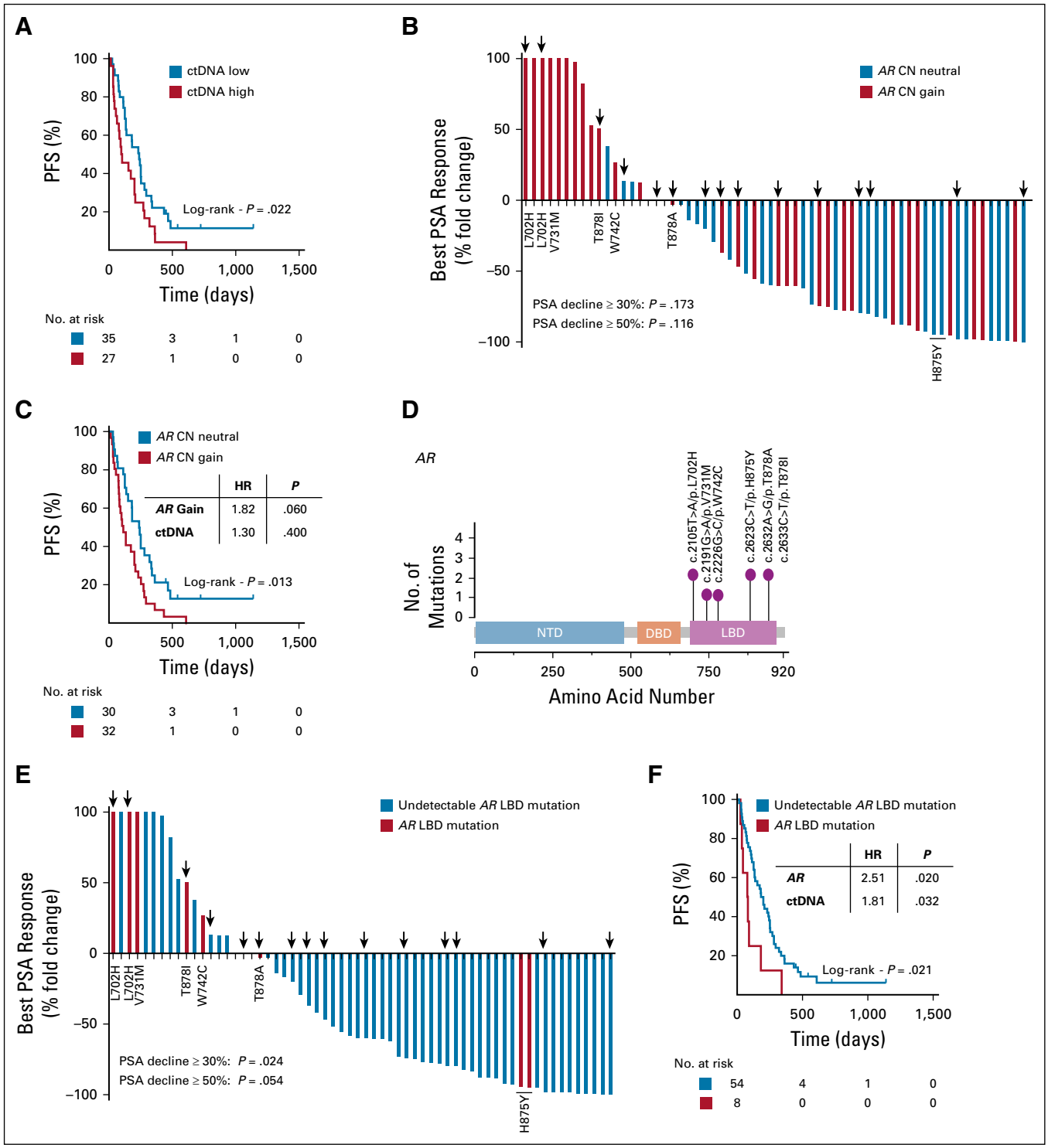


FIG 2. Progression-free survival (PFS): Pathogenic androgen receptor (*AR*) ligand-binding domain (LBD) mutations are associated with a shorter time to progression. (A) Kaplan-Meier method and log-rank test were used to determine median time to progression for patients who had high versus low circulating tumor DNA (ctDNA) before therapy. (B) Waterfall plot of best prostate-specific antigen (PSA) response for all patients (N = 62) after therapy as determined by best percentage fold change in PSA. *AR* copy number (CN) gain and *AR* LBD missense mutations were determined by deep next-generation sequencing (NGS) of cell-free DNA (cfDNA) before therapy. *AR* LBD missense mutations were included for patients with detectable mutations. Black arrow indicates patients with prior abiraterone or enzalutamide therapy. χ^2 analyses for a 30% or greater and 50% or greater PSA decrease. (C) Kaplan-Meier method and log-rank test were used to determine median time to progression for patients who had a gain in *AR* CN compared with patients who were *AR* CN neutral before therapy. The association of *AR* CN gain with PFS controlled for ctDNA burden using multivariable proportional hazards regression modeling. (C) Gene schematic illustrating pathogenic *AR* LBD mutations detected by targeted NGS of cfDNA before abiraterone and enzalutamide therapies. (E) Waterfall plot

(35.4 weeks *v* 77.4 weeks *v* 157.7 weeks; $P < .001$; Fig 3E). *TP53* defects in conjunction with *RB1* defects were associated with worse OS (HR, 4.50; 95% CI, 1.79 to 11.28; $P = .001$) that remained significant after adjusting for other variables (Table 3 and Data Supplement).

PI3K and Wingless-Type MMTV Integration Site Pathways

PI3K pathway defects involving genetic alterations in *PTEN*—CN loss and/or truncating mutations—and *PIK3CA*—CN gain and/or pathogenic missense mutation—were detected in nearly one quarter of patients before therapy (Figs 1C and 1D). Patients with PI3K pathway defects before therapy had a significantly shorter median survival (49.4 weeks *v* 134.9 weeks; $P < .001$) and worse OS (HR, 3.64; 95% CI, 1.69 to 7.86; $P = .001$), even after controlling for ctDNA burden ($P = .026$; Fig 3F, Table 3, and Data Supplement). PI3K pathway alterations were also associated with a worse PSA response that remained significant after adjusting for ctDNA burden (HR, 8.53; 95% CI, 2.09 to 34.81; $P = .003$; Table 2). Wingless-type MMTV integration site pathway defects involving genetic alterations in adenomatous polyposis coli—CN loss and/or truncating mutations—and β-catenin—CN gain and pathogenic missense mutations—were detected in nearly 15% of patients before to therapy (Figs 1C and 1D). Wingless-type MMTV integration site pathway defects were associated with a worse OS (HR, 2.92; 95% CI, 1.28 to 6.68; $P = .011$) using proportional hazards regression modeling; however, significance was lost after controlling for ctDNA burden ($P = .051$; Fig 3G and Table 3).

BRCA1, BRCA2, and ATM

Men with lethal prostate cancer are more likely to have germline mutations in DNA repair genes[33,34]; however, the association of HDR gene defects with response to abiraterone and enzalutamide is conflicting.[17,29,30] Approximately one third of patients had germline and/or somatic deleterious mutations in or CN loss of *BRCA1*, *BRCA2*, or *ATM* before therapy, with some patients having more than one mutation (Figs 1C and 1D and Data Supplement). Collective ClinVar deleterious missense mutations, truncating mutations, and/or CN loss in *BRCA2, BRCA1*, or *ATM* were not significantly associated with PSA response ($P = .417$), PFS ($P = .855$), or OS ($P = .326$; Tables 2 and 3). Analysis of truncating mutations alone in *BRCA1*, *BRCA2*, and *ATM* did not increase prognostic significance.

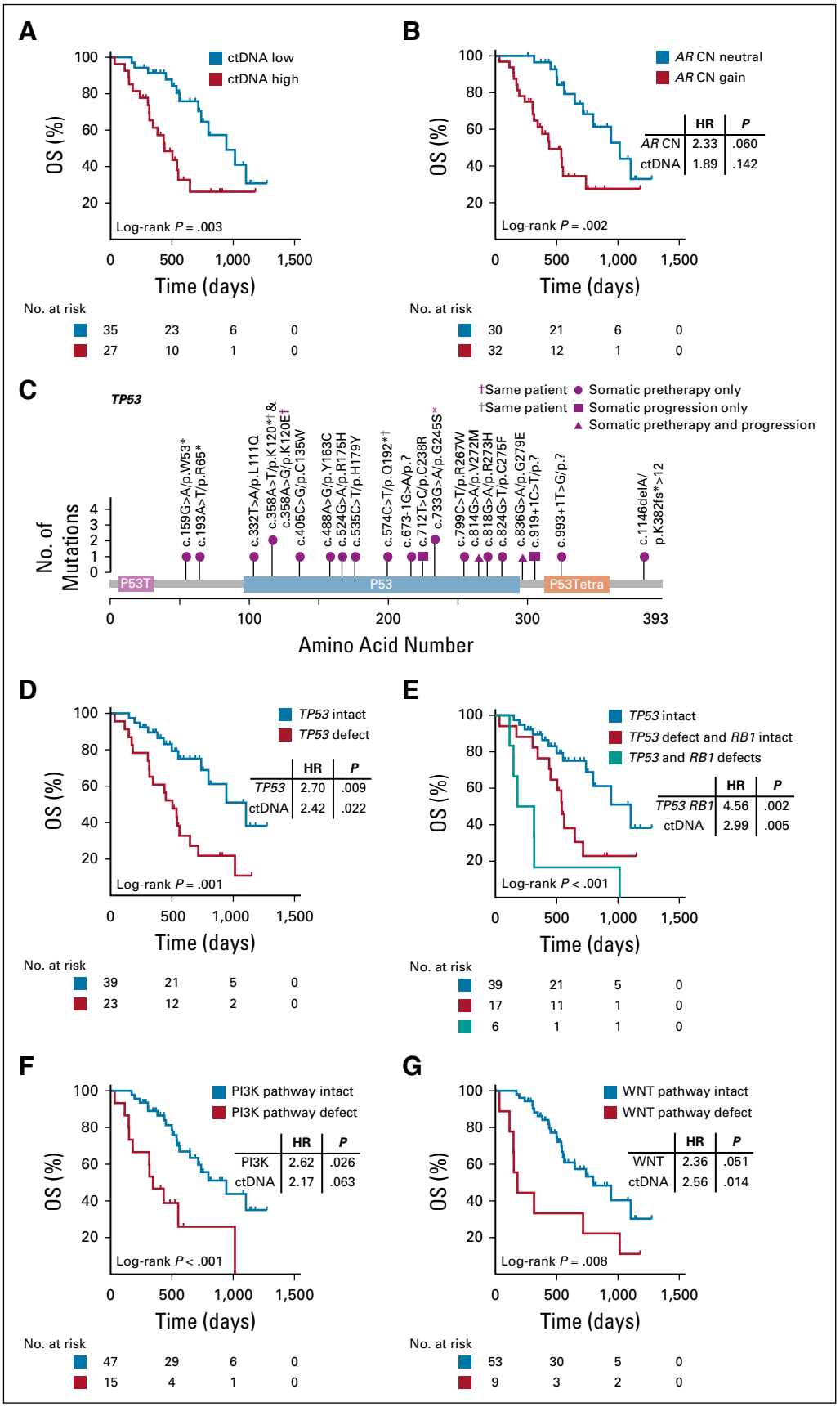
DISCUSSION

Liquid biopsies using cfDNA as a tumor analyte are rapidly being developed for cancer diagnostics of solid tumors.[35-37] When obtained concurrently, plasma-derived cfDNA is highly concordant with tissue biopsies for tumor-specific genetic alterations.[38,39] As a result of their advantages over traditional tissue biopsies, including the ease of accessibility for sequential monitoring of cancer dynamics and recapitulation of tumor heterogeneity, clinical development of cfDNA has the potential to advance prostate cancer precision medicine.[40]

Mechanisms of resistance to abiraterone and enzalutamide likely involve alterations to androgen–AR axis signaling. Previous studies have indicated that collective genetic aberrations to *AR,* including CN gain and mutations, are associated with worse outcomes in patients on abiraterone or enzalutamide therapies.[14,15] The value of *AR* LBD mutations alone as a predictive marker for response to enzalutamide and abiraterone in patients with mCRPC has yet to be fully established. A previous study demonstrated that patients with mCRPC harboring two or more *AR* mutations had worse outcomes on enzalutamide.[20] An additional retrospective study showed that *AR* mutations—L702H and T878A—were associated with shorter PFS and OS in postdocetaxel patients with mCRPC on abiraterone.[19] In contrast, a large prospective study reported that *AR* LBD mutations were not associated with time to progression on abiraterone or enzalutamide therapies in treatment-naïve patients with mCRPC.[17] In the current study, we found that *AR* LBD missense mutations detected in cfDNA before enzalutamide and abiraterone therapies were associated with a shorter PFS, but not PSA response or OS. Lack of a strong association with PSA response and OS lessens the likelihood that *AR* LBD mutations will be rigorous biomarkers for therapeutic decision making. Discrepancies between study findings may be a result of several factors, including prior therapies, study therapy, study design, specific *AR* LBD mutation, *AR* amplification, and disease burden. Prior therapies likely change the repertoire and incidence of *AR* LBD mutations.[19,41] As a result of their low individual prevalence, *AR* LBD mutations are often combined for analyses; however, studies support the idea that *AR* LBD mutations have distinct functional properties, including ligand promiscuity and agonistic activity, that mediate selective-therapy resistance.[32,41] Furthermore, the coincidence of other genetic alterations, including *AR* amplification or *TP53* defects, and overall disease burden may be confounders. Future large-scale and multicenter prospective validation will be necessary to determine fully the roles of individual mutations in drug resistance.

AR CN gain as a single marker has been demonstrated to be associated with worse outcomes in patients with mCRPC

FIG 2. (Continued). of best PSA response for all patients (N = 62) after therapy as determined by best percentage fold change in PSA. *AR* LBD mutations were determined by deep NGS of cfDNA before therapy and listed below. Black arrow indicates patients with prior abiraterone or enzalutamide therapy. χ^2 analyses for a 30% or greater and 50% or greater PSA decrease. (F) Kaplan-Meier method and log-rank test were used to determine median time to progression for patients who were positive versus negative for *AR* LBD mutations before therapy. The association of *AR* LBD mutations with PFS controlled for ctDNA burden using multivariable proportional hazards regression modeling. DBD, DNA-binding domain; NTD, N-terminal domain.


A
ctDNA low
ctDNA high
OS (%)
Log-rank P = .003
Time (days)
No. at risk
35 23 6 0
27 10 1 0
B
AR CN neutral
AR CN gain
HR P
AR CN 2.33 .060
ctDNA 1.89 .142
Log-rank P = .002
No. at risk
30 21 6 0
32 12 1 0
C
TP53
†Same patient
†Same patient
Somatic pretherapy only
Somatic progression only
Somatic pretherapy and progression
No. of Mutations
c.159G>A/p.W53*
c.193A>T/p.R65*
c.332T>A/p.L111Q
c.358A>T/p.K120*†
c.358A>G/p.K120E†
&
c.405C>G/p.C135W
c.488A>G/p.Y163C
c.524G>A/p.R175H
c.535C>T/p.H179Y
c.574C>T/p.Q192*†
c.673-1G>A/p.?
c.712T>C/p.C238R
c.733G>A/p.G245S*
c.799C>T/p.R267W
c.814G>A/p.V272M
c.818G>A/p.R273H
c.824G>T/p.C275F
c.836G>A/p.G279E
c.919+1C>T/p.?
c.993+1T>G/p.?
c.1146delA/p.K382fs*>12
P53T
P53
P53Tetra
Amino Acid Number
D
TP53 intact
TP53 defect
HR P
TP53 2.70 .009
ctDNA 2.42 .022
Log-rank P = .001
No. at risk
39 21 5 0
23 12 2 0
E
TP53 intact
TP53 defect and RB1 intact
TP53 and RB1 defects
HR P
TP53 RB1 4.56 .002
ctDNA 2.99 .005
Log-rank P < .001
No. at risk
39 21 5 0
17 11 1 0
6 1 1 0
F
PI3K pathway intact
PI3K pathway defect
HR P
PI3K 2.62 .026
ctDNA 2.17 .063
Log-rank P < .001
No. at risk
47 29 6 0
15 4 1 0
G
WNT pathway intact
WNT pathway defect
HR P
WNT 2.36 .051
ctDNA 2.56 .014
Log-rank P = .008
No. at risk
53 30 5 0
9 3 2 0
OS (%)
Time (days)

on abiraterone and enzalutamide.[19,20] A retrospective study reported that *AR* CN gain was associated with worse PFS and OS in men who were treated with enzalutamide or abiraterone for mCRPC.[19] Similarly, *AR* CN gain was also reported to be associated with a worse PSA response and PFS in patients on enzalutamide.[20] Our study also demonstrated an association of *AR* CN gain with PFS and OS; however, significance was lost in multivariable modeling, which is consistent with a previous report.[17] Clearly, additional prospective studies are needed to assess the clinical strength of *AR* CN gain as a predictive biomarker for therapeutic response to enzalutamide and abiraterone in patients with mCRPC.

In the current study, *TP53* and PI3K pathway defects were associated with worse OS. Deregulation of these pathways likely mediates resistance to androgen–AR axis therapies. Concurrent *TP53* and *RB1* defects are highly enriched in AR-independent neuroendocrine mCRPC compared with adenocarcinoma mCRPC.[42] Combined *TP53* and *RB1* loss has been shown to promote lineage switching from an AR-dependent to an AR-independent state[41,43,44] and consequent resistance to AR-targeted therapies. Similar to *TP53*, genetic alterations in *PTEN* are enriched in mCRPC compared with metastatic castration-sensitive prostate cancer and localized prostate cancer.[11]

Studies suggest that *PTEN* loss may mediate castration resistance by downregulating AR,[25-28] thereby supporting a rationale for combined inhibition of PI3K and AR- in *PTEN*-deficient mCRPCs.[45,46]

Association of pathogenic mutations in HDR genes with response to abiraterone and enzalutamide therapy is conflicting. A clinical trial in patients with mCRPC suggested that genetic alterations in HDR genes that were detected in metastatic biopsy tissue may be associated with longer PFS when on abiraterone therapy.[29] Concordant findings were observed in a second study that supported the idea that patients with mCRPC harboring a germline *BRCA1/2* or *ATM* mutation may also have improved outcomes to abiraterone and enzalutamide.[30] In contrast, another study showed that truncating mutations in *BRCA2* and *ATM* detected in cfDNA were associated with a shorter time to progression on abiraterone and enzalutamide therapies in treatment-naïve patients with mCRPC.[17] In our study, collective somatic and germline genetic alterations were also not associated with worse outcomes to enzalutamide and abiraterone. Association differences may reflect variables, such as sample size, prior treatment status, disease burden, disease heterogeneity, somatic versus germline, and single versus dual loss. Certainly, additional prospective investigation is needed to determine the clinical significance of HDR mutations as predictive markers to abiraterone and enzalutamide therapies.

In the current study, many patients had detectable alterations that could serve as potential therapeutic targets. Previous studies have shown that patients with mCRPC with either germline or somatic mutations in HDR genes achieved significant responses to olaparib[47] and to abiraterone plus veliparib.[29] More than one quarter of patients in our study had a deleterious germline or somatic *BRCA1*, *BRCA2*, or *ATM* mutation detected before therapy or at disease progression, which suggests that these patients may benefit from therapies that target poly (ADP-ribose) polymerase or platinum-based chemotherapy.[29,47,48] In addition, immunotherapy trials have been largely unsuccessful in men with mCRPC[49]; however, rare responders have been reported.[50] A seminal clinical trial demonstrated that microsatellite instable cancers caused by mismatch repair (MMR) gene deficiency were sensitive to programmed death-1 blockade, perhaps because of the formation of neoantigens resulting from increased mutational burden.[51] Inactivation of MMR genes and elevated mutational burden have been detected in some men with aggressive prostate cancers.[33,52,53] One patient in our study had a detectable noncanonical MMR gene mutation in his cfDNA and a correspondingly high

FIG 3. Overall survival (OS): Tumor protein p53 (*TP53*) and phosphoinositide 3-kinase (PI3K) pathway defects are associated with worse OS. (A) Kaplan-Meier method and log-rank test were used to determine median OS for patients who had high versus low circulating tumor DNA (ctDNA) before therapy. (B) Kaplan-Meier method and log-rank test were used to determine median OS for patients who had androgen receptor (AR) copy number (CN) gain before therapy. Association of *AR* CN gain with OS controlled for ctDNA burden using multivariable proportional hazards regression modeling. (C) Gene schematic illustrating deleterious *TP53* mutations detected by deep next-generation sequencing (NGS) of cell-free DNA (cfDNA) before abiraterone and enzalutamide therapies and at disease progression while on therapy. (D) Kaplan-Meier method and log-rank test were used to determine median OS for patients who had *TP53* defects—CN loss and or ClinVar pathogenic/likely pathogenic mutations—before therapy. Association of *TP53* defects with OS controlled for ctDNA burden using multivariable proportional hazards regression modeling. (E) Kaplan-Meier method and log-rank test were used to determine median OS for patients who had both *TP53* and retinoblastoma-associated protein 1 (*RB1*) defects compared with patients who had *TP53* defects but were *RB1* intact—CN loss and or ClinVar pathogenic/likely pathogenic mutations—before therapy. Association of dual *TP53* and *RB1* defects with OS controlled for ctDNA burden using multivariable proportional hazards regression modeling. (F) Kaplan-Meier method and log-rank test were used to determine median OS for patients who had PI3K pathway defects—CN loss and/or truncating mutations in phosphatase and tensin homolog and/or CN gain of *PIK3CA*—before therapy. Association of *PI3K* defects with OS controlled for ctDNA burden using multivariable proportional hazards regression modeling. (G) Kaplan-Meier method and log-rank test were used to determine median OS for patients who had wingless-type MMTV integration site (WNT) pathway defects—CN loss and/or truncating mutations in adenomatous polyposis coli and/or CN gain and/or pathogenic missense mutations in β-catenin—before therapy. Association of WNT defects with OS controlled for ctDNA burden using multivariable proportional hazards regression modeling. P53, P53 DNA-binding domain; P53T, P53 transactivation motif; P53Tetra, P53 tetramerisation motif.

mutational burden that suggested that he may be an ideal candidate for checkpoint immunotherapy. This study supports the idea that cfDNA may be a useful analyte for directing clinical decisions in prostate cancer precision medicine.

Several limitations to our study exist. Of note, the small sample size precluded multivariable analyses that incorporated more than two variables and analyses by therapy subgroup. Consistent with other reports, patients with prior exposure to abiraterone and enzalutamide experienced worse outcomes.[54-59] Statistical significance was not reached, likely because of the small overall sample size and the few patients with prior therapy. In addition, the small size precluded any definitive conclusions pertaining to the association of *AR* LBD mutations with outcomes. Larger prospective studies will be needed to validate our findings. Samples were obtained from two hospitals, and future prospective studies would benefit from the inclusion of a larger number of institutions. Future prospective studies would also be strengthened by radiologic confirmation of progression for every patient. An additional limitation was the variability of cfDNA input for NGS among patients. NGS protocols were adjusted on the basis of total input, but for patients with low input the lack of genetic alteration detection was considered indeterminate as opposed to negative. In addition, mutations in such genes as *TP53* and *ATM* detected in cfDNA at low allelic frequencies may be false positives as a result of clonal hematopoiesis.[60] Corresponding tissue was not available for all samples to confirm *TP53* status, and future studies will examine both prostate tumor tissue and blood leukocytes for genetic alterations. A final limitation was our inability to evaluate *AR* splice variants, including *AR*-V7, because of the requirement of circulating tumor cells or whole-blood RNA. The presence of AR-V7 is certainly another established mechanism of primary and acquired resistance to next-generation hormonal therapies.[21-23] Future studies should aim to simultaneously analyze the full complement of *AR* aberrations, including gene mutations, amplifications, genomic structural rearrangements, and mRNA splice variants, from a single liquid biopsy.

In summary, our findings indicate ctDNA burden was highly associated with worse outcomes to enzalutamide and abiraterone. Association of *AR* status with outcomes was not robust and will need additional prospective validation. *TP53* loss, especially in the context of concurrent *RB1* defects, and PI3K pathway defects were associated with worse OS. These studies provide the rationale for larger prospective multi-institutional studies to additionally assess the clinical utility of integrating genetic alterations detected in cfDNA for the optimal management of metastatic prostate cancer.

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