original report

Multimodal Approach to Outcome Prediction in Metastatic Castration-Resistant Prostate Cancer by Integrating Functional Imaging and Plasma DNA Analysis

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abstract

PURPOSE Biomarkers for treatment personalization in metastatic castration-resistant prostate cancer (mCRPC) could help improve patient outcomes. Multiple tests on blood have reported associations with poorer outcome, including serum lactate dehydrogenase (LDH), chromogranin A (CGA), neutrophil:lymphocyte ratio (NLR), and, recently, copy number (CN) of androgen receptor (AR) in plasma DNA. Biologic data suggest an association between choline uptake and AR signaling. We aimed to integrate 18^{F}-fluorocholine (FCH) uptake on positron emission tomography/computed tomography (PET/CT) scanning with plasma *AR* CN and other routinely obtained circulating biomarkers to evaluate their association with outcome.

MATERIALS AND METHODS We determined plasma *AR* CN by digital droplet polymerase chain reaction from 105 mCRPC samples collected before abiraterone (n = 65) or enzalutamide (n = 40) therapy in the before (n = 26) and after (n = 79) chemotherapy settings. Pretreatment serum LDH, CGA, and NLR were also measured. FCH-PET/CT scan was performed at baseline, and maximum standardized uptake value (SUV_{max}), total lesion activity (TLA), and metabolic tumor volume (MTV) were calculated. Main end points were the correlation of FCH-PET/CT parameters with circulating biomarkers and their impact on outcome.

RESULTS Plasma *AR* CN gain was observed in 27 patients (25.7%), and it correlated significantly with higher median SUV_{max}, TLA, and MTV values ($P < .001$). Kaplan-Meier curves showed significantly worse progression-free survival and overall survival in patients with plasma *AR* gain and higher SUV_{max}, TLA, and MTV values ($P < .001$ in each prognostic group). Conversely, no association was reported for prostate-specific antigen response. On multivariable analysis of overall survival, we showed as independent factors *AR* gain (hazard ratio [HR], 1.92; 95% CI, 1.07 to 3.47; $P = .029$), presence of visceral metastasis (HR, 3.04; 95% CI, 1.66 to 5.58; $P = < .001$), LDH (HR, 2.95; 95% CI, 1.72 to 5.05; $P < .001$), NLR (HR, 3.51; 95% CI, 2.14 to 5.74; $P < .001$), serum CGA (HR, 3.36; 95% CI, 1.99 to 5.67; $P < .001$), and MTV (HR, 2.09; 95% CI, 1.25 to 3.50; $P = .005$).

CONCLUSION Our results indicate the potential usefulness of integrating functional imaging with plasma DNA analysis and other noninvasive biomarkers as a tool to improve treatment selection for CRPC. A larger prospective evaluation is warranted.

ASSOCIATED CONTENT

Data Supplement

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

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INTRODUCTION

Prostate cancer is the second most common malignancy among men worldwide.[1,2] The standard treatment for metastatic disease is androgen deprivation therapy,[3] but cancer cells eventually acquire resistance, and castration-resistant prostate cancer (CRPC) develops. It is now widely accepted that the majority of CRPCs are still dependent on the androgen receptor (AR) signaling pathway,[4,5] and new AR pathway inhibitors such as enzalutamide and abiraterone have shown efficacy against CRPC.[6-9]

Biomarkers for personalizing treatment in metastatic CRPC (mCRPC) could improve patient outcomes. Multiple tests have revealed associations with a poorer outcome, including serum lactate dehydrogenase (LDH),[10] visceral metastasis (especially liver involvement[10-12]), chromogranin A (CGA),[13,14] and neutrophil:lymphocyte ratio (NLR).[15,16]

During the past few years, the potential for plasma DNA analysis to improve clinical practice has substantially increased, with real-time molecular characterization that permits patient stratification and thus facilitates treatment decision making. Plasma *AR* gain

CONTEXT

Key Objective

In the context of multiple biomarkers strategy, we assessed the role of plasma DNA analysis in combination with functional imaging and other routinely obtained circulating biomarkers to perform a better prognostication of metastatic castration-resistant prostate cancer (mCRPC) in patients.

Knowledge Generated

Androgen receptor copy number detected in plasma, choline-positron emission tomography parameters, and other clinical factors can be considered as independent predictors of overall survival. Our final parsimonious prognostic model from the multivariable analysis of overall survival permitted classification of patients with mCRPC into three risk groups according to the independent prognostic role of these biomarkers.

Relevance

Our results suggested the potential usefulness of integrating functional imaging with plasma DNA analysis and other noninvasive biomarkers as a tool to improve treatment selection for patients with mCRPC.

is now acknowledged as a therapy-guiding predictive and prognostic biomarker.[17-21,22]

Furthermore, recent biologic data suggest an association between choline uptake and AR copy number (CN).[23] Increased AR protein expression and/or signaling has been indirectly related to the upregulation of choline kinase alpha (CHKA), which can bind directly to the AR ligand-binding domain. CHKA could be considered a sort of chaperone for AR, resulting in the ubiquitination and activation of AR-dependent transcription and displaying different functions involved in tumor growth and progression.[23] The overexpression of CHKA protein in multiple tumors, including prostate cancer, leads to an increased uptake of choline and provides the rationale for using ^{18}F-fluorocholine positron emission tomography/computed tomography (FCH-PET/CT) in this patient setting. In addition, early FCH-PET/CT has been associated with clinical outcome, independently of the decline in prostate-specific antigen (PSA) in patients with mCRPC treated with abiraterone or enzalutamide.[24,25]

Our aim was to integrate functional imaging, represented as choline uptake in FCH-PET/CT, with plasma *AR* status, clinical data, and other routinely obtained circulating biomarkers and to evaluate their association with outcome. We did this by combining multiple biomarker approaches to improve the patient treatment selection and to personalize CRPC treatment by stratifying patients according to a specific prognostic score.

MATERIALS AND METHODS

Patients and Molecular Analyses

Between October 2011 and June 2016, 105 patients with CRPC (65 treated with abiraterone and 40 treated with enzalutamide) were considered evaluable for this study. Twenty-six (24.8%) were chemotherapy naive and 79 (75.2%) had previously undergone chemotherapy. All had a histologically confirmed diagnosis of prostate adenocarcinoma treated with abiraterone or enzalutamide in pre- or postchemotherapy settings. Additional selection criteria are specified in the Data Supplement. The study included patients participating in a protocol approved by the Institutional Review Board of Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy (REC 2192/2013).

Patients were treated with abiraterone 1,000 mg plus prednisone 5 mg twice per day or enzalutamide 160 mg alone once per day. Treatment was continued until there was evidence of disease progression or unacceptable toxicity. The choice of therapy was at the discretion of the treating physician.

Serum PSA was assessed within 1 week of starting treatment and once per month thereafter. In addition, serum LDH, alkaline phosphatase, complete blood count (used to determine NLR), and serum CGA were also measured within 1 week of starting therapy. Radiographic disease was assessed with CT and bone scans at the time of screening and once every 12 weeks during treatment. In this study, FCH-PET/CT was performed after 12 $\pm$ 4 weeks of treatment.

Peripheral blood samples were collected from each patient at baseline and at the first radiologic evaluation. Circulating DNA was extracted from 1 to 2 mL of plasma by using the QIAamp Circulating Nucleic Acid Kit (QIAGEN, Santa Clarita, CA). Total extracted plasma DNA was quantified using the Quant-iT high sensitivity PicoGreen double-stranded DNA Assay Kit (Invitrogen) or by spectrophotometric evaluation (NanoDrop ND-1000; Celbio, Milan, Italy). Given the previously demonstrated strong agreement between targeted next-generation sequencing and digital droplet polymerase chain reaction (ddPCR) for CN quantification,[20] we performed an analysis of *AR* CN using ddPCR on a QX200 ddPCR system (Bio-Rad). For this, we used the *AR* gene and at least two different reference genes (*NSUN3*, *EIF2C1*, or *AP3B1*) and *ZXDB* at Xp11.21 as the control gene[20] (Data Supplement).

PET/CT Imaging

FCH-PET/CT was performed on an integrated PET/CT system, and the images were read sequentially using a Xeleris III workstation. Semiquantitative criteria based on the maximum standardized uptake value (SUV_{max}) and the target:background ratio were used to aid the visual analysis.[26] The metabolic tumor volume (MTV) was calculated as the sum of each 3-dimensional volume of interest. Moreover, for each lesion volume and SUV, the mean was multiplied and then summed to generate the total lesion activity (TLA). MTV is a purely volumetric entity, whereas TLA also takes into account the metabolic activity of the lesion leading to an estimate of tumor activity (Data Supplement).

Statistical Analysis

Data were summarized by frequency for categorical variables and by cutoff using receiver operating characteristic (ROC) curves and median and range for continuous variables. The association between categorical variables was assessed by using the χ^2 test or Fisher's exact test, as appropriate. A comparison between baseline imaging and clinical, laboratory, and molecular features in patients with or without *AR* gain was performed using the median test (Wilcoxon). ROC curve analysis was performed to estimate the best cutoff value of FCH-PET/CT parameters (TLA, MTV, and SUV_{max}) and serum CGA level considered as continuous variables.

Progression-free survival (PFS) was measured as the time between the first day of abiraterone or enzalutamide treatment and the date of disease progression or death (whichever came first) or last tumor evaluation. Overall survival (OS) was defined from the date of the start of therapy until death as a result of any cause or last follow-up visit. Survival curves were traced by the Kaplan-Meier method, and comparisons were based on the log-rank test. Univariable and multivariable Cox regression models were used to investigate potential predictors of PFS and OS and to estimate hazard ratios (HRs) and their 95% CIs. All P values were two-sided, and a $P < .05$ was considered statistically significant. Statistical analyses were performed with SAS 9.4 software (SAS Institute, Cary, NC).

A Weibull multiple regression model was adopted to evaluate the combined impact of molecular, laboratory, and imaging features on survival. From a full model that contained all the variables, we obtained a final parsimonious model by using a backward selection procedure. The prognostic score was based on the final model, which included six variables. Partial scores were obtained by dividing the value of each regression coefficient by the smallest regression coefficient. The total score for a given patient was obtained by summing the patient's appropriate partial scores, and three groups of patients with different median survival probabilities were identified. If the total score was 2.0 or below, the patient was classified in group I, group II required a score between 2.1 and 4.0, and group III required a score of 4.0 or greater. The choice of time and number of groups with different prognoses was made in terms of clinical consideration, such as time at 15 months (group I at 15 months: OS was greater than 70%; group II at 15 months: OS was 30% to 70%; group III at 15 months: OS was less than 30%).

RESULTS

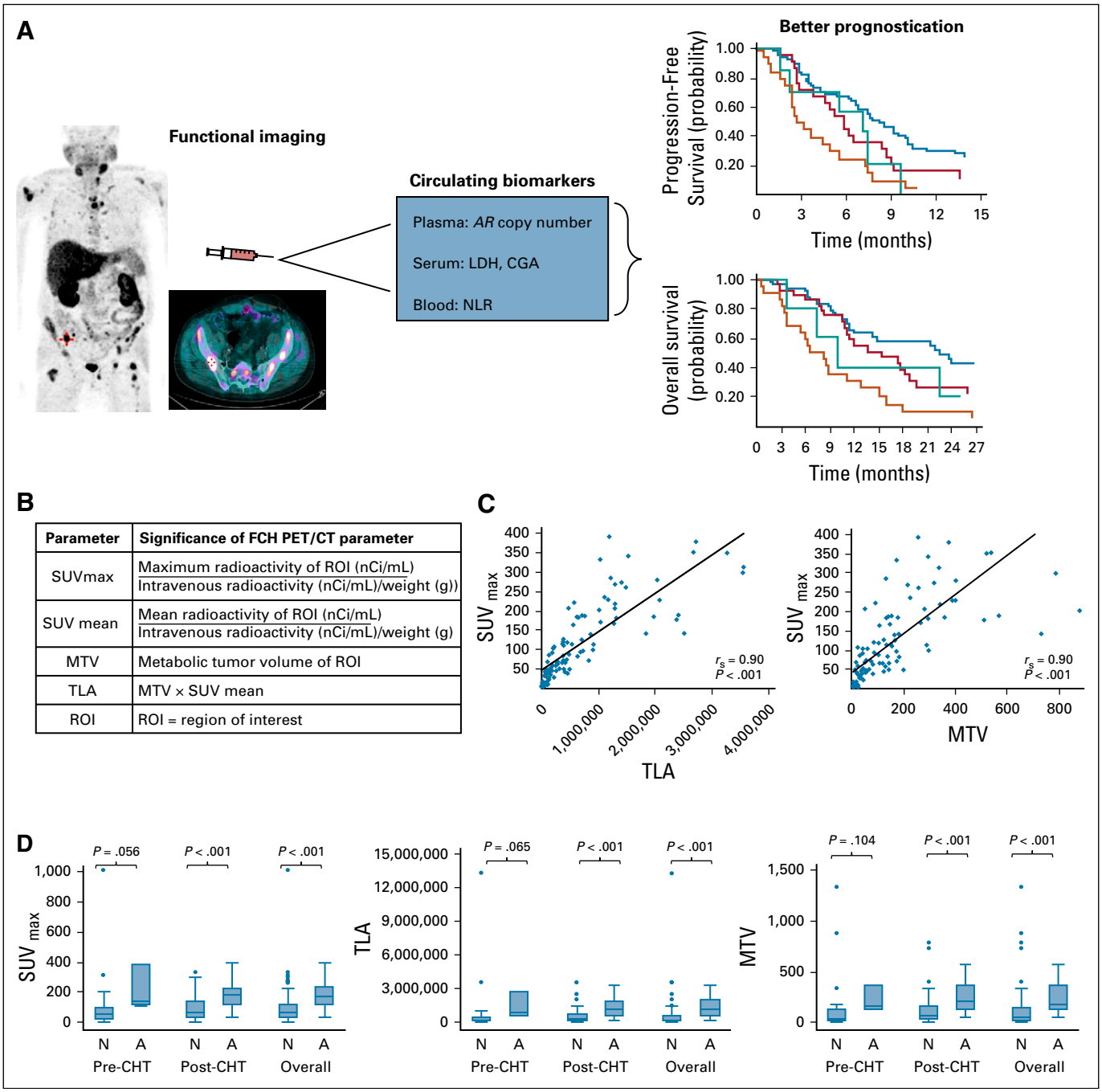
Association Among Plasma *AR* Gain, Baseline Increased Metabolic Activity on PET/CT Scan, and Circulating Routine Biomarkers

We detected *AR* CN gain in plasma from 27 patients with mCRPC (25.7%), three chemotherapy-naive patients (11.5%), and 24 docetaxel-treated patients (30.4%). A summary of overall patient characteristics by *AR* status is available in the Data Supplement. Patients with *AR* gain had significantly higher TLA, MTV, and SUV_{max} values ($P < .001$), number of lesions on FCH-PET/CT scan greater than median value of 12 lesions ($P < .001$), LDH greater than upper normal value of 225 mU/mL ($P = .013$), PSA level greater than median value of 29.3 ng/mL ($P = .004$), and cell-free DNA concentration greater than median value of 38.5 ng/mL ($P < .001$).

Direct Correlation Between Choline Uptake and PET/CT With Molecular Alterations

We assessed all FCH-PET/CT parameters for each patient and correlated them with other circulating prognostic biomarkers (Fig 1A). We identified SUV_{max} as the standard uptake volume, MTV as a pure volumetric entity, and TLA as an indicator of tumor activity (Fig 1B). The reliability of the data are shown in Figure 1C and the Data Supplement. A strong correlation was observed between SUV_{max} and TLA and MTV ($r_s = 0.90$; $P < .001$ for both parameters) and between MTV and TLA in both *AR* normal ($r_s = 0.94$; $P < .001$) and *AR* gain patients ($r_s = 0.85$; $P < .001$). Moreover, the reliability of these data were confirmed, which revealed a particularly evident correlation between TLA and MTV. Figure 1D and the Data Supplement show the median baseline values of TLA, MTV, and SUV_{max} as a function of *AR* CN in the overall population ($P < .001$), revealing only a trend in chemotherapy-naive patients, perhaps because of the small number of patients with *AR* gain.

The ROC curves for the best cutoff value for TLA, MTV, and SUV_{max} were 563,979, 112, and 50, respectively, with 42 (40.0%), 50 (47.6%), and 68 (64.8%) patients showing TLA, MTV, and SUV_{max} values above the cutoff value, respectively. In addition, pretreatment median values for cell-free DNA were also significantly higher in patients with higher TLA, MTV, and SUV_{max} both before and after chemotherapy (Data Supplement).



Parameter	Significance of FCH PET/CT parameter
SUVmax	Maximum radioactivity of ROI (nCi/mL) / Intravenous radioactivity (nCi/mL)/weight (g))
SUV mean	Mean radioactivity of ROI (nCi/mL) / Intravenous radioactivity (nCi/mL)/weight (g)
MTV	Metabolic tumor volume of ROI
TLA	MTV × SUV mean
ROI	ROI = region of interest

FIG 1. Functional imaging in patients with castration-resistant prostate cancer (CRPC). (A) Utility of functional imaging for identifying circulating biomarkers for better prognostication in patients with metastatic CRPC who have been treated with abiraterone or enzalutamide. (B) Description and significance of ^{18}F-fluorocholine positron emission tomography/computed tomography (FCH-PET/CT) parameters. (C) Linear regression (diagonal line) between maximum standardized uptake value (SUVmax), total lesion activity (TLA), and metabolic tumor volume (MTV). (D) Association between choline uptake measured as SUV_{max}, TLA, MTV, and circulating androgen receptor (*AR*) copy number. A box plot is shown with the thick horizontal line depicting the median value of SUVmax, TLA, and MTV in overall, pre-, and postchemotherapy patients. A, *AR* copy number amplified; CGA, chromogranin A; CHT, chemotherapy, LDH, lactate dehydrogenase; N, *AR* copy number normal; nCi, nanoCurie; NLR, neutrophil:lymphocyte ratio.

Ability of Functional Imaging and Plasma *AR* Status to Predict Clinical Outcomes

At the time of the analysis, 90 (85.7%) of the overall 105 patients (15 [57.7%] of 26 before chemotherapy and 75 [92.4%] of 79 patients after chemotherapy) had progressive disease and 82 patients (78.1%) had died (11 [42.3%] before chemotherapy and 71 [88.6%] after chemotherapy). Median follow-up was 44 months (range, 1 to 67 months). The median PFS and OS for the overall population were 6.7 months (95% CI, 5.0 to 7.4 months)

and 15.3 months (95% CI, 11.0 to 19.0 months), respectively. In the prechemotherapy group, the median PFS was 7.2 months (95% CI, 4.2 months to not reached), whereas OS was not reached. In the postchemotherapy group, the median PFS was 6.7 months (95% CI, 4.6 to 7.5 months) and the median OS was 13.9 months (95% CI, 10.8 to 18.3 months).

Kaplan-Meier curves were generated to show PFS and OS in patients with CRPC who were treated with chemotherapy (Fig 2) according to *AR* CN, TLA, and MTV. Chemotherapy-treated patients were divided into four prognostic groups according to baseline *AR* status and TLA, MTV, and SUV_{max} values: *AR* normal, TLA, MTV, and SUV_{max} low; *AR* gain, TLA, MTV, and SUV_{max} low; *AR* normal, TLA, MTV, and SUV_{max} high; and *AR* gain, TLA, MTV, and SUV_{max} high. They were characterized by a significant difference in PFS ($P < .001$ for TLA, $P = .003$ for MTV, and $P < .001$ for SUV_{max}) and OS ($P < .001$ for TLA, $P < .001$ for MTV, and $P < .001$ for SUV_{max}; Data Supplement).

Identification of Imaging, Molecular, and Clinical Features as Independent Predictors of Treatment Outcome

We performed univariable and multivariable Cox proportional hazards regression analysis of OS to evaluate the prognostic impact of different factors. In the univariable analysis, clinical variables such as the presence of visceral metastasis, PSA, alkaline phosphatase, LDH, NLR, serum CGA, previous radical radiotherapy, and the presence of pain were significantly associated with shorter OS in addition to molecular factors (*AR* CN, cell-free DNA concentration [corresponding to double-stranded DNA (dsDNA)] detected in plasma) and FCH-PET/CT features (MTV, TLA, SUV_{max}, and number of lesions; Table 1).

We confirmed these data in the multivariable analysis of OS using a backward stepwise procedure. We showed that *AR* CN (HR, 1.92; 95% CI, 1.07 to 3.47; $P = .029$), the presence of visceral metastasis (HR, 3.04; 95% CI, 1.66 to 5.58; $P < .001$), LDH (HR, 2.95; 95% CI, 1.72 to 5.05; $P < .001$), NLR (HR, 3.51; 95% CI, 2.14 to 5.74; $P < .001$), CGA (HR, 3.36; 95% CI, 1.99 to 5.67; $P < .001$), and MTV (HR, 2.09; 95% CI, 1.25 to 3.50; $P = .005$; Table 2) were significant independent factors. It is possible that the strong correlation among all PET/CT parameters abrogated the statistical significance of TLA and MTV in this analysis.

Combination of Functional Imaging With Circulating Biomarker Analysis to Improve mCRPC Prognostication

Patients were randomly divided into one training set (n = 69) and one validation set (n = 36). The larger two-thirds group of patients was used as a training set to construct the score, and the remaining one third was used as a validation set.

Table 2 shows the partial score value for each category, which was obtained by subdividing each regression coefficient by the smallest one as described in Statistical Analysis. The total score for a given patient was obtained by summing the patient's appropriate partial scores, and three groups of patients with different median survival probabilities were identified. If the total score was 2.0 or lower, the patient was classified in group I, group II required a score between 2.1 and 4.0, and group III required a score of 4.0 or greater (Data Supplement). The choice of time and number of groups with different prognoses was made in terms of clinical consideration, such as time at 15 months (group I, 15 months: OS was greater than 70%; group II, 15 months: OS was 30% to 70%; group III, 15 months: OS was less than 30%). In the training set, 27 patients (39.1%) were attributed to risk group I, 13 (18.8%) to risk group II, and 29 (42.0%) to risk group III.

The survival experience of the three patient groups was identified by the score. Survival probabilities were estimated by the exponential model and by the Kaplan-Meier method ($P < .001$; Data Supplement). In the training set, median OS was significantly different among the three risk groups (risk group I, 31.0 months [95% CI, 21.8 to 39.8 months]; risk group II, 17.6 months [95% CI, 11.4 to 22.5 months]; and risk group III, 7.3 months [95% CI, 6.0 to 9.8 months]; $P < .001$; Fig 3A). Similar results were observed in the validation set (Fig 3B).

Correlation Between Plasma DNA Analysis and Metabolic Response Assessed by FCH-PET/CT

The prognostic role of plasma *AR* gain was strongly evident before starting abiraterone or enzalutamide, whereas no variation in *AR* CN was reported in patients during treatment or upon progression. Figure 4 shows FCH-PET/CT images at baseline and at the first re-evaluation of four patients from different prognostic groups according to *AR* status and TLA, MTV, and SUV_{max}, confirming the impact on survival of the association between baseline *AR* CN and FCH-PET/TC. A change in dsDNA concentration was reported in relation to the type of response to abiraterone or enzalutamide. We also evaluated PSA response in these groups on the basis of pretreatment *AR* CN and choline uptake, and we observed no significant association (Data Supplement).

DISCUSSION

This study examined the feasibility of using functional imaging to assess tumor burden and also to evaluate its predictive and prognostic role in CRPC treated with abiraterone or enzalutamide. We confirmed evidence from preclinical and clinical studies showing that CHKA expression and choline production are androgen regulated and that the relationship between CHKA expression, choline uptake, and *AR* signaling could be used in managing prostate cancer.[23] Within the context of multiple biomarker strategies, including plasma AR, FCH-PET/CT

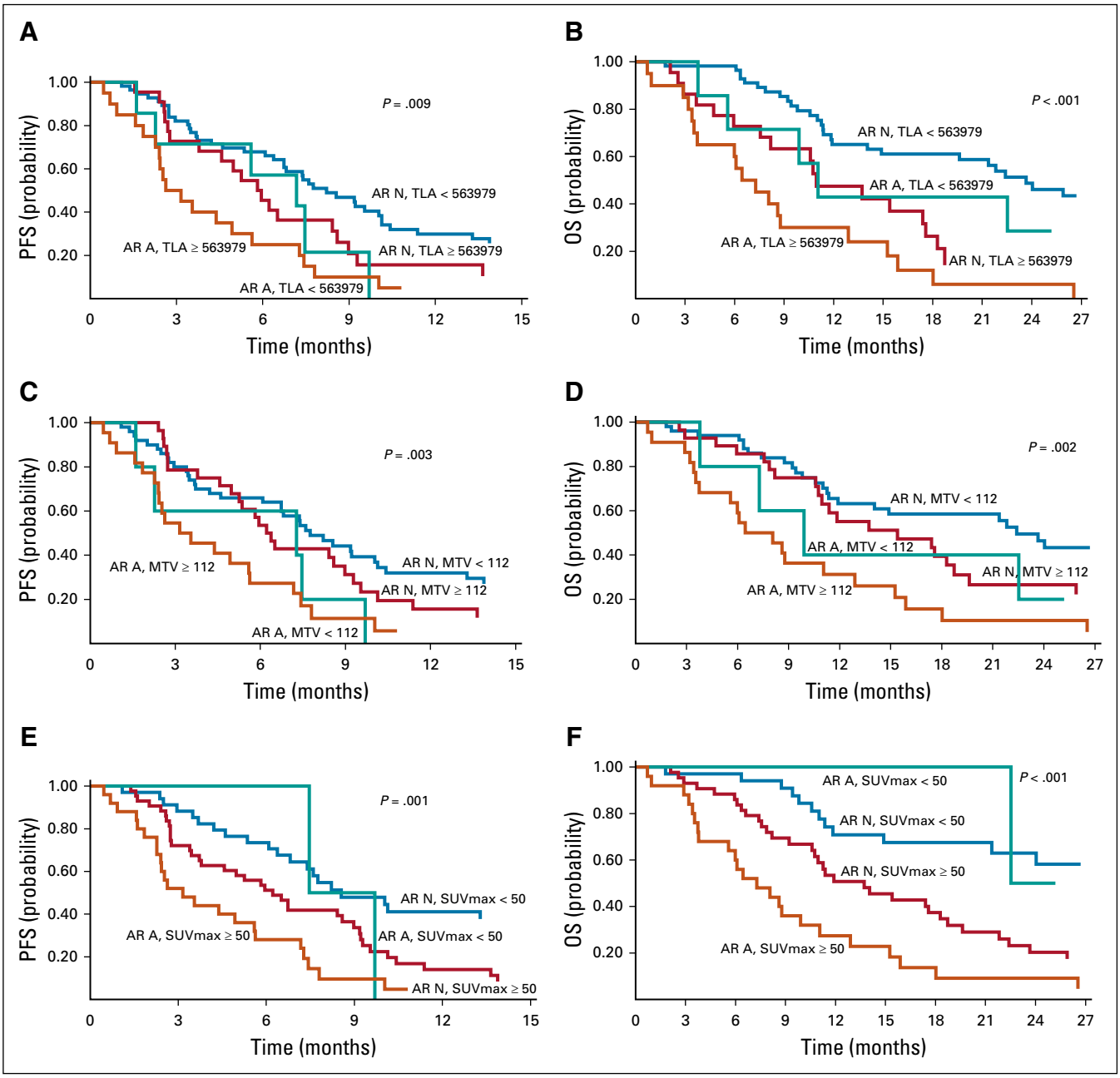


FIG 2. Progression-free survival (PFS) and overall survival (OS) according to copy number (CN) of androgen receptor (AR) and functional imaging in overall population of patients with castration-resistant prostate cancer who were treated with abiraterone or enzalutamide. (A) PFS and (B) OS according to *AR* CN and tumor lesion activity (TLA). (C) PFS and (D) OS according to AR CN and metabolic tumor volume (MTV). (E) PFS and (F) OS according to *AR* CN and maximum standardized uptake value (SUV_{max}). In these Kaplan-Meier curves, baseline *AR* CN was divided into *AR* normal (AR N) and *AR* amplified (AR A). TLA, MTV, and SUV_{max} values were grouped into high and low groups according to cutoff identified by receiver operating characteristic curves. High and low TLA values were 563,979 or greater and less than 563,979, high and low MTV values were 112 or greater and less than 112, and high and low SUV_{max} values were 50 or greater and less than 50.

scans, and other well-established prognostic factors in patients with mCRPC, our results show that molecular functional imaging is capable of assessing disease extent and distribution, and it can also contribute to identifying biologic features of overall lesions from patients with CRPC. In this study, we also showed, to our knowledge for the first time, that combining functional imaging and circulating molecular and laboratory parameters can be used to develop a new prognostic stratification tool for CRPC treated with AR-directed therapies.

Currently, numerous drugs in this setting have shown antitumor activity and often lead to a benefit in survival.

TABLE 1. Univariable Analysis of OS

Variable	No. of Patients	No. of Events	Median OS (months)	95% CI	*P*	HR	95% CI	*P*
Overall	105	82	15.3	11.0 to 19.0	—	—		—
Age, years								
< 74	49	40	12.9	8.7 to 21.8		1.00		
≥ 74	56	42	15.9	11.1 to 22.5	.438	0.84	0.55 to 1.30	.439
Clinical variables								
Gleason score								
6-7	42	30	19.0	11.0 to 26.6		1.00		
8-10	51	42	11.3	7.4 to 17.6	.285	1.30	0.80 to 2.09	.287
Presence of visceral metastasis								
No	84	63	18.7	12.9 to 24.0		1.00		
Yes	21	19	9.8	6.3 to 11.2	< .001	2.84	1.62 to 4.97	< .001
No. of previous lines of therapy								
1-2	69	49	18.7	11.8 to 24.0		1.00		
> 2	36	33	11.0	8.1 to 15.9	.104	1.44	0.93 to 2.25	.106
ECOG PS								
0-1	102	79	15.3	11.0 to 19.6		1.00		
≥ 2	3	3	12.9	11.4 to 17.4	.458	1.55	0.48 to 4.96	.462
Median prostate-specific antigen, ng/mL								
< 29.3	52	38	21.8	14.0 to 28.3		1.00		
≥ 29.3	52	43	11.0	7.6 to 15.3	< .001	2.32	1.45 to 3.72	< .001
Alkaline phosphatase, U/L								
< 129	64	46	18.0	11.1 to 26.5		1.00		
≥ 129	36	32	11.6	6.4 to 17.6	.033	1.63	1.03 to 2.58	.035
Serum lactate dehydrogenase, U/L								
< 225	76	54	18.3	11.9 to 24.0		1.00		
≥ 225	24	24	9.3	6.1 to 12.9	< .001	2.54	1.55 to 4.18	< .001
Neutrophil:lymphocyte ratio								
< 3	56	38	22.5	18.0 to 29.9		1.00		
≥ 3	49	44	7.4	6.0 to 8.8	< .001	3.71	2.35 to 5.88	< .001
Serum chromogranin A, μg/L								

(Continued on following page)

TABLE 1. Univariable Analysis of OS (Continued)

Variable	No. of Patients	No. of Events	Median OS (months)	95% CI	*P*	HR	95% CI	*P*
< 120	49	33	27.7	21.4 to 35.1		1.00		
120-360	30	23	14.9	8.8 to 18.0		3.23	1.75 to 5.95	< .001
> 360	26	26	5.1	3.4 to 6.3	< .001	12.74	6.89 to 23.57	< .001
Hemoglobin, g/dL								
> 12.5	42	33	14.0	11.0 to 21.4		1.00		
≤ 12.5	63	49	15.4	9.8 to 22.5	.914	1.02	0.66 to 1.59	.914
Albumin, g/dL								
> 4	36	26	21.8	11.8 to 29.9		1.00		
≤ 4	50	39	11.6	10.6 to 17.4	.126	1.48	0.89 to 2.44	.128
Time from start of ADT to initiation of abiraterone or enzalutamide, months								
> 36	59	43	13.7	10.6 to 24.0		1.00		
≤ 36	46	39	15.3	9.4 to 19.6	.329	1.24	0.80 to 1.92	.330
Previous prostatectomy								
No	63	51	18.3	11.8 to 23.7		1.00		
Yes	42	31	11.1	7.9 to 17.6	.871	1.04	0.66 to 1.63	.871
Previous radical radiotherapy								
No	61	45	18.7	11.4 to 27.3		1.00		
Yes	44	37	11.1	7.4 to 15.9	.029	1.62	1.05 to 2.52	.030
Presence of pain								
No	96	73	17.4	11.2 to 22.4		1.00		
Yes	9	9	8.2	3.7 to 15.9	.005	2.67	1.31 to 5.43	.007
Molecular variables								
AR copy number								
Normal	78	58	18.7	11.9 to 24.0		1.00		
Gain	27	24	8.1	3.8 to 12.9	< .001	2.64	1.60 to 4.35	< .001
Double-stranded DNA concentration, ng/mL								
< 38.5	52	32	25.9	21.4 to 30.6		1.00		
≥ 38.5	53	50	7.9	6.1 to 10.8	< .001	4.24	2.64 to 6.79	< .001
Functional imaging variables								

(Continued on following page)

TABLE 1. Univariable Analysis of OS (Continued)

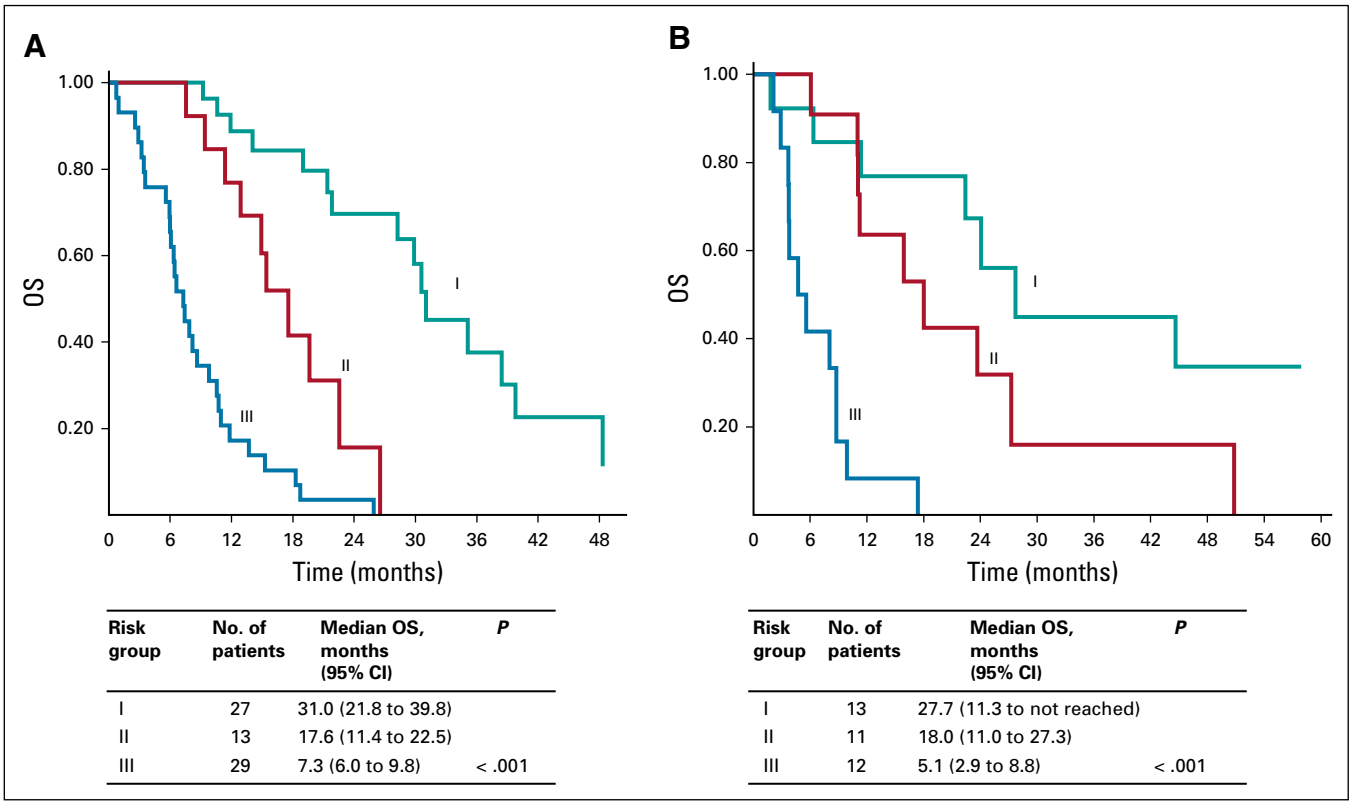
Variable	No. of Patients	No. of Events	Median OS (months)	95% CI	*P*	HR	95% CI	*P*
Metabolic tumor volume								
< 112	55	37	22.5	11.9 to 29.9		1.00		
≥ 112	50	45	11.0	7.9 to 15.4	< .001	2.17	1.39 to 3.38	< .001
SUV_{max}								
< 50	36	20	28.3	21.4 to 48.3		1.00		
≥ 50	68	61	10.9	7.9 to 14.0	< .001	3.28	1.95 to 5.52	< .001
Total lesion activity								
< 563,979	63	43	22.5	14.0 to 29.9		1.00		
≥ 563,979	42	39	8.4	6.0 to 12.9	< .001	3.03	1.93 to 4.78	< .001
Median No. of lesions on FCH-PET/CT								
< 12	52	34	22.5	14.0 to 30.6		1.00		
≥ 12	53	48	10.8	7.6 to 15.4	< .001	2.57	1.63 to 4.04	< .001

Abbreviations: ADT, androgen deprivation therapy; *AR*, androgen receptor; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; FCH-PET/CT, ^{18}F-fluorocholine positron emission tomography/computed tomography; HR, hazard ratio; OS, overall survival; SUV_{max}, maximum standardized uptake value.

TABLE 2. Multivariable Analysis of OS After Backward Stepwise Procedure

	Factor Estimate	SE	*P*	HR	95% CI	Partial Score
AR copy number						
Normal	0.00		—	1.00		0
Gain	0.655	0.300	.029	1.92	1.07 to 3.47	1.0
Presence of visceral metastasis						
No	0.00		—	1.00		0
Yes	1.112	0.310	< .001	3.04	1.66 to 5.58	1.7
LDH, U/L						
< 225	0.00		—	1.00		0
≥ 225	1.081	0.275	< .001	2.95	1.72 to 5.05	1.7
NLR						
< 3	0.00		—	1.00		0
≥ 3	1.255	0.251	< .001	3.51	2.14 to 5.74	1.9
Serum CGA, ng/mL						
< 120	0.00		—	1.00		0
≥ 120	1.211	0.267	< .001	3.36	1.99 to 5.67	1.8
MTV						
< 112	0.00		—	1.00		0
≥ 112	0.740	0.261	.005	2.09	1.25 to 3.50	1.1

Abbreviations: *AR*, androgen receptor; CGA, chromogranin A; HR, hazard ratio; LDH, lactate dehydrogenase; MTV, metabolic tumor volume; NLR, neutrophil:lymphocyte ratio; OS, overall survival; SE, standard error.



Risk group	No. of patients	Median OS, months (95% CI)	P
I	27	31.0 (21.8 to 39.8)	
II	13	17.6 (11.4 to 22.5)	
III	29	7.3 (6.0 to 9.8)	< .001

Risk group	No. of patients	Median OS, months (95% CI)	P
I	13	27.7 (11.3 to not reached)	
II	11	18.0 (11.0 to 27.3)	
III	12	5.1 (2.9 to 8.8)	< .001

FIG 3. Kaplan-Meier curves for overall survival (OS) and relative survival (months) by risk group in the training set (A) and validation set (B). The survival curves were estimated by the final stepwise model of multivariable analysis for patients with metastatic castration-resistant prostate cancer who were treated with abiraterone or enzalutamide. In both treatment groups, survival experience of the three groups of patients was identified by the score.

However, the identification of biomarkers could facilitate personalized next-line systemic therapy in a more accurately selected population. During the past decade, molecular stratification from tissue biopsy has provided several therapeutic targets and prognostic markers, but numerous factors can limit the usefulness of tissue biomarkers (from both primary and secondary tumors). Primary prostate cancer is usually multifocal at the time of diagnosis and often only one tumor clone is responsible for metastasis. This clone may not necessarily be from the largest lesion or the lesion with the highest Gleason grade, as confirmed by the lack of significance of the Gleason score with clinical outcome in our univariable analysis. Moreover, a polyclonal metastasis-to-metastasis seeding may occur during disease progression.[27] Given the complex tumor heterogeneity, assessment of the archival primary tumor or metastatic biopsies for the clinical management of patients with CRPC may not be adequate for or entirely representative of current tumor status. Consequently, alternative approaches, such as blood-based assays and functional imaging may be more feasible because of the possibility of performing serial evaluations during the course of the disease aimed at monitoring tumor dynamics and treatment outcome.

The advantages of using prognostic markers detected by liquid biopsy before starting treatment is now widely acknowledged, especially for *AR* CN which, unlike *AR* mutation status, does not noticeably change during AR-directed treatment.[19] For this reason, we considered circulating biomarkers at baseline and did not include the detection of *AR* mutations, whose frequency is usually too low in pretherapy samples, especially from chemotherapy-naive patients.[19,20]

The significant prognostic impact on treatment outcome through the combination of *AR* status and choline uptake assessed by FCH-PET/CT could lead to the reassessment of prognostic index models for survival that became available over the years in patients with mCRPC treated with standard chemotherapy and hormonal agents.[10,11] These nomograms use readily available clinical and laboratory factors. In this study, we presented functional molecular imaging as a future potential candidate for developing an updated prognostic model for predicting clinical outcome. The lack of association between *AR* CN, TLA, MTV, SUV_{max}, and PSA response was likely a result of the characteristic action mechanisms of abiraterone or enzalutamide, which often lead to an unusual PSA modulation.[28,29]

This study has some limitations, the most significant being the variability in the inclusion of chemotherapy-naive and postdocetaxel patients treated with AR-directed therapies (which may have influenced outcome estimates). In

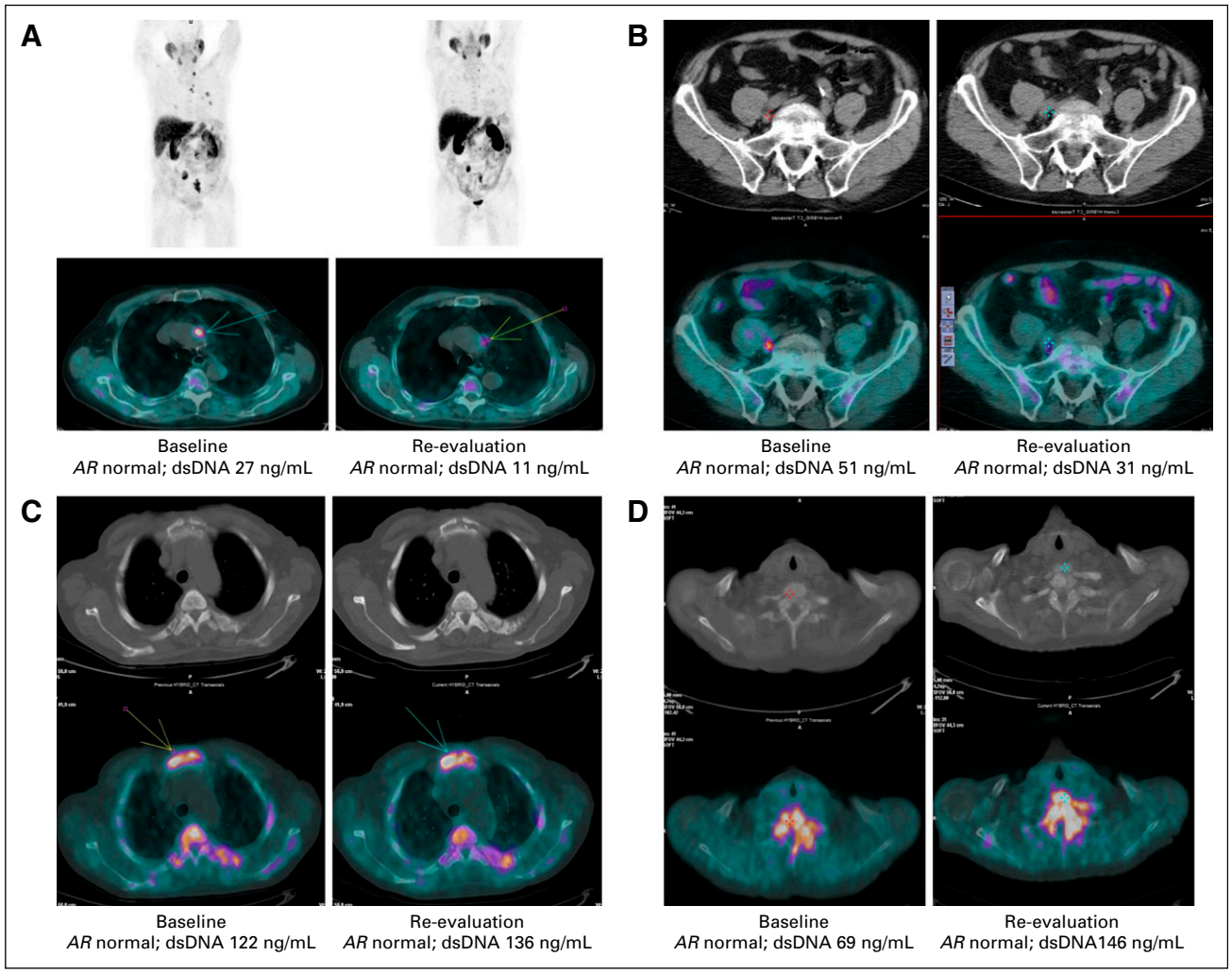


FIG 4. Impact of baseline androgen receptor (*AR*) copy number, cell-free DNA, and choline uptake by ^{18}F-fluorocholine positron emission tomography/computed tomography on clinical outcome in patients with castration-resistant prostate cancer who were treated with abiraterone or enzalutamide. dsDNA, double-stranded DNA. (A) Patient 1; 78 years of age; Gleason score 9 (4+5); 10 sites of nodal localization; baseline *AR* normal; TLA = 290576.3, MTV = 50.26; SUV_{max} = 68.52: treatment with abiraterone postdocetaxel for 7 months. Re-evaluation after 45 days of therapy: early good FCH-PET/CT response in mediastinal and abdominal nodal metastases. (B) Patient 2; 80 years of age; Gleason score 7 (3+4); four sites of nodal localization; baseline *AR* gain; TLA = 23745.7; MTV = 14.67; SUV_{max} = 12.97; treatment with abiraterone postdocetaxel for 30 months. Re-evaluation after 80 days of therapy: partial FCH-PET/CT response. (C) Patient 3; 85 years of age; Gleason score 8 (4+4); 26 sites of bone localization; baseline *AR* normal; TLA = 1292423.8; MTV = 279.85; SUV_{max} = 206.49; treatment with abiraterone postdocetaxel for 12 months. Re-evaluation after 90 days of therapy: stable disease. (D) Patient 4; 87 years of age; Gleason score 8 (4+4); 10 sites of bone localization; baseline *AR* gain; TLA = 599060; MTV = 142; SUV_{max} = 49.25; treatment with enzalutamide postdocetaxel for 5 months. Re-evaluation after 40 days of therapy: progressive disease.

addition, this study was a retrospective single-center work. Additional limitations are the small sample size, especially in the prechemotherapy group, and the presence of PET/CT indices as nonabsolute measures, which can be affected by technical issues such as PET scanner calibration, imaging reconstruction, image acquisition, and timing of tracer administration. Moreover, FCH-PET/CT is still controversial as an imaging tool in clinical practice because of its costs and uncertain diagnostic accuracy. Despite these limitations, our study provided a valuable preliminary insight into the potential usefulness of functional molecular imaging for managing patients with mCRPC. Future biomarker studies will include evaluation of the performance of PET/CT with new promising tracers such as ^{68}Ga-prostate-specific membrane antigen, which recently proved useful for detecting relapse in patients with biochemical recurrence of prostate cancer after radical treatment[30] and as a prognostic factor for survival outcome in CRPC.[31]

Apart from detecting *AR* mutations, we did not explore other circulating *AR* aberrations such as AR splice variant-7 (which is associated with outcome in patients with CRPC treated with abiraterone or enzalutamide[32]) because of the lack of concurrent appropriately collected samples and genomic alterations in DNA defect repair[33]

and neuroendocrine differentiation genes,[34,35] which have recently been shown to drive resistance to androgen suppression.

Another limitation to the interpretation of our data is the clinical utility of cell-free DNA as a prognostic biomarker because of different proportions of tumor DNA constituting the total cell-free DNA concentration (range, 0.01% to 95%).[36] Estimation of tumor content by DNA sequencing and bioinformatic algorithms is warranted in our samples, which could potentially increase the utility of cell-free DNA as a biomarker that, for this reason, we did not consider in our multivariable analysis,[19,21] and as a reliable complementary approach for disease monitoring.

In conclusion, our study integrated the innovative biomarker approaches of functional imaging and genomics, which contributed to better prognostication, as recently seen for patients with metastatic melanoma[37] and colorectal cancer.[38] Combining different predictive and prognostic biomarker strategies could help to overcome the inter- and intrapatient heterogeneity of CRPC, thus improving patient risk stratification and treatment selection. A larger prospective evaluation is warranted.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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