original report

Plasma Androgen Receptor Copy Number Status at Emergence of Metastatic Castration-Resistant Prostate Cancer: A Pooled Multicohort Analysis

Anuradha Jayaram, MD[1]; Anna Wingate, MSc[1]; Daniel Wetterskog, PhD[1]; Vincenza Conteduca, MD, PhD[2]; Daniel Khalaf, MD[3]; Mansour Taghavi Azar Sharabiani, PhD[4]; Fabio Calabrò, MD[5]; Lorraine Barwell, MD[6]; Susan Feyerabend, MD[7]; Enrique Grande, MD[8]; Alberto Martinez-Carrasco, MsC[9]; Albert Font, MD, PhD[10]; Alfredo Berruti, MD[11]; Cora N. Sternberg, MD[12]; Rob Jones, MA, MD, PhD[6]; Florence Lefresne, MD[13]; Marjolein Lahaye, MSc[13]; Shibu Thomas, PhD[14]; Shilpy Joshi, PhD[15]; Dong Shen, MD, PhD[14]; Deborah Ricci, PhD[14]; Michael Gormley, PhD[14]; Axel S. Merseburger, MD[16]; Bertrand Tombal, MD, PhD[17]; Matti Annala, MSc[3,18]; Kim N. Chi, MD[3,19]; Ugo De Giorgi, MD, PhD[2]; Enrique Gonzalez-Billalabeitia, MD, PhD[9]; Alexander W. Wyatt, MD, PhD[3]; and Gerhardt Attard, MD, PhD[1]

abstract

PURPOSE Increases in androgen receptor (*AR*) copy number (CN) can be detected in plasma DNA when patients develop metastatic castration-resistant prostate cancer. We aim to evaluate the association between *AR* CN as a continuous variable and clinical outcome.

PATIENTS AND METHODS PCR2023 was an international, multi-institution, open-label, phase II study of abiraterone acetate plus prednisolone (AAP) or abiraterone acetate plus dexamethasone that included plasma *AR* assessment as a predefined exploratory secondary end point. Plasma *AR* CN data (ClinicalTrials.gov identifier: NCT01867710) from this study (n = 133) were pooled with data from the following three other cohorts: cohort A, which was treated with either AAP or enzalutamide (n = 73); the PREMIERE trial (ClinicalTrials.gov identifier: NCT02288936) of biomarkers for enzalutamide (n = 94); and a phase II trial from British Columbia (ClinicalTrials.gov identifier: NCT02125357) that randomly assigned men to either AAP or enzalutamide (n = 201). The primary outcome measures for the biomarker analysis were overall survival and progression-free survival.

RESULTS Using multivariable fractional polynomials analysis using Cox regression models, a nonlinear relationship between plasma *AR* CN and outcome was identified for overall survival, where initially for small incremental gains in CN there was a large added hazard ratio that plateaued at higher CN. The CN cut point associated with the highest local hazard ratio was 1.92. A similar nonlinear association was observed with progression-free survival. In an exploratory analysis of PCR2023, the time from start of long-term androgen-deprivation therapy to start of AAP or abiraterone acetate plus dexamethasone was significantly shorter in patients with plasma *AR* CN of 1.92 or greater than patients with plasma *AR* CN of less than 1.92 (43 *v* 130 weeks, respectively; $P = .005$). This was confirmed in cohort A ($P = .003$), the PREMIERE cohort ($P = .03$), and the British Colombia cohort ($P = .003$).

CONCLUSION Patients with metastatic castration-resistant prostate cancer can be dichotomized by a plasma *AR* CN cut point of 1.92. Plasma *AR* CN value of 1.92 or greater identifies aggressive disease that is poorly responsive to AR targeting and is associated with a prior short response to primary androgen-deprivation therapy.

ASSOCIATED CONTENT

Appendix

Data Supplement

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

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INTRODUCTION

Standard of care treatment at development of metastatic castration-resistant prostate cancer (mCRPC) is inhibition of androgen receptor (*AR*) signaling with either abiraterone acetate administered with prednisone (AAP; 5 mg twice a day) or enzalutamide. The duration of benefit is variable, with some patients experiencing rapid progression and some responding for several years.[1,2] There is a clinical need to develop biomarker strategies to stratify patients at emergence of mCRPC on the basis of their predicted benefit from AR targeting.

Obtaining metastatic tumor biopsies is challenging in clinical practice because most mCRPC is restricted to bone, especially in men with lower volume disease. Studies across multiple tumor types have demonstrated potential clinical utility of plasma tumor DNA for identifying somatic genomic aberrations (rearrangements, mutations, or copy number [CN]) relating to resistance in the metastatic setting, especially at key clinical decision points.[3-6]

AR gene aberrations are rare before treatment with primary androgen-deprivation therapy (ADT) but progressively increase in prevalence in patients with

CONTEXT

Key Objective

Several studies have assessed plasma DNA, including the androgen receptor (*AR*), as a biomarker for patient stratification. Variable plasma *AR* copy number (CN) parameters have been used, making it challenging to interpret differences in associations with outcome observed across multiple analyses. We aimed to define the most appropriate framework for outcome-based stratification using plasma *AR* CN in patients with metastatic castration-resistant prostate cancer starting therapy with abiraterone acetate or enzalutamide.

Knowledge Generated

This pooled analysis demonstrates a nonlinear relationship of plasma *AR* CN with outcome, where an *AR* CN cut point of 1.92 or greater has the strongest association with shorter progression-free survival and overall survival on AR-targeting therapies as well as a shorter duration of benefit from prior primary androgen-deprivation therapy.

Relevance

A fixed cut point for plasma *AR* CN of 1.92 is a pragmatic way to clinically implement *AR* assessment as a liquid biomarker for metastatic castration-resistant prostate cancer.

mCRPC.[7] *AR* CN amplification in prostate cancer cells is associated with increased *AR* expression[8] and has a wide variable continuous range, with some mCRPC tumors harboring an average of 25 copies.[9] It has been proposed that plasma *AR* gain or mutations are associated with worse outcomes with second-generation AR-targeting agents.[10-12] However, it is unclear how this biomarker performs because the presence or absence of *AR* CN gain is likely insufficient to robustly predict benefit from AR-targeting agents. We aimed to evaluate the association between *AR* CN as a continuous variable and clinical outcome. For this evaluation, we performed a pooled analysis of *AR* CN in chemotherapy-naïve patients with mCRPC receiving abiraterone acetate or enzalutamide in four independent clinical studies that included biomarker evaluation as a secondary objective.

PATIENTS AND METHODS

Participants and Study Design

This was a pooled analysis of four cohorts. The PCR2023 cohort (ClinicalTrials.gov identifier: NCT01867710) was an international, multi-institutional, open-label, parallel-arm, phase II study of abiraterone acetate in asymptomatic or minimally symptomatic, chemotherapy-naïve patients with mCRPC.[13] Patients were randomly assigned 1:1:1:1 to abiraterone acetate and one of four different glucocorticoid regimens (prednisone 5 mg twice a day or once a day or 2.5 mg twice a day or dexamethasone 0.5 mg once a day) to evaluate tolerability. The trial was not designed to detect differences in clinical outcome between corticosteroid doses, so for the purposes of biomarker analysis, all patients were grouped together. Clinical outcome data were obtained after completion of the main study and extension protocols, with a closure date of June 5, 2018.

The other three cohorts have been described previously (ClinicalTrials.gov identifier: NCT02288936).[10,12] Cohort A is a subset of patients that included patients treated with either abiraterone acetate (in combination with prednisone 5 mg twice a day) or enzalutamide and recruited to biomarker protocols in the Royal Marsden (London, United Kingdom; Protocol No. REC 04/Q0801/6) or the Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (Meldola, Italy; Protocol No. REC 2192/2013).[10] The PREMIERE trial (European Union Clinical Trial Register identifier: 2014-003192-28; ClinicalTrials.gov identifier: NCT02288936) was sponsored and conducted by the Spanish Oncology Genitourinary Group and was a biomarker-driven study in men treated with enzalutamide. The final cohort, the British Columbia (BC) cohort, was from a randomized phase II trial of AAP versus enzalutamide (ClinicalTrials.gov identifier: NCT02125357) that was sponsored and conducted by the BC Cancer Agency.[12]

For the biomarker analyses from all four studies, only patients with histologically confirmed prostate adenocarcinoma and no neuroendocrine differentiation; progressive disease despite castrate levels of serum testosterone (< 50 ng/dL); ongoing medical or prior surgical castration; and no prior treatment with chemotherapy, AAP, or enzalutamide were included. Specific selection criteria by cohort are specified in the Data Supplement. All four studies obtained institutional review board and ethics committee approval and were conducted in accordance with the Declaration of Helsinki and the Good Clinical Practice Guidelines of the International Conference on Harmonization. All patients provided written informed consent for the biomarker research.

Procedures

Plasma was collected 30 days before starting treatment (Data Supplement). DNA from PCR2023, cohort A, and PREMIERE was analyzed by droplet digital polymerase chain reaction (ddPCR), as described previously[10] (Data Supplement). In PCR2023 and cohort A, *AR* CN was also measured using two different targeted next-generation

sequencing (NGS) approaches (Data Supplement). A high agreement was observed between ddPCR and targeted NGS (Data Supplement). *AR* CN calls from ddPCR were used for the analyses in PCR2023 and Cohort A. *AR* CN in the BC cohort was estimated using targeted capture NGS, as described previously.[12]

In PCR2023, serum prostate-specific antigen (PSA) was assessed at screening, at cycle 1, every month for the first 6 months, and every 3 months thereafter. PSA levels in cohort A and the PREMIERE and BC cohorts were measured as previously described.[10] Disease was evaluated radiographically using computed tomography scans of the chest,

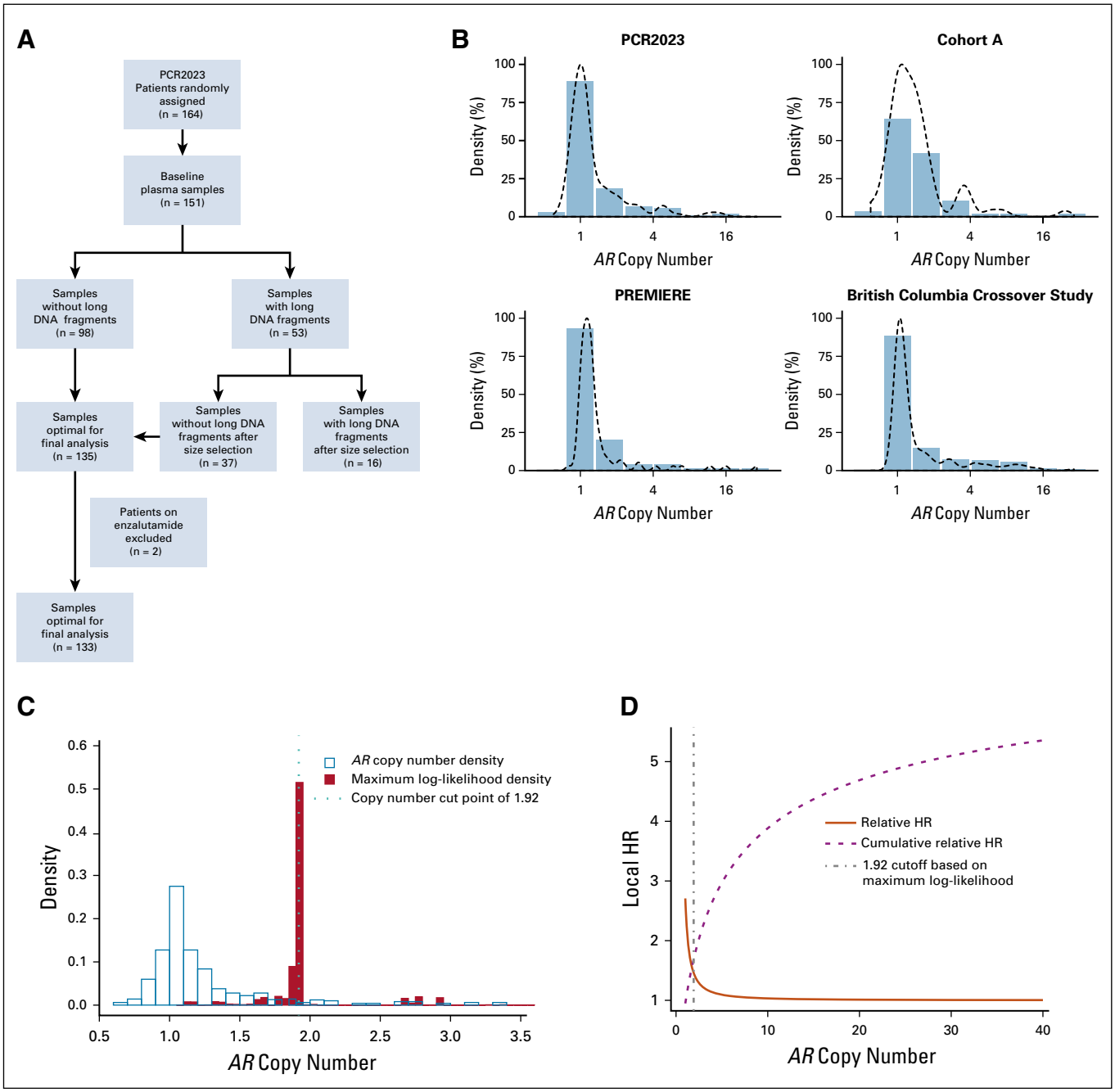

FIG 1. Clinical cohorts, markers of tumor volume, and association with plasma androgen receptor (*AR*) status. (A) Flow diagram of the patient selection for biomarker evaluation in PCR2023. (B) *AR* copy number (CN) distribution across the four clinical cohorts (PCR2023, cohort A, PREMIERE, and British Colombia crossover study) included in the pooled analysis (N = 501). Multivariable fractional polynomials analysis was performed using Cox regression models on data pooled from four cohorts including age and CN and stratified by trial. (C) Distributions of *AR* CN versus maximum log-likelihood statistics. Maximum log-likelihood statistics were used as a correlative measure to identify the optimal cut point of *AR* CN in association with overall survival outcome. (D) Visual inspection showed that the cut point region overlaps with the turning point of decreasing local hazard ratio (HR) identified via the maximum log-likelihood statistics approach.

abdomen, and pelvis and technetium whole-body bone scans at the time of screening and every 12 weeks on treatment. Serum lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) were measured at screening or on day 1 of cycle 1.

Outcomes

Plasma samples were collected prospectively in all four studies with the primary aim of studying associations between plasma DNA aberrations and clinical outcome as secondary exploratory objectives. This analysis evaluating plasma *AR* CN was defined after sample collection. The primary end point for this biomarker analysis in all four cohorts was overall survival (OS). For full definitions of outcome measures, see the Data Supplement.

For exploratory analysis of the association between *AR* status at development of mCRPC and time on ADT, the outcome measure of time from start of uninterrupted ADT to start of AAP, abiraterone acetate plus dexamethasone (AAD), or enzalutamide was used. Patients on combined androgen blockade and antiandrogens were not excluded.

Statistical Analyses

Multivariable fractional polynomials (MFP) analysis using Cox regression models was performed using STATA/SE version 15.1 (STATA, College Station, TX) to include ALP, LDH, PSA, age, and CN stratified by trial.[14] To address *AR* CN as a continuous variable, maximum log-likelihood statistics were used as a correlative measure along with bootstrapping and cross-validation. Respective CN cut points were superimposed over local hazard ratios (HRs). Local HRs were obtained by exponentiating the differences between log-hazard functions of x (for a given CN) and $x + 1$. This was determined using RStudio (https://www.rstudio.com/). Time-to-event outcomes were evaluated using Kaplan-Meier survivor estimates and the log-rank test. The association of clinically relevant baseline factors (previously shown to be associated with prognosis[15-17] for OS and progression-free survival [PFS]) was examined using univariable and multivariable Cox regression models that were performed with stepwise variable selection to identify the prognostic factors for OS and PFS. Odds ratios of PSA response at 12 weeks from baseline were determined using a 2×2 contingency table, and significant differences were determined using Fisher's exact test. The time on ADT by *AR* status at mCRPC was compared using the Mann-Whitney *U* test. All tests were two sided, and an α error of 5% was considered significant.

RESULTS

Clinical Cohorts and Plasma *AR* Status

Between June 2013 and October 2014, 164 patients were recruited onto the PCR2023 study at 22 centers in five countries. Of the 164 intent-to-treat patients, 151 patients

TABLE 1. Baseline Patient Characteristics Across the Four Clinical Trial Cohorts

Characteristic	PCR2023 Cohort	Cohort A	PREMIERE Cohort	British Columbia Cohort
No. of patients	133	73	94	201
Treatment	Abiraterone acetate plus prednisone or dexamethasone	Abiraterone acetate or enzalutamide	Enzalutamide	Abiraterone acetate or enzalutamide
Randomization	Yes	No	No	Yes
Median follow-up time, months	46	39.16	32	14
PFS, months	33.08	NA	33.15	7.5
OS, months	46.03	28.02	38.34	20.3
Median age, years (range)	70 (53-88)	73 (56-91)	77 (57-95)	75 (49-94)
Median pretreatment PSA, mg/L (range)	50.75 (0.67-1,537)	32 (1.4-1,555)	24.95 (1.99-4,319)	36.1 (1.7-2,817)
Median pretreatment ALP, ULN (range)	0.81 (0.19-8.95)	0.68 (0.34-4.12)	0.72 (0.24-17.46)	0.81 (0.29-47.8)
Median pretreatment LDH, ULN (range)	0.88 (0.44-4.52)	0.83 (0.4-4.74)	0.84 (0.29-3.36)	0.79 (0.31-12.9)
Bone-only metastases, no. (%)	106 (79.6)	44 (60.3)	66 (70.2)	157 (78)
Bone and visceral metastases, No. (%)	33 (2.3)	2 (2.7)	1,212 (12.8)	39 (19.4)
Visceral-only metastases, No. (%)	33 (2.3)	1 (0.8)	4 (4.3)	22 (11)

Abbreviations: ALP, alkaline phosphatase; LDH, lactate dehydrogenase; NA, not applicable; OS, overall survival; PFS, progression-free survival; PSA, prostate-specific antigen; ULN, upper limit of normal.

consented to the optional biomarker study for the predefined exploratory secondary analysis and donated plasma before start of treatment. In total, 133 patients were available for biomarker analysis. Patient and treatment characteristics of this biomarker population at the time of sample collection are provided in Figure 1A and Appendix Table A1. The median follow-up time for assessment of PFS and OS was 46.03 months. Median radiographic PFS (rPFS) and OS times were 18.66 months (range, 0.5 to 56.64 months) and 40.34 months (range, 0.5 to 56.77 months), respectively.

We included *AR* CN data from 501 patients, including new data from the PCR2023 cohort described earlier (n = 133); cohort A, as described previously[10] (n = 73); the PREMIERE trial (n = 94); and the BC trial[12] (n = 201). The four cohorts have relatively similar eligibility criteria and clinical characteristics (Table 1 and Data Supplement). *AR* CN distribution for the four cohorts followed a right-skewed distribution (Fig 1B). The median values of *AR* CN for PCR2023, cohort A, PREMIERE, and the BC cohort were 1.06, 1.23,1.17, and 1.09, respectively; the range across all four cohorts was 0.6 to 28.8 (Appendix Table A1).

Multitrial and Multivariable Assessment Identifies Nonlinear Relationship Between *AR* CN and Outcome

To evaluate the relationship between *AR* CN as a continuous distribution and OS, we performed an MFP analysis using Cox regression models including age, ALP, LDH, and PSA and stratified by trial. At lower levels of *AR* CN, we found that small incremental increases in CN were associated with large increases to the local HR. However, the added HR was not constant, and the additional impact of

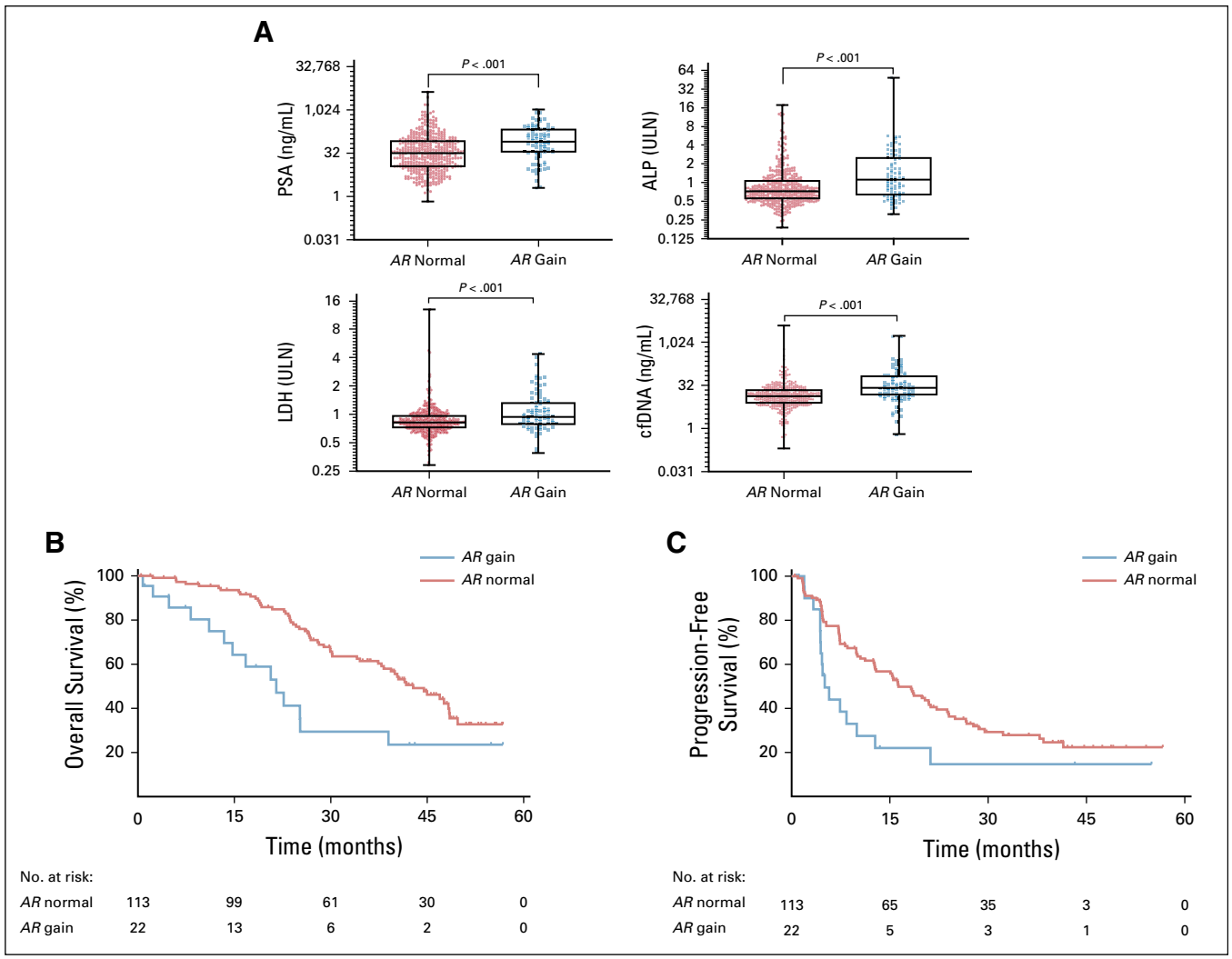

FIG 2. Plasma androgen receptor (*AR*) copy number (CN) and outcome. (A) Association between estimated plasma *AR* CN status and four circulating clinical indices of tumor volume markers. Groups were split into plasma *AR* CN normal or plasma *AR* CN gain. Cell-free DNA (cfDNA) yield, prostate-specific antigen (PSA), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) levels are reported; ALP and LDH levels are reported relative to upper limit of normal (ULN) The association between outcome and patients dichotomized by a plasma *AR* CN of 1.92 or greater in PCR2023 is included as an example, showing (C) overall survival and (D) progression-free survival for patients with normal *AR* CN and *AR* CN gain.

increasing *AR* CN decreased at higher CNs. Using maximum log-likelihood statistics along with bootstrapping and cross-validation, we identified an *AR* CN of 1.92 as the most optimal cut point associated with poor outcome for OS (Fig 1C). Visual inspection showed that the cut point region overlapped with the turning point of decreasing local HR identified via the maximum log-likelihood statistics approach.[14] At increasing CNs, the decreasing trend became so prominent that it reached a plateau close to an HR of 1 (Fig 1D). A similar effect on local HR was seen for PFS (Data Supplement). Using the defined cut-off, we found a statistically significant correlation between plasma *AR* CN and clinical indices of tumor volume, namely, serum LDH, ALP, PSA, and total circulating DNA yield (Mann-Whitney *U* test, $P \leq .001$ for all four parameters; Fig 2A).

Plasma *AR* CN Is Independently Associated With Poor Clinical Outcomes

We constructed a multivariable model to evaluate the association between OS and PFS and five clinical prognostic factors (LDH, ALP, PSA, age, and disease site) and plasma *AR* CN (gain $\geq$ 1.92) or normal CN as covariates for the PCR2023 cohort. The clinical prognostic factors selected have been previously demonstrated to be strong independent predictors of OS and PFS for AR-targeted therapies.[15,17-20] Using stepwise backward elimination, plasma *AR* gain; high ALP, LDH, and PSA; and the presence of bone and visceral metastases were all independently associated with poorer OS (Table 2). Similarly, in the multivariable model for PFS, plasma *AR* gain and high LDH and ALP remained independently significantly associated with PFS (Appendix Table A2).

On an individual cohort level, the HRs for *AR* CN gain versus normal CN are listed in Appendix Table A3. Including the PCR2023 cohort as an example because it has not been reported as a stand-alone study previously, 22 patients (16.5%) were categorized as having *AR* CN gain (Appendix Table A4). Patients harboring *AR* gain had a significantly shorter OS when compared with *AR* normal patients (median OS, 21.52 *v* 42.81 months, respectively; HR, 2.37; 95% CI, 1.07 to 5.25; $P \leq .001$; Fig 2C). We also observed significantly shorter PFS and rPFS in the patients with *AR* gain compared with those who were *AR* normal (median PFS, 5.1 *v* 16.3 months, respectively; HR, 1.94; 95% CI, 0.897 to 3.87; $P = .01$; and median rPFS, 7.4 *v* 21.2 months, respectively; HR, 2.0; 95% CI, 0.97 to 4.405; $P = .005$; Fig 2D; Data Supplement). PSA decline of 50% or greater at 12 weeks was not significantly different for plasma *AR* normal patients versus patients with *AR* gain (odds ratio, 1.78; 95% CI, 0.63 to 4.71; $P = .29$; Data Supplement).

TABLE 2. Multivariable Cox Proportional Hazards Analysis of Predictors for Overall Survival for PCR2023 Cohort

	Overall Survival		
Variable	**HR**	**95% CI**	***P***
AR gain (yes *v* no)	3.1	2.2 to 4.3	< .001
Pretreatment ALP/ULN (continuous variable)	1.1	1.1 to 1.2	< .001
Pretreatment LDH/ULN (continuous variable)	1.6	1.4 to 1.9	< .001
Disease site: bone and visceral metastasis	3.4	1.9 to 6.0	< .001
Pretreatment PSA (continuous variable)	1	1 to 1	< .001

Abbreviations: ALP, alkaline phosphatase; *AR*, androgen receptor; HR, hazard ratio; LDH, lactate dehydrogenase; PSA, prostate-specific antigen; ULN, upper limit of normal.

Plasma *AR* Status at Development of mCRPC and Duration of Benefit From ADT

Finally, we performed an exploratory analysis in the PCR2023 cohort evaluating the time from initiation of continuous long-term ADT to start of AA. Start dates of primary ADT were available for 123 patients (92.4%), and all patients were treated with a first-generation antiandrogen before AAP or AAD. The median time on ADT for this population was 128.3 weeks, and disease stage at diagnosis was available for 89 of 123 patients with ADT data. Of these 89 patients, 53 (59.6%) were stage M0 at diagnosis. We observed that *AR* CN gain (CN $\geq$ 1.92) in pretreatment AAP or AAD samples was associated with a significantly shorter time on ADT compared normal *AR* CN (median, 43.1 *v* 130.2 weeks, respectively; $P = .005$; Fig 3). We repeated this analysis independently in each of the three cohorts included in the pooled *AR* CN analysis. Data were available for 52 (70%) of 74 patients from cohort A, all the PREMIERE patients, and 105 (52%) of 201 patients in the BC cohort. For cohort A and the PREMIERE cohort, 69.2% (36 of 52 patients) and 60% (56 of 94 patients) of patients were stage M0 at diagnosis, respectively, whereas in the BC cohort, only men who had metastatic disease detected on computed tomography of the chest, abdomen, and pelvis or whole-body bone scan at start of continuous ADT were included. The median times from start of ADT to second-generation hormone treatment of cohort A and the PREMIERE and BC cohorts were 91, 165, and 64 weeks, respectively. This was significantly shorter for patients with plasma *AR* gain compared with *AR* normal patients in all three cohorts (cohort A: median, 41 *v* 98 weeks, respectively; $P = .0026$; PREMIERE trial: median, 99 *v* 198 weeks, respectively; $P = .03$; and BC cohort: median, 41 *v* 74 weeks, respectively; $P = .003$; Mann-Whitney *U* test; Fig 3).

DISCUSSION

Our pooled analysis confirms a nonlinear relationship between *AR* CN and outcome, with an incremental increase in HR to a CN value of 1.92 that then becomes progressively less at higher values. This supports the use of a fixed cut point as a pragmatic way to clinically implement *AR* CN as a liquid biopsy biomarker to dichotomize patients into

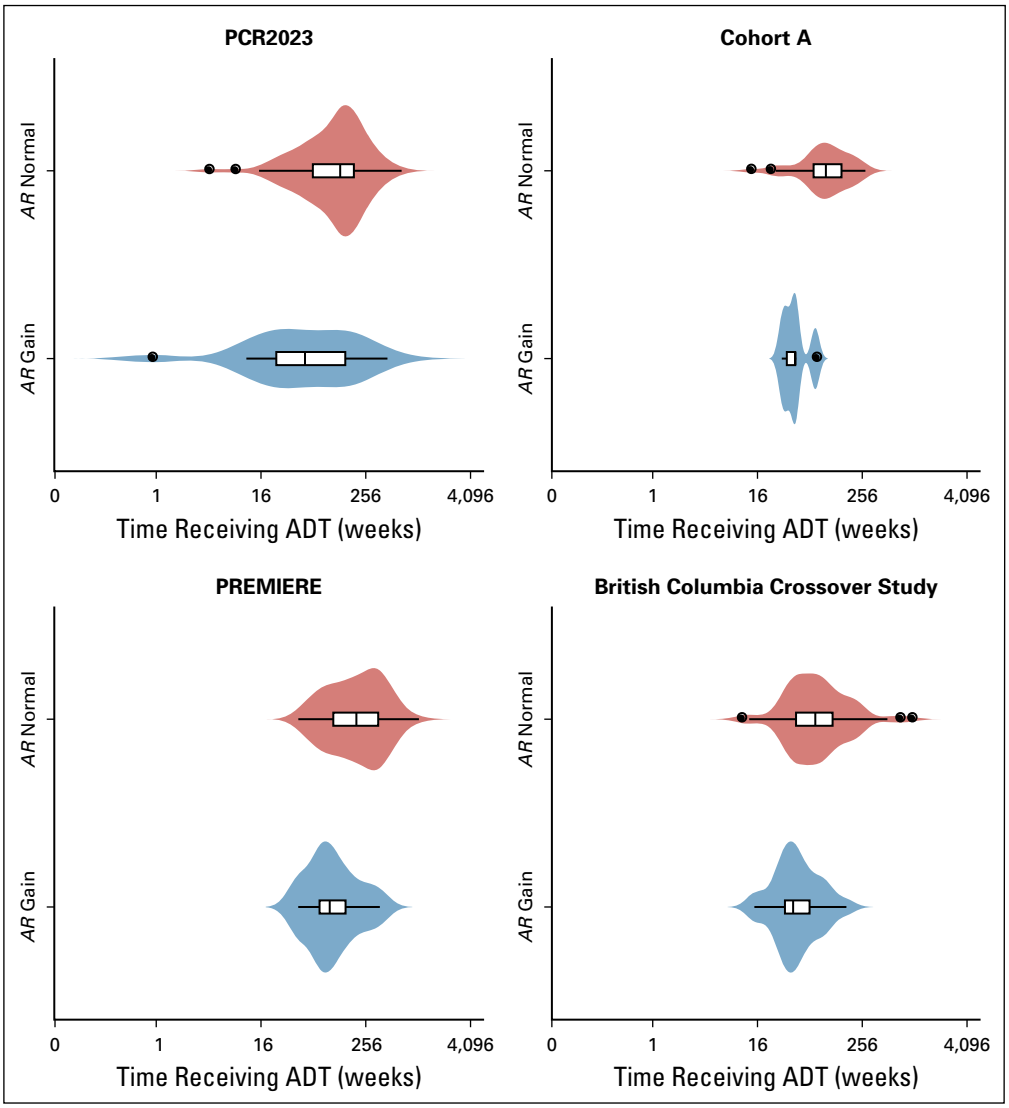

FIG 3. Time from start of androgen-deprivation therapy (ADT) to start of abiraterone acetate or enzalutamide divided by androgen receptor (*AR*) copy number (CN) of 1.92 or greater (*AR* CN gain) or *AR* CN of less than 1.92 (*AR* CN normal). *AR* CN normal is demonstrated by the blue violin plots, whereas *AR* CN gain is demonstrated by the light red violin plots. Box plots within the violin plots indicate the median and the upper and lower quartiles, with whiskers extending from the shortest to longest time on ADT. The *x*-axis (weeks) is scaled as $\log_2$.

prognostically distinct groups. Importantly, this analysis identifies a cut point higher than would be expected using the technical limits for defining gain that have been used in most previous studies.[10,12,21,22] Using the technical limits would result in misclassification of patients with a better prognosis who have an *AR* CN between approximately 1.3 and 1.92. Furthermore, multivariable analyses suggest that plasma *AR* provides information independent from well-established poor prognostic biomarkers and, as a result of the underlying biology, could provide predictive information. The plasma *AR* CN cut point generated from this analysis incorporates data from both targeted NGS and ddPCR because we have demonstrated a strong agreement between these two techniques; thus, this study robustly confirms the applicability of this cut point regardless of methodology undertaken to obtain *AR* CN. In addition, to account for differences in frequency of PSA and imaging measurements, end points, follow-up times, and treatment, the MFP was stratified by trial. Despite the clinical heterogeneity of the cohorts, the association between plasma *AR* CN and outcome was seen across all of the cohorts.

The wide variability in the median time on ADT in the four cohorts is likely attributed to the differences in the proportion of patients with metastatic disease at diagnosis. Importantly, we nonetheless observed a shorter time on primary ADT for men who had plasma *AR* CN gain at development of mCRPC. This finding may offer a biologic explanation for the observation of a lower response rate to

second-generation AR-targeting agents when the response to ADT was shorter than 12 months.[23-25]

To appropriately interrogate our main aim, we focused our genomic analysis solely on *AR*. Future studies could integrate *AR* CN dichotomization with other molecular markers that have been suggested to be associated with worse outcome, including aberrations of TP53[12,21] or WNT signaling[26] or AR splice variant detection.[27,28] These studies would require larger cohorts of patients to allow multiple testing. ARV-7 status may be more challenging to detect in lower volume patients similar to the population in our study as a result of the rarity of circulating tumor cells.[27] In addition, we did not include *AR* somatic point mutations because they are rarely detected (at allelic frequencies > 0.01) in this population.[10,12]

We used *AR* CN values not controlled for circulating tumor DNA (ctDNA) fraction. *AR* CN has a wide range in a patient and is heterogenous across metastatic clones.[8,12,21,29] In this disease setting, low-volume disease often precludes biopsy of multiple metastases, and it is not feasible to derive tumor biopsy CN values for comparison. Plasma *AR* is a representation of the overall CN with putatively varying contributions from individual clones. We have hypothesized that correcting *AR* CN on the basis of total circulating tumor fraction would not be reflective of true *AR* CN in individual clones. Accepting this limitation of plasma tumor DNA assessment, we propose to use actual *AR* CN values for classification. It is probable that the actual *AR* CN at a cellular level that results in treatment resistance is manyfold higher than our cut point; the latter may be the value that is most likely to detect the presence of a high CN clone that rapidly expands after treatment initiation, leading to resistance.

Patients with *AR* gain have a higher ctDNA fraction, and in fact, in this prechemotherapy setting where overall ctDNA fractions are lower, the two are closely linked and do not provide independent prognostic information.[12,21,29] Nonetheless, *AR* CN gain can be detected at low fractions, probably as a result of the high level of AR gain observed in mCRPC. In liquid biopsy tests for a molecular aberration, there is an increased chance of detection at higher tumor load. This introduces a prognostic bias derived from the worse outcomes expected as a result of high ctDNA fraction.[11,12] This could be the case for *AR* CN. Most importantly for clinical utility is whether a test is prognostic or predictive. This analysis and previous studies of *AR* CN are single-arm studies and, therefore, have not tested the predictive value of *AR* CN. In an exploratory analysis in two similar but nonrandomized cohorts, we reported that the outcome of patients with mCRPC at a similar stage as patients in our study population but who were treated with taxane chemotherapy was worse in patients with higher circulating DNA but not those with plasma *AR* gain.[30] Randomized studies are required to investigate whether *AR* gain can be used for treatment selection. Our pooled analyses identify a higher cut point than used in previous studies as most strongly associated with outcome. Although technically justified by detection limits of the assays used, splitting patients into *AR* CN gain or CN normal groups using lower cut points may be less sensitive for detecting clinically relevant associations. Future studies that evaluate the utility of assessing plasma *AR* CN should use the higher CN cut point for dichotomization of patients.

AFFILIATIONS

[1]University College London Cancer Institute, London, United Kingdom
[2]Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori, Istituto di Ricovero e Cura a Carattere Scientifico, Meldola, Italy
[3]Vancouver Prostate Centre, University of British Columbia, Vancouver, British Columbia, Canada
[4]The School of Public Health, Imperial College London, London, United Kingdom
[5]San Camillo and Forlanini Hospitals, Rome, Italy
[6]University of Glasgow, The Beatson West of Scotland Cancer Centre, Glasgow, United Kingdom
[7]Studienpraxis Urologie, Nürtingen, Germany
[8]Hospital Ramón y Cajal, Madrid, Spain
[9]Hospital Universitario Morales Meseguer, Biobanco Nodo 3, Instituto Murciano de Investigación Biosanitaria–Universidad de Murcia, Murcia, Spain
[10]Institut Catala d'Oncologia-Hospital Germans Trias i Pujol, Badalona, Spain
[11]University of Brescia, Spedali Civili Hospital, Brescia, Italy
[12]Englander Institute for Precision Medicine, Weill Cornell Medicine, New York–Presbyterian Hospital, New York, NY
[13]Janssen Research and Development, Beerse, Belgium
[14]Janssen Research and Development, Spring House, PA
[15]HireGenics, Duluth, GA
[16]University Hospital Schleswig-Holstein, Lübeck, Germany
[17]Institut de Recherche Clinique, Université Catholique de Louvain, Brussels, Belgium
[18]Prostate Cancer Research Center, University of Tampere, Tampere, Finland
[19]BC Cancer Agency, Vancouver, British Columbia, Canada

CORRESPONDING AUTHOR

Gerhardt Attard, MD, PhD, University College London Cancer Institute, Paul O'Gorman Building, 72 Huntley St, London WC1E 6BT, United Kingdom; Twitter: @attardlab; e-mail: g.attard@ucl.ac.uk.

EQUAL CONTRIBUTION

A.J. and A.W. contributed equally to this work.

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AUTHOR CONTRIBUTIONS

Conception and design: Anuradha Jayaram, Gerhardt Attard

Financial support: Shibu Thomas, Dong Shen, Kim N. Chi, Ugo De Giorgi, Enrique Gonzalez-Billalabeitia, Alexander Wyatt, Gerhardt Attard

Administrative support: Florence Lefresne, Kim N. Chi, Ugo De Giorgi, Enrique Gonzalez-Billalabeitia, Alexander W. Wyatt, Gerhardt Attard

Provision of study materials or patients: Vincenza Conteduca, Lorraine Barwell, Susan Feyerabend, Enrique Grande, Albert Font, Alfredo Berruti, Cora N. Sternberg, Rob Jones, Shibu Thomas, Dong Shen, Kim N. Chi, Ugo De Giorgi, Enrique Gonzalez-Billalabeitia, Alexander W. Wyatt, Gerhardt Attard

Collection and assembly of data: Anuradha Jayaram, Anna Wingate, Daniel Wetterskog, Vincenza Conteduca, Lorraine Barwell, Susan Feyerabend, Alberto Martinez-Carrasco, Albert Font, Alfredo Berruti, Cora N. Sternberg, Rob Jones, Dong Shen, Deborah Ricci, Michael Gormley, Axel S. Merseburger, Bertrand Tombal, Kim N. Chi, Enrique Gonzalez-Billalabeitia, Alexander W. Wyatt, Gerhardt Attard

Data analysis and interpretation: Anuradha Jayaram, Anna Wingate, Daniel Wetterskog, Daniel Khalaf, Mansour Taghavi Azar Sharabiani, Fabio Calabrò, Susan Feyerabend, Enrique Grande, Cora N. Sternberg, Florence Lefresne, Marjolein Lahaye, Shibu Thomas, Shilpy Joshi, Dong Shen, Deborah Ricci, Michael Gormley, Axel S. Merseburger, Bertrand Tombal, Matti Annala, Ugo De Giorgi, Gerhardt Attard

Manuscript writing: All authors

Final approval of manuscript: All authors

Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Anna Wingate

Research Funding: Janssen (Inst)

Travel, Accommodations, Expenses: Janssen

Vincenza Conteduca

Consulting or Advisory Role: Bayer, Astellas Pharma, Janssen-Cilag, Sanofi

Travel, Accommodations, Expenses: Bayer, Astellas Pharma, Janssen-Cilag, Sanofi

Daniel Khalaf

Consulting or Advisory Role: Bayer

Fabio Calabrò

Consulting or Advisory Role: Pfizer

Susan Feyerabend

Consulting or Advisory Role: Janssen-Cilag

Enrique Grande

Honoraria: Pfizer, Bristol-Myers Squibb, Ipsen, Roche, Eisai, Eusa Pharma, MSD, Genzyme, Adacap, Novartis, Pierre Fabre, Lexicon, Celgene, Janssen-Cilag, Astellas Pharma

Consulting or Advisory Role: MSD, Pfizer, Ipsen

Research Funding: MTEM/Threshold (Inst), Roche, Pfizer (Inst), AstraZeneca (Inst), Ipsen (Inst)

Travel, Accommodations, Expenses: Bristol-Myers Squibb, Genentech, Pfizer, Janssen-Cilag

Alfredo Berruti

Consulting or Advisory Role: Janssen-Cilag, Astellas Pharma

Speakers' Bureau: Janssen-Cilag

Research Funding: Astellas Pharma (Inst), Janssen-Cilag (Inst)

Travel, Accommodations, Expenses: Janssen-Cilag, Sanofi

Cora N. Sternberg

Honoraria: Pfizer, Astellas Pharma, Sanofi, Ipsen, AstraZeneca. Janssen

Consulting or Advisory Role: Bristol-Myers Squibb, Novartis, Bayer, Eisai, MSD, Clovis Oncology, Pfizer, Roche, Ipsen, Incyte, AstraZeneca, Sanofi, Merck, Medscape, UroToday

Research Funding: Janssen (Inst), Genentech (Inst), Bayer (Inst), Sanofi (Inst), Medivation (Inst), Exelixis (Inst), Genzyme (Inst), Aragon Pharmaceuticals (Inst), Array BioPharma (Inst), Aveo (Inst), AstraZeneca (Inst), Bristol-Myers Squibb (Inst), Boehringer Ingelheim (Inst), Clovis Oncology (Inst), Eisai (Inst), Genentech (Inst), GlaxoSmithKline (Inst), Eli Lilly (Inst), Incyte (Inst), Merck (Inst), Millennium (Inst), Myovant Sciences (Inst), Nektar (Inst), Pfizer (Inst), Clovis Atlas (Inst)

Rob Jones

Honoraria: Astellas Pharma, Janssen, AstraZeneca, MSD Oncology, Bristol-Myers Squibb, Pfizer, Novartis, Ipsen, Seattle Genetics, Sanofi, Bayer, Genentech, Eusa Pharma, Pharmacyclics

Consulting or Advisory Role: Clovis Oncology

Research Funding: Roche (Inst), Astellas Pharma (Inst), AstraZeneca (Inst), Pfizer (Inst), Novartis (Inst), Exelixis (Inst), Clovis Oncology (Inst)

Travel, Accommodations, Expenses: Ipsen, Bayer, Janssen, Astellas Pharma, MSD Oncology

Florence Lefresne

Employment: Johnson & Johnson

Stock and Other Ownership Interests: Johnson & Johnson

Honoraria: Johnson & Johnson

Research Funding: Johnson & Johnson

Travel, Accommodations, Expenses: Johnson & Johnson

Marjolein Lahaye

Employment: Janssen-Cilag

Leadership: Janssen-Cilag

Stock and Other Ownership Interests: Janssen-Cilag

Honoraria: Janssen-Cilag

Consulting or Advisory Role: Janssen-Cilag

Shibu Thomas
Employment: Janssen Research & Development
Leadership: Janssen Research & Development
Stock and Other Ownership Interests: Janssen Research & Development
Research Funding: Janssen Oncology
Patents, Royalties, Other Intellectual Property: Four patents (Inst)
Travel, Accommodations, Expenses: Janssen Research & Development

Dong Shen
Employment: Janssen Research & Development
Stock and Other Ownership Interests: Janssen Research & Development

Deborah Ricci
Employment: Janssen
Stock and Other Ownership Interests: Janssen
Patents, Royalties, Other Intellectual Property: Janssen (Inst)
Travel, Accommodations, Expenses: Janssen

Michael Gormley
Employment: Janssen Research & Development
Stock and Other Ownership Interests: Johnson & Johnson
Patents, Royalties, Other Intellectual Property: I have several patents pending where I am listed as the inventor of things that have been developed in the course of my work at Janssen Research and Development (Inst)
Travel, Accommodations, Expenses: Janssen Research & Development

Axel S. Merseburger
Honoraria: Janssen-Cilag, Astellas Pharma, Ipsen, Roche, Bristol-Myers Squibb, Eisai, Takeda, Pfizer, Novartis
Consulting or Advisory Role: MSD Oncology, Bristol-Myers Squibb, Janssen-Cilag, Astellas Pharma, Ipsen
Speakers' Bureau: Ipsen
Research Funding: Novartis (Inst), AstraZeneca (Inst), Janssen-Cilag (Inst), Bristol-Myers Squibb (Inst), Clovis Oncology (Inst)
Travel, Accommodations, Expenses: Janssen-Cilag, Astellas Pharma, Ipsen

Bertrand Tombal
Honoraria: Amgen, Astellas Pharma, Bayer, Ferring, Sanofi, Janssen, Pfizer, Myovant Sciences
Consulting or Advisory Role: Astellas Pharma, Bayer, Ferring, Janssen, Takeda, Steba Biotech, Sanofi
Speakers' Bureau: Amgen, Janssen
Research Funding: Ferring (Inst)
Travel, Accommodations, Expenses: Amgen, Astellas Pharma, Bayer, Ferring, Janssen, Sanofi

Kim N. Chi
Honoraria: Sanofi, Janssen, Astellas Pharma, Bayer
Consulting or Advisory Role: Essa, Astellas Pharma, Janssen, Sanofi, Amgen, Bayer, AstraZeneca, Roche
Research Funding: Janssen (Inst), Astellas Pharma (Inst), Bayer (Inst), Sanofi (Inst), Tokai Pharmaceuticals (Inst), Eli Lilly/ImClone (Inst), Bristol-Myers Squibb (Inst), Merck (Inst), Roche (Inst)

Ugo De Giorgi
Consulting or Advisory Role: Pfizer, Janssen, Astellas Pharma, Sanofi, Bristol-Myers Squibb, Bayer, Ipsen, Merck
Research Funding: Sanofi (Inst), AstraZeneca (Inst), Roche (Inst)
Travel, Accommodations, Expenses: Bristol-Myers Squibb, Ipsen, Janssen, Pfizer

Enrique Gonzalez-Billalabeitia
Travel, Accommodations, Expenses: Bristol-Myers Squibb, Pfizer, Bristol-Myers Squibb, Janssen-Cilag, Astellas Pharma, Sanofi, Roche, Pfizer

Alexander W. Wyatt
Consulting or Advisory Role: Genzyme
Speakers' Bureau: Janssen
Research Funding: Janssen

Gerhardt Attard
Honoraria: Janssen, Astellas Pharma, Janssen (I)
Consulting or Advisory Role: Janssen-Cilag, Veridex, Ventana Medical Systems, Astellas Pharma, Medivation, Novartis, Millennium, Abbott Laboratories, ESSA, Bayer, Pfizer
Speakers' Bureau: Janssen, Astellas Pharma, Takeda, Sanofi, Ventana Medical Systems
Research Funding: Janssen (Inst), Arno Therapeutics (Inst), Innocrin Pharma (Inst)
Patents, Royalties, Other Intellectual Property: I am on the Institute of Cancer Research Rewards to Inventors list of abiraterone acetate
Travel, Accommodations, Expenses: Janssen, Astellas Pharma, Medivation, Ventana Medical Systems, Abbott Laboratories, Bayer, ESSA, Janssen (I), Astellas Pharma (I), Pfizer
Other Relationship: Institute of Cancer Research

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APPENDIX

TABLE A1. Plasma *AR* CN Across All Four Clinical Cohorts

AR CN Value	PCR2023	Cohort A	PREMIERE	British Columbia Cohort
Range	0.7-14.7	0.6-24.3	0.8-28	0.92-28.3
Median	1.06	1.23	1.17	1.09
Mean	1.66	1.85	1.95	2.11

Abbreviations: *AR*, androgen receptor; CN, copy number.

TABLE A2. Multivariate Cox Proportional Hazards Analysis of Predictors for Progression-Free Survival for PCR2023 Cohort

	Progression-Free Survival		
Variable	**HR**	**95% CI**	***P***
AR CN gain (yes *v* no)	2.6	2.0 to 3.5	< .001
Pretreatment ALP/ULN (continuous variable)	1.1	1.0 to 1.1	< .001
Pretreatment LDH/ULN (> *v* < 1)	1.5	1.2 to 1.9	< .001
Age (continuous variable)	1	1 to 1	< .01

Abbreviations: ALP, alkaline phosphatase; *AR*, androgen receptor; CN, copy number; HR, hazard ratio; LDH, lactate dehydrogenase; ULN, upper limit of normal.

TABLE A3. Plasma Androgen Receptor Copy Number Status and Clinical Outcome in the Four Cohorts Used for Pooled Analysis

	Overall Survival			Progression-Free Survival		
Cohort	**Hazard Ratio**	***P***	**95% CI**	**Hazard Ratio**	***P***	**95% CI**
PCR2023	2.37	< .001	1.07 to 5.25	1.94	.01	0.897 to 3.87
Cohort A	3.22	< .001	1.17 to 8.85	2.08	.01	0.92 to 4.72
PREMIERE	5.62	< .001	1.42 to 22.17	3.9	< .001	1.27 to 12.03
British Columbia cohort	5.4	< .001	2.63 to 10.94	2.45	< .001	1.44 to 4.18

TABLE A4. Baseline Characteristics of PCR2023 Cohort by *AR* Status

	Abiraterone Chemotherapy Naive (n = 133)		
Characteristic	***AR* Normal (n = 111, 83.5%)**	***AR* Gain (n = 22, 16.5%)**	***P***
Median age, years (range)	71 (53-88)	67 (56-88)	.56
Median pretreatment PSA, mg/L (range)	41.3 (0.67-1,537)	104.7 (8.7-792.7)	< .01
Median pretreatment LDH, ULN (range)	0.88 (0.44-4.52)	1.02 (0.73-4.47)	.01
Median pretreatment ALP, ULN (range)	0.79 (0.19-8.95)	1.16 (0.38-5.45)	.01
Site of metastases, No. (%)			
Bone only	89 (66.9)	5 (3.7)	.77
Visceral only	5 (3.7)	0 (0)	.59
Visceral and bone	4 (3)	0 (0)	NS

Abbreviations: ALP, alkaline phosphatase; *AR*, androgen receptor; LDH, lactate dehydrogenase; NS, not significant; PSA, prostate-specific antigen; ULN, upper limit of normal.