CANCER

Plasma *AR* and abiraterone-resistant prostate cancer

Alessandro Romanel,[1]* Delila Gasi Tandefelt,[2]* Vincenza Conteduca,[2,3] Anuradha Jayaram,[2,4] Nicola Casiraghi,[1] Daniel Wetterskog,[2] Samanta Salvi,[3] Dino Amadori,[3] Zafeiris Zafeiriou,[2,4] Pasquale Rescigno,[2,4] Diletta Bianchini,[2,4] Giorgia Gurioli,[3] Valentina Casadio,[3] Suzanne Carreira,[2] Jane Goodall,[2] Anna Wingate,[2,4] Roberta Ferraldeschi,[2,4†] Nina Tunariu,[2,4] Penny Flohr,[2] Ugo De Giorgi,[3] Johann S. de Bono,[2,4] Francesca Demichelis,[1,5,6‡§] Gerhardt Attard[2,4‡§]

Androgen receptor (*AR*) gene aberrations are rare in prostate cancer before primary hormone treatment but emerge with castration resistance. To determine *AR* gene status using a minimally invasive assay that could have broad clinical utility, we developed a targeted next-generation sequencing approach amenable to plasma DNA, covering all *AR* coding bases and genomic regions that are highly informative in prostate cancer. We sequenced 274 plasma samples from 97 castration-resistant prostate cancer patients treated with abiraterone at two institutions. We controlled for normal DNA in patients' circulation and detected a sufficiently high tumor DNA fraction to quantify *AR* copy number state in 217 samples (80 patients). Detection of *AR* copy number gain and point mutations in plasma were inversely correlated, supported further by the enrichment of nonsynonymous versus synonymous mutations in *AR* copy number normal as opposed to *AR* gain samples. Whereas *AR* copy number was unchanged from before treatment to progression and no mutant *AR* alleles showed signal for acquired gain, we observed emergence of T878A or L702H AR amino acid changes in 13% of tumors at progression on abiraterone. Patients with *AR* gain or T878A or L702H before abiraterone (45%) were 4.9 and 7.8 times less likely to have a ≥50 or ≥90% decline in prostate-specific antigen (PSA), respectively, and had a significantly worse overall [hazard ratio (HR), 7.33; 95% confidence interval (CI), 3.51 to 15.34; $P = 1.3 \times 10^{-9}$) and progression-free (HR, 3.73; 95% CI, 2.17 to 6.41; $P = 5.6 \times 10^{-7}$) survival. Evaluation of plasma *AR* by next-generation sequencing could identify cancers with primary resistance to abiraterone.

INTRODUCTION

Patients with metastatic prostate cancer commonly respond to androgen deprivation therapy. When patients relapse, a disease state known as castration-resistant prostate cancer (CRPC) develops, against which agents such as abiraterone or enzalutamide, targeting the androgen receptor (AR) axis, are highly effective (*1*, *2*). Abiraterone- and enzalutamide-resistant CRPC is now common and a major challenge in the management of prostate cancer. Ongoing whole-exome and transcriptome sequencing studies of metastatic tumor biopsies obtained in this setting are starting to give insights into the complexity and distribution of genomic aberrations (*3*, *4*). Metastatic biopsies have provided important information on the emergence of resistance mutations in other diseases, such as *EGFR* mutations in lung cancer (*5*). However, monitoring for mutations of resistance with serial samples is challenging in CRPC as well as other tumor types because of the logistics of obtaining repeated tumor biopsies and the observation of genomic heterogeneity in prostate cancer metastases (*6*). Our group and others have advocated using "liquid biopsies" (*7*–*11*). By using next-generation sequencing on circulating tumor DNA obtained from plasma through a minimally invasive blood test, we have demonstrated the capacity to interrogate for disease evolution and identify genomic aberrations that emerge with drug resistance (*10*). This approach allows surveillance of genetic material potentially representative of multiple metastases (*12*).

Progression to CRPC is associated with *AR* copy number gain or somatic point mutations (*13*–*15*). We hypothesized that genomic aberrations involving the *AR* were associated with resistance to abiraterone, potentially through allowing reactivation of AR signaling. We therefore aimed to use circulating tumor DNA extracted from repeated plasma samples to interrogate the circulating *AR* genomic landscape in CRPC resistant to potent AR-targeting drugs while controlling for circulating tumor DNA fraction through a combination of assay design and computational means. Key to this strategy is the concurrent sequencing and analysis of common prostate cancer somatic variants and deletions, similar to the approach we designed to assess tumor tissue purity to account for cell admixture (*16*). This reduces bias secondary to missing circulating *AR* genomic lesions caused by low circulating tumor DNA or normal DNA artifact. To evaluate associations with clinical outcome that would support the clinical utility of this strategy, we used samples prospectively collected independently at two institutions in correlative biomarker protocols that stipulated the molecular analysis of plasma DNA to identify associations with treatment resistance in prostate cancer.

RESULTS

Selection of CRPC patients for evaluation of *AR* genomic aberrations

We selected patients starting standard-of-care abiraterone between January 2011, after the reporting of the abiraterone phase 3 regulatory trial (*1*), and October 2014. We performed sequencing on all patients who had available for analysis a minimum of 6 ng of DNA from plasma (three patients had <6 ng of DNA in 2 ml of plasma) collected

[1]Centre for Integrative Biology, University of Trento, Trento 38123, Italy. [2]The Institute of Cancer Research, London SW7 3RP, UK. [3]Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST), IRCCS, Meldola 47014, Italy. [4]The Royal Marsden NHS Foundation Trust, London SM2 5PT, UK. [5]Institute for Computational Biomedicine, Weill Cornell Medicine, NY 10021, USA. [6]Institute for Precision Medicine, Weill Cornell Medicine, NY 10021, USA.
*These authors contributed equally to this work.
†Present address: Astex Therapeutics, 436 Cambridge Science Park, Cambridge CB4 0QA, UK.
‡Co-senior authors.
§Corresponding author. E-mail: demichelis@science.unitn.it (F.D.); gerhardt.attard@icr.ac.uk (G.A.)

within 30 days of starting treatment. To have a population representative of patients commonly seen in clinical practice, we allowed patients who had received prior treatment with docetaxel or new AR-targeting agents (namely, enzalutamide or orteronel). We obtained targeted next-generation sequencing data from 274 samples from 97 patients: 71 patients treated at the Royal Marsden (RM), London, UK, and 26 at the Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST), Meldola, Italy (characteristics described in Table 1). We included at least one sample obtained 8 weeks after treatment initiation from 70 patients (Fig. 1A). Across all samples, we achieved a median coverage of 1434X (table S1).

Detection of *AR* gain in CRPC patients with circulating tumor DNA

We used the abundance of highly informative lesions in prostate cancer to calculate circulating tumor DNA fraction in 270 of 274 study samples (table S2). On the basis of false-positive distributions built from in silico simulations and empirical data for point mutations and common genomic monoallelic deletions, we identified a threshold of 0.075 as the lower circulating tumor DNA fraction amenable to accurate estimation of absolute *AR* copy number. Of 274 evaluable samples, 217 had a confirmed circulating tumor DNA fraction greater than 0.075, including pre-abiraterone samples from 80 of 97 patients (82%) (table S3). We observed a significantly lower circulating tumor DNA fraction in samples collected at the time of initial treatment response compared to that after disease progression (fig. S1, $P = 0.02$). Using sequence data that covered all the *AR* coding bases (table S2), we proceeded to interrogate *AR*. Samples with evidence of *AR* gained copy number state were classified as *AR* gain (table S4). In total, 81 of 217 samples had *AR* gain, including pre-abiraterone samples from 32 of 80 patients. We used digital droplet polymerase chain reaction (PCR) to validate next-generation sequencing calls of *AR* gain, and by finding that *ZXDB* at Xp11.21 was copy number neutral in these cases, we

Table 1. Patient characteristics and treatment history. ND, not determined.

Characteristics	Overall (*n* = 97)	IRST (*n* = 26)	RM (*n* = 71)
Age, years			
Median (range)	73 (41–92)	76 (63–92)	72 (41–87)
Gleason sum at diagnosis, *n* (%)			
≤7	26 (26.8)	3 (11.6)	23 (32.4)
≥8	57 (58.7)	18 (69.2)	39 (54.9)
Not available	14 (14.4)	5 (5.2)	9 (12.7)
Prior treatment for CRPC, *n* (%)			
Bicalutamide	97 (100)	26 (100)	71 (100)
Docetaxel	82 (84.5)	25 (96.1)	57 (80.3)
Enzalutamide	19 (19.6)	6 (23)	13 (18.3)
Cabazitaxel	6 (6.2)	2 (7.7)	4 (5.6)
Orteronel	4 (5.1)	0	4 (5.6)
Other	4 (4.1)	3 (11.5)	1 (1.4)
Pretreatment PSA, μg/liter			
Median (range)	127 (2–3211)	63.7 (2–1229)	141 (2–3211)
Pretreatment LDH, U/liter			
Median (range)	181 (97–968)	177.5 (97–968)	183 (98–950)
Pretreatment ALP, U/liter			
Median (range)	105 (39–1040)	99 (46–688)	124 (39–1040)
Sites of metastases, *n* (%), *n* with visceral metastases (%)			
≤5 bone metastases	39 (40.2), 13 (13.4)	12 (46.2), 4 (15.4)	27 (38), 9 (12.7)
≥6 bone metastases	50 (51.6), 7 (7.2)	13 (50), 5 (19.2)	37 (52.1), 3 (4.2)
Lymph node, no bone metastases	8 (8.2), 2 (2.0)	1 (3.8), 1 (3.8)	7 (9.9), 1 (1.4)
Time of follow-up, days			
Median (range)	608 (47–1439)	460 (47–608)	983 (78–1439)
Progression-free survival, days			
Median (range)	196 (41–900)	224 (41–577)	171 (62–900)
Overall survival, days			
Median (range)	533 (48–1439)	ND (48–609)	415 (78–1439)

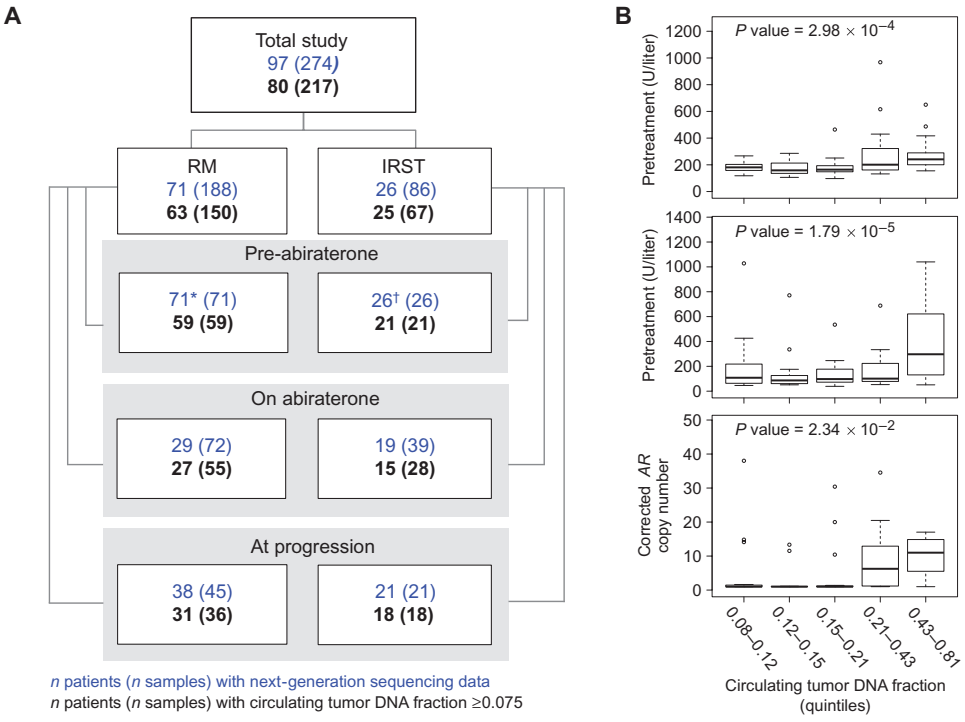

Fig. 1. Detection of circulating tumor DNA in CRPC patients. (**A**) Study profile showing the number of patients and samples with next-generation sequencing data and with a circulating tumor DNA fraction ≥0.075. Twenty-six patients (*) and one patient (†) had pre-abiraterone samples only. (**B**) Correlation of circulating tumor DNA fraction with serum LDH, serum ALP, and *AR* copy number.

confirmed that the area of gain did not involve the whole arm of chromosome X (fig. S2). We observed a significant dosage effect between circulating tumor DNA fraction and serum indices of tumor burden [lactate dehydrogenase (LDH) and alkaline phosphatase (ALP)] (Fig. 1B; $P = 2.98 \times 10^{-4}$ and $P = 1.79 \times 10^{-5}$, respectively), total circulating cell-free DNA (fig. S3, $P = 1.58 \times 10^{-6}$), and plasma *AR* copy number (Fig. 1B, $P = 2.34 \times 10^{-2}$). Nonetheless, we also observed instances of high *AR* copy number state in the presence of low circulating tumor DNA fraction (Fig. 1B). We also confirmed that circulating tumor DNA fraction was associated with a significantly worse overall and progression-free survival (fig. S4A; $P = 0.008$ and $P = 0.011$, respectively), but patients with a fraction <0.075 did not represent a prognostically distinct group (fig. S4B).

Mutant *AR* alleles not associated with copy number gain

We detected somatic *AR* nonsynonymous point mutations described recently in sequencing studies of CRPC tissue (*3*) in 41 plasma samples (15%) from 16 patients (table S5). W742C and W742L *AR* mutations were observed in the same sample collected before initiation of abiraterone in a patient who had progressed on and discontinued bicalutamide 36 days previously. L702H was only observed in patients (five) receiving prednisolone. The L702H, H875Y, and T878A mutations were validated using digital droplet PCR (fig. S5). Among samples with a circulating DNA fraction ≥0.075, we observed a significant inverse correlation between detection of *AR* copy number gain and *AR* point mutations (Fig. 2A, $P = 0.004$), and no instances where the fraction of reads suggested gain of a mutant *AR* allele. Because we had sequence data on all bases in the coding regions of the *AR*, we proceeded to identify a significantly higher rate of nonsynonymous with respect to synonymous *AR* point mutations in the samples with no *AR* gain compared to those with gain (Fig. 2B, $P = 0.028$), supporting selection of nonsynonymous mutations in the absence of gain (*17*, *18*). To identify *AR* point mutations that are specifically associated with resistance to abiraterone, we selected lesions that were consistently detected and showed an increase or persistence in circulating abundance with disease progression. We included 59 patients with both baseline and progression samples (Fig. 1A). Two *AR* point mutations (L702H, three patients; T878A, four patients) were the only ones that met these criteria (Fig. 2C). Both mutations are activated by nonandrogenic ligands present in increased concentrations in patients treated with abiraterone (*10*, *19*).

No change in *AR* copy number at progression on abiraterone

We then identified 44 patients with detectable tumor DNA in circulation sufficient for *AR* copy number assessments in both baseline and progression samples. We observed that 77% of patients with gain at baseline and an equal fraction with no gain showed no change in *AR* status in their progression samples (Fig. 3). The equal conversion rate (23%) in both groups is overall in keeping with the heterogeneous genomic nature of CRPC metastases and their dynamic representation in circulation (*10*) and suggests that *AR* copy number status in individual metastases does not noticeably change with abiraterone.

Plasma *AR* gene aberrations strongly associated with clinical outcome on abiraterone

We then proceeded to evaluate the rate of prostate-specific antigen (PSA) decline for patients with either *AR* gain or an *AR* (L702H or T878A) point mutation in the pre-abiraterone plasma sample [*AR* aberrant, 36 of 80 patients (45%)]. Plasma *AR* aberrant patients were 4.9 and 7.8 times less likely to have a ≥50 or ≥90% decline in PSA, respectively (Fig. 4A; $P = 0.002$ and $P = 0.004$, respectively). No trend for PSA decline was observed when considering circulating tumor DNA fraction (fig. S6). Patients with normal plasma *AR* also had a significantly longer overall and progression-free survival when compared to patients with *AR* gain or mutation (Fig. 4, B and C; $P = 1.3 \times 10^{-9}$ and $P = 5.6 \times 10^{-7}$, respectively). Similar significance was observed comparing overall and progression-free survival between *AR* normal and *AR* gain (fig. S7; $P = 5.7 \times 10^{-9}$ and $P = 3.1 \times 10^{-6}$, respectively), and, although unconfirmed because of limited patient numbers, a similar trend was also observed for *AR* mutants (table S6). Moreover, in support of these data, a swimmers' plot suggests that among patients with both *AR* gain and a decline in PSA, resistance that may be secondary to *AR* gain clones present before treatment emerged within a shorter time frame than for *AR* normal patients; both patients with *AR* gain and a ≥90% decline

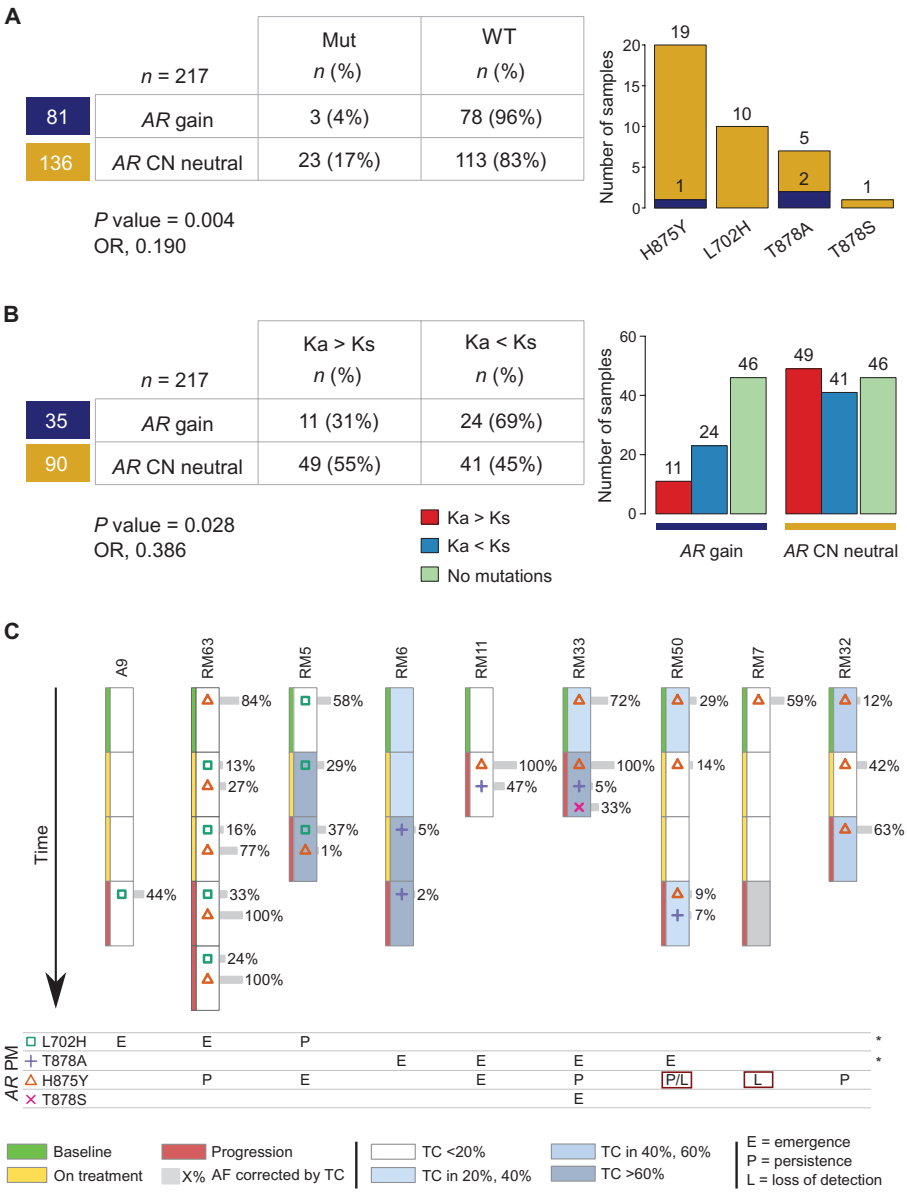

Fig. 2. *AR* gain in nonmutant *AR* alleles. (**A**) Distribution of *AR* point mutations in all samples, stratified by *AR* copy number (CN) status. OR, odds ratio. (**B**) The prevalence of nonsynonymous (Ka) and synonymous (Ks) substitutions in *AR* gain and *AR* CN neutral samples. Fisher's exact test was applied to test differences between the number of mutated (Mut) versus wild-type (WT) samples across *AR* gain and *AR* CN neutral (A) and nonsynonymous versus synonymous substitutions in *AR* gain versus *AR* copy number neutral samples (B). (**C**) Presence of *AR* point mutations (PM) in serial plasma samples from study patients. For every patient, the temporal pattern of mutation detection is shown, distinguishing baseline (green), on-treatment (yellow), and progression (red) samples, along with fractions of circulating tumor DNA (TC). Mutations are marked with different colors and symbols, and the corresponding allelic fractions (AF) corrected for tumor DNA fraction are reported. Temporal patterns observed for each specific patient/mutation combination are annotated as emergence (E), persistence (P), or loss of detection (L and marked with a red box). Stars are used to mark *AR* point mutations that are consistently detected with disease progression.

in PSA developed radiological progression within 35 weeks (fig. S8). In multivariate regression analysis considering *AR* gene status, serum LDH and ALP, total circulating DNA (categorized into quartiles), and prior use of enzalutamide or orteronel (binary), aberrant *AR* remained the only significant variable that was associated with worse overall (HR, 6.85; 95% CI, 3.21 to 14.60) and progression-free survival (HR, 3.58; 95% CI, 1.92 to 6.69) in the final models (table S7).

DISCUSSION

We sequenced all the *AR* coding regions in plasma from CRPC patients immediately before starting abiraterone, on treatment, and after progression, concurrently evaluating both copy number and somatic point mutations. We provide clinical qualification of preliminary data reported by us supporting an association between *AR* copy number gain and resistance to abiraterone (*10*, *20*). A smaller study has also suggested an association between *AR* gain and resistance to enzalutamide (*21*). In contrast, fluorescence in situ hybridization performed on prostate biopsies reported an association between *AR* amplification and sensitivity to first-generation anti-androgens (*13*). Future studies may shed light on the explanation for this discordance, including possible genomic differences between prostate tumors and plasma DNA.

Although we observe a strong correlation between circulating tumor DNA fraction and indices of tumor load and outcome, our strategy may underestimate circulating tumor DNA fraction in some patients because of the emergence of more abundant lesions not included in our custom panel. Concurrent sequencing of more targets could therefore increase the proportion of patients with and the estimated concentrations of circulating tumor DNA. It is also possible that patients with normal plasma *AR* copy number harbor a subclone with *AR* gain, which is

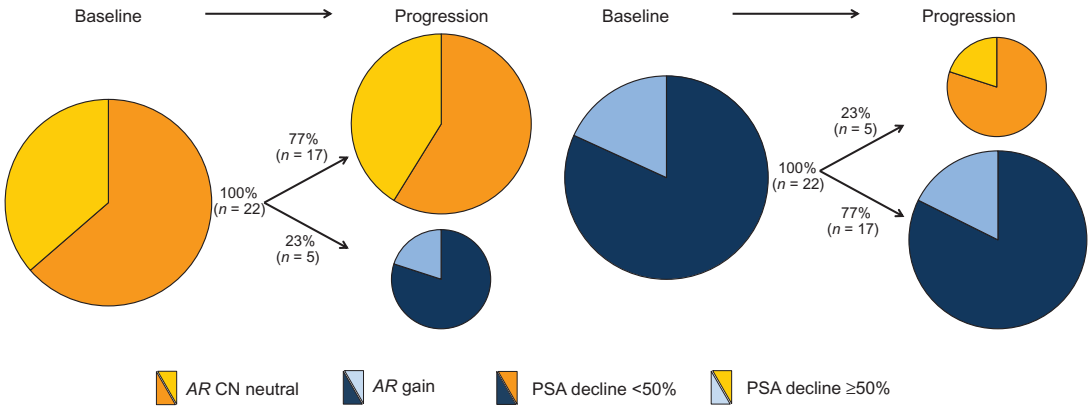

Fig. 3. No change in *AR* copy number on abiraterone. An illustration showing the changes in *AR* gene status for patients who had a tumor DNA fraction greater than 0.075 in both baseline and progression samples. Samples that are *AR* copy number neutral (left) or have *AR* copy number gain (right) pre-abiraterone are split into *AR* copy number neutral and *AR* copy number gain at progression. The areas of the circles are sized to represent the magnitude of the fractions. Orange represents *AR* copy number neutral, and blue represents *AR* copy number gain. The split of patients with a PSA decline ≥50% (light) or not (dark) is shown for every group.

present in too low a frequency relative to overall circulating tumor DNA to be detected. This could explain a proportion of the cases with normal plasma *AR* who did not respond to abiraterone. Nonetheless, after controlling for circulating tumor DNA fraction, emergence of *AR* gain at progression in patients with normal pretreatment *AR* copy number was uncommon, also suggesting that resistance to abiraterone commonly develops secondary to mechanisms that do not involve *AR* gain in these patients.

Resistance in up to 30% of patients with no detectable *AR* gain at progression was associated with an *AR* somatic point mutation, which is often observed several months before confirmed clinical progression and putatively activated by nonandrogenic ligands. This suggests that analysis of plasma *AR* could complement other modalities for evaluating CRPC patients and allow early treatment change before overt radiological progression. The recent report of an association between AR splice variants and resistance to abiraterone (*22*) introduces the possibility of a link between a gain in *AR* copy number, increased AR transcripts, and the presence of AR splice variants. Also, a recent study reports that AR splice variants and *AR* point mutations are generally mutually exclusive (*23*), similar to our observation for *AR* copy number gain. We have not studied AR splicing variants in this study because we did not have concurrent appropriately collected samples. *AR* gain has been previously shown to be associated with increased AR expression in prostate cancers (*24*), which can drive resistance to androgen suppression in vitro (*25*, *26*). Future studies could shed light on the association between *AR* gene state and AR transcriptional profiles and their association with other aberrations that could drive abiraterone resistance.

Among patients who had previously received enzalutamide or orteronel, 10 of 20 had *AR* gain and only 3 (all plasma *AR* normal) had a PSA decline ≥50%, in keeping with previous reports of cross-resistance (*22*). In addition to the prognostic importance, the association with a lower rate of PSA decline suggests that *AR* gene state may be predictive for abiraterone resistance. This could suggest that patients with aberrant plasma *AR* should be selected for treatments such as chemotherapy or radiopharmaceuticals. A similar association may also be observed with other potent AR-targeting strategies such as enzalutamide or the experimental agents ODM-201, ARN-509, and galeterone. These data now warrant prospective validation in randomized clinical trials. Overall, the association of genomic aberrations with clinically meaningful end points in our study suggests that circulating tumor DNA is representative of tumor clones that are driving disease progression in CRPC.

MATERIALS AND METHODS

Study design

The primary aim was to determine the association between *AR* genomic aberrations in plasma from CRPC patients starting abiraterone and a ≥50 or ≥90% decline in serum PSA. Secondary aims included the association with radiological progression-free survival, overall survival, and changes in *AR* genomic status after disease progression. Samples were collected prospectively under two biomarker protocols, separately approved by the RM (REC 04/Q0801/6) and IRST (REC 2192/2013). The aims of the study were defined after sample collection. We aimed to analyze samples from the two institutions separately, but we grouped them together after confirming no substantial differences between the two data sets. Patients needed to have histologically or biochemically confirmed prostate adenocarcinoma and were planning to initiate a new treatment for progressive CRPC, as defined by a minimum of three or more rising serum PSA values obtained two or more weeks apart, with the last value being 2.0 ng/ml or higher in the presence of castrate levels of serum testosterone [<50 ng/dl (1.73 nM)], consistent with the Prostate Cancer Clinical Trials Working Group 2 (PCWG2) guidelines (*27*). Patients were required to receive abiraterone until disease progression as defined by at least two of the following: a rise in PSA, worsening symptoms, or radiological progression as defined previously (*28*), namely, progression in soft tissue lesions measured by computed tomography (CT) imaging according to the modified Response Evaluation Criteria in Solid Tumors or progression on bone scanning according to criteria adapted from the PCWG2. All patients provided written informed consent.

Procedures

Peripheral blood samples for plasma DNA extraction were obtained within 30 days of treatment initiation, and patients were given the option for further blood draws every 8 weeks on treatment and after progression. Serum PSA, LDH, and ALP were checked before the start of abiraterone treatment, after 12 weeks of treatment, and every 4 weeks thereafter. CT scans of the chest, abdomen, and pelvis, and technetium-99m

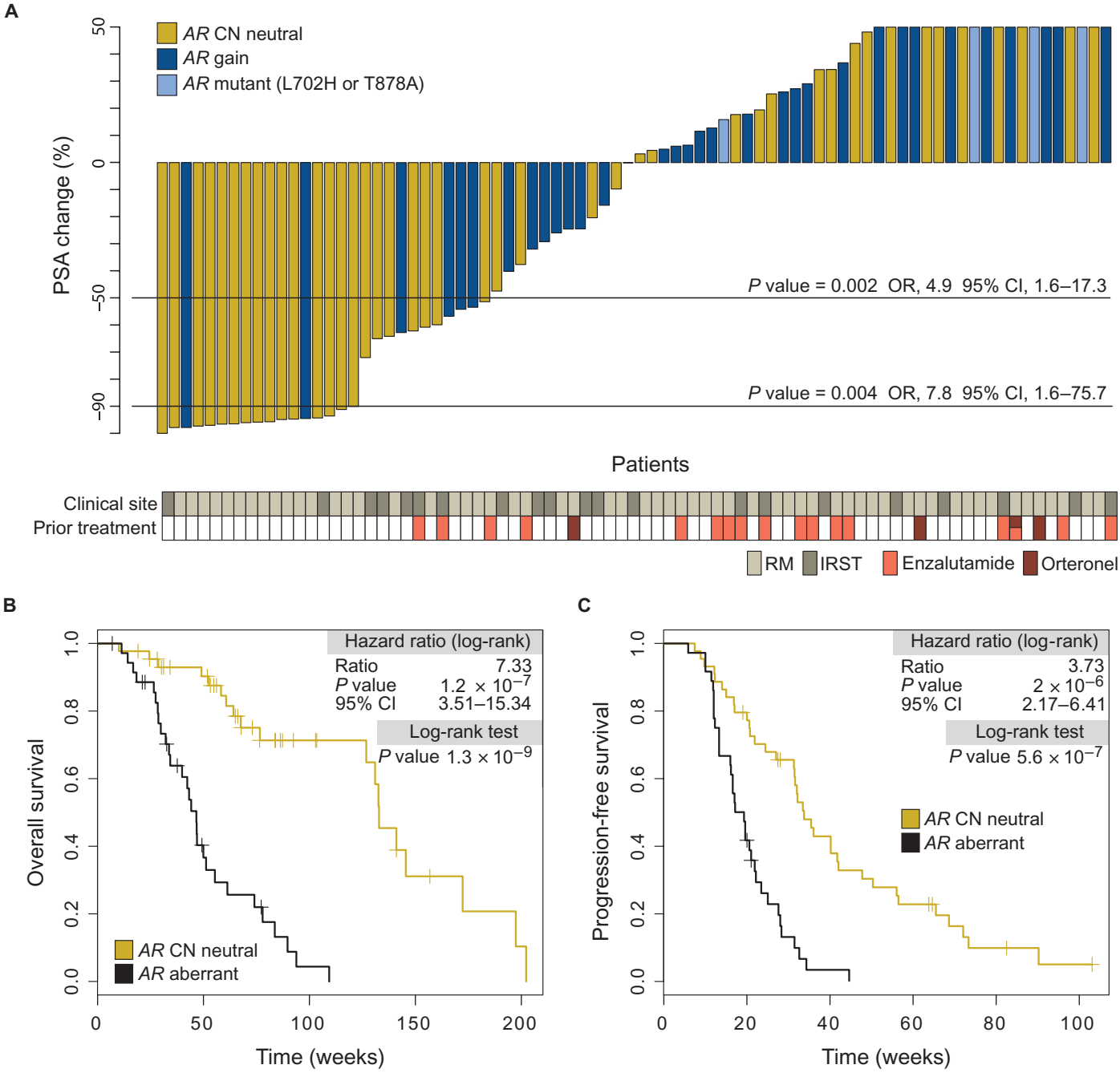

Fig. 4. Association of *AR* gene status before abiraterone with treatment outcome. (**A**) Waterfall plot showing the magnitude of PSA decline in patients with *AR* gain, an L702H or T878A *AR* point mutation, or *AR* copy number neutral. The odds ratios for *AR* copy number neutral having a ≥50 or ≥90% decline in PSA were calculated using Fisher's exact test. Clinical site and prior treatment with the potent AR-targeting agents enzalutamide or orteronel are identified in the matrix. (**B** and **C**) Overall survival (B) and progression-free survival (C) for *AR* copy number neutral versus *AR* aberrant (*AR* gain or point mutation, L702H or T878A) cancers are shown with results of univariate analysis in the inset.

bone scans were performed up to 30 days before treatment initiation and every 2 to 4 months.

Circulating DNA was extracted from 1 to 2 ml of plasma using the QIAamp Circulating Nucleic Acid Kit (Qiagen) and quantified using the high-sensitivity Quant-iT PicoGreen double-stranded DNA Assay Kit (Invitrogen). Germline DNA was extracted from buccal swabs, saliva, or white blood cells using the QIAamp DNA kit (Qiagen). We used our custom AmpliSeq library panel (table S2) in combination with the Ion AmpliSeq Library Preparation Kit version 2.0 (Life Technologies), with a total DNA input of 10 ng. The samples were barcoded with IonXpress barcodes (Life Technologies) to enable sample pooling. Sequencing was performed on samples that passed quality control on

the Personal Genome Machine (PGM) Ion Torrent using a 316 or 318 Chip version 2.0 to account for 1000X expected coverage per target (n = 6 of 8 pooled samples) (tables S1 and S8).

Computational analysis

Data preprocessing included read counts for all genomic positions in the assay, which included informative single-nucleotide polymorphisms (SNPs) (polymorphic positions at which the individual has a heterozygous genotype) necessary for data analysis as previously described (*10*), using ASEQ (*29*). In silico simulations with germline samples from study patients and healthy individuals were performed to assess sensitivity and specificity detecting point mutations and tumor DNA fraction in circulation (tumor content) (table S3). Tumor content was estimated using a strategy similar to (*10*, *16*) but relying on a modified approach for assessing the percentage of aberrant tumor reads to improve both computational and detection performances. Specifically, the algorithm combined control sample data features, in silico simulation, and Mann-Whitney statistics to provide a fast and precise tumor content estimation. For 50 or more informative SNPs and coverage greater than 1000X, mean sensitivity values of 97 and 99.9% were achieved for 8 and 10% of aberrant tumor reads, respectively, with a mean specificity of 99% (figs. S9 and S10). To ensure unbiased *AR* gain calls, we first implemented a procedure modeling the stochastic noise of the copy number estimation with parameter distribution derived from control samples and used it to call *AR* copy number state in all the study samples. An extensive description of the procedures to estimate plasma DNA tumor fraction, copy number call, and point mutation detection is provided in Supplementary Materials and Methods.

Statistical analysis

Pearson correlation statistics with significance level of 5% was used to measure the association between circulating tumor DNA fraction, serum indices of tumor burden, plasma *AR* copy number, and total circulating cell-free DNA. Fisher's exact test with a significance level of 5% was used to measure the association between *AR* copy number gain and *AR* point mutation, nonsynonymous to synonymous *AR* point mutations in *AR* gain compared to no *AR* gain, and PSA decline for patients with either *AR* gain or *AR* point mutation (T878A or L702H). Univariate overall survival and progression-free survival analyses were performed using the Kaplan-Meier estimator (log-rank test). Multivariate overall survival analysis was performed using a proportional hazard model with stepwise model selection by Akaike information criterion using forward and backward directions. Continuous variables were categorized as per distribution quartiles.

SUPPLEMENTARY MATERIALS

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Materials and Methods

Fig. S1. Circulating tumor DNA fraction at response compared to after-disease progression.

Fig. S2. Validation of next-generation sequencing calls of *AR* gain.

Fig. S3. Total cell-free DNA concentration versus tumor DNA fraction.

Fig. S4. Overall and progression-free survival analysis of circulating tumor DNA fraction.

Fig. S5. Validation of *AR* mutations.

Fig. S6. PSA change waterfall plot for tumor DNA fraction.

Fig. S7. Overall and progression-free survival analysis of *AR* normal versus *AR* gain.

Fig. S8. Swimmer plot of patients with a PSA decline.

Fig. S9. Sensitivity analysis of aberrant tumor read percentage estimation.

Fig. S10. Specificity analysis of aberrant tumor read percentage estimation and local tumor content (LTC) median false-positive analysis.

Table S1. Coverage statistics summary (provided as an Excel file).

Table S2. Content of the custom AmpliSeq panel IAD44450_30 (provided as an Excel file).

Table S3. Tumor content (TC) of study samples (provided as an Excel file).

Table S4. *AR* gene copy number call data (provided as an Excel file).

Table S5. Somatic point mutation data (GRCh37/hg19) (provided as an Excel file).

Table S6. Survival data for patients with L702H or T878A *AR* mutations (provided as an Excel file).

Table S7. Multivariate analyses (provided as an Excel file).

Table S8. Germline sample sequencing statistics (provided as an Excel file).

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Plasma *AR* and abiraterone-resistant prostate cancer

Alessandro Romanel, Delila Gasi Tandefelt, Vincenza Conteduca, Anuradha Jayaram, Nicola Casiraghi, Daniel Wetterskog, Samanta Salvi, Dino Amadori, Zafeiris Zafeiriou, Pasquale Rescigno, Diletta Bianchini, Giorgia Gurioli, Valentina Casadio, Suzanne Carreira, Jane Goodall, Anna Wingate, Roberta Ferraldeschi, Nina Tunariu, Penny Flohr, Ugo De Giorgi, Johann S. de Bono, Francesca Demichelis and Gerhardt Attard

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Detecting resistance before it starts

Androgen receptor targeting is the cornerstone of prostate cancer treatment. Even when the tumors become "castration-resistant" or no longer sensitive to androgen deprivation, androgen signaling can still be effectively targeted by newer drugs such as abiraterone and enzalutamide, which also inhibit the androgen signaling axis. Romanel *et al*. analyzed tumor DNA samples from the blood of 97 patients with castration-resistant prostate cancer at different times during the course of treatment with abiraterone. Although some new mutations emerged during therapy, the authors found that androgen receptor amplifications were present from the beginning and correlated with abiraterone resistance, suggesting that detection of these amplifications should be useful for identifying abiraterone-resistant cancers before starting treatment.