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ORIGINAL ARTICLE

Androgen receptor mutations in patients with castration-resistant prostate cancer treated with apalutamide

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Background: Mutations in the androgen receptor (AR) ligand-binding domain (LBD), such as F877L and T878A, have been associated with resistance to next-generation AR-directed therapies. ARN-509-001 was a phase I/II study that evaluated apalutamide activity in castration-resistant prostate cancer (CRPC). Here, we evaluated the type and frequency of 11 relevant AR-LBD mutations in apalutamide-treated CRPC patients.

Patients and methods: Blood samples from men with nonmetastatic CRPC (nmCRPC) and metastatic CRPC (mCRPC) pre- or post-abiraterone acetate and prednisone (AAP) treatment (≥6 months' exposure) were evaluated at baseline and disease progression in trial ARN-509-001. Mutations were detected in circulating tumor DNA using a digital polymerase chain reaction-based method known as BEAMing (beads, emulsification, amplification and magnetics) (Sysmex Inostics' GmbH).

Results: Of the 97 total patients, 51 had nmCRPC, 25 had AAP-naïve mCRPC, and 21 had post-AAP mCRPC. Ninety-three were assessable for the mutation analysis at baseline and 82 of the 93 at progression. The overall frequency of detected AR mutations at baseline was 7/93 (7.5%) and at progression was 6/82 (7.3%). Three of the 82 (3.7%) mCRPC patients (2 AAP-naïve and 1 post-AAP) acquired *AR* F877L during apalutamide treatment. At baseline, 3 of the 93 (3.2%) post-AAP patients had detectable *AR* T878A, which was lost after apalutamide treatment in 1 patient who continued apalutamide treatment for 12 months.

Conclusions: The overall frequency of detected mutations at baseline (7.5%) and progression (7.3%) using the sensitive BEAMing assay was low, suggesting that, based on this assay, AR-LBD mutations such as F877L and T878A are not common contributors to *de novo* or acquired resistance to apalutamide.

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Key words: apalutamide, ARN-509, castration-resistant prostate cancer, androgen receptor, mutations

Introduction

Castration-resistant prostate cancer (CRPC) is the lethal form of the disease that carries a poor prognosis [1, 2]. Molecular profiling studies have shown that androgen receptor (AR) overexpression is associated with resistance to conventional antiandrogens, and preclinical experiments confirm that AR overexpression contributes

to CRPC progression [3]. This insight and the demonstration that androgen ligands persist in CRPC patient tumors despite medical castration led to the eventual clinical development of novel androgen-AR axis–signaling inhibitors, including most recently, apalutamide [4, 5].

Although the majority of patients respond to these next-generation AR-targeted agents, the durability of response is limited [6], and only a subset benefit from sequential AR-directed therapies [7–10]. Several potential mechanisms have been proposed to explain resistance to these agents, including DNA alterations in the AR gene, the production of AR mRNA splice variants such as AR-V7 [11, 12], increased mitogen-activated protein kinase signaling and alternative signaling pathways [3]. Point mutations in the AR ligand-binding domain (AR-LBD) have also been associated with resistance to AR-targeted therapy [13–20], including *AR* F877L and *AR* T878A (formerly *AR* F876L and *AR* T877A) [21], which have been associated with resistance to apalutamide, enzalutamide or the androgen biosynthesis inhibitor abiraterone acetate (hereafter abiraterone), respectively. Additionally, although all AR mutations alter the specificity of ligand binding, there are 2 types of AR mutations, those that convert AR antagonists to agonists (e.g. F877L, W742L/C) and those that result in broadened ligand specificity and a 'promiscuous AR' that can bind to other endogenous steroids [17].

To evaluate the relationship of AR-LBD mutations and resistance to next-generation antiandrogens, Balbas et al. [14] screened for human prostate cancer cell populations with persistent AR transcriptional activity, proliferative ability and tumorigenic potential in the presence of enzalutamide using an AR-regulated enhanced green fluorescent protein reporter and a randomly mutagenized AR library. These investigators identified a novel mutation, *AR* F877L, that spontaneously arose in cells with prolonged treatment with enzalutamide and apalutamide [14]. Joseph et al. [18] and Korpal et al. [19] confirmed these findings with *AR* F877L-expressing prostate cancer cell lines in castrated mice. Neither enzalutamide nor apalutamide inhibited tumor growth in the *AR* F877L-expressing tumors, but both drugs exhibited robust antitumor activity in wild-type AR-expressing tumors [18, 19]. Based on these preclinical data, Joseph et al. [18] used the BEAMing (beads, emulsification, amplification and magnetics) technique to evaluate serial circulating tumor DNA (ctDNA) samples from 29 patients with metastatic CRPC (mCRPC) treated on a phase I study of apalutamide. As expected, *AR* F877L was not found in pretreatment samples but the mutation was detected in 3 (10%) post-apalutamide patients with a rising prostate-specific antigen (PSA), suggesting a possible mechanism for acquired treatment resistance [18]. There is biochemical evidence based on engineered cell line models that enzalutamide is only a weak partial agonist of *AR* F877L, but a strong partial agonist of the double mutant *AR* F877L/T878A [22, 23].

The *AR* T878A mutation has been associated with resistance to abiraterone in a xenograft model [15], which was subsequently detected in metastatic tumor biopsies from CRPC patients relapsing on the CYP17A1 inhibitors abiraterone or ketoconazole [16]. In a recent study, men harboring the *AR* T878A mutation in ctDNA showed inferior PSA response rates and shorter overall survival with abiraterone compared with men with a wild-type AR gene [24]. These studies and others underscore the need to further investigate predictive biomarkers for resistance to AR-targeted therapies.

The aim of the present study was to evaluate the frequency of F877L, T878A and other AR-LBD mutations at baseline and disease progression in nonmetastatic (nm) and mCRPC patients who were abiraterone plus prednisone naïve (AAP-naïve) or who had previously received abiraterone plus prednisone (post-AAP) [25, 26]. Eleven somatic AR-LBD mutations were evaluated at baseline and disease progression in ctDNA using BEAMing, a digital polymerase chain reaction (PCR)-based method (supplementary Table S1, available at *Annals of Oncology* online).

Methods

Patients with nmCRPC and mCRPC were enrolled in a phase II trial of apalutamide (ARN-509-001) [25, 26]. All patients had pathologically confirmed prostate cancer, had been medically or surgically castrated (serum testosterone of ≤50 ng/dl) and had an Eastern Cooperative Oncology Group performance status of 0–1. Patients were excluded if they had received prior enzalutamide, ketoconazole or chemotherapy for mCRPC or had distant metastases with nmCRPC. Patients in the mCRPC cohort had disease progression based on either PSA progression (≥2 ng/ml within 2 weeks of study enrollment) or radiographic progression (≥2 new bone lesions, Prostate Cancer Working Group 2 criteria) [27] and had no prior exposure to abiraterone plus prednisone (i.e. AAP-naïve cohort) or received ≥6 months of abiraterone plus prednisone treatment before disease progression (i.e. post-AAP cohort).

Plasma samples were sent to Sysmex Inostics' GmbH (Hamburg, Germany) analytical facility on dry ice; samples were stored at –70 °C until they were analyzed. Samples were thawed at room temperature for 15–30 min before DNA preparation. BEAMing (Sysmex Inostics' GmbH), which combines emulsion PCR using magnetic beads coated with gene-specific primers to detect and quantify known mutations in ctDNA [28], was used to detect 11 possible somatic AR-LBD mutations in the patient samples (i.e. 11 of >30 known AR-LBD mutations available to assay via BEAMing at the time of the analysis) (supplementary Table S1, available at *Annals of Oncology* online). These 11 mutations affect 6 key amino acid residues (V716, W742, H875, F877, T878 and M896). Detection, quantification and validation are discussed in the supplementary methods, available at *Annals of Oncology* online).

Results

Baseline data ($N = 97$) were similar among cohorts, with the exception of percentage of black and Asian patients, baseline PSA and Gleason score (supplementary Table S2, available at *Annals of Oncology* online). Ninety-three of 97 (96%) patients in the phase II study were assessable for the AR mutation analysis at baseline (nmCRPC, $n = 50$; AAP-naïve mCRPC, $n = 24$; post-AAP mCRPC, $n = 19$); 82 of the 93 (88%) patients assessable at baseline were assessable for the mutation analysis at progression (nmCRPC, $n = 47$; AAP-naïve mCRPC, $n = 20$; post-AAP mCRPC, $n = 15$). The median (range) treatment duration was 26.9 (0.03–37.84) months for the nmCRPC cohort, 20.97 (2.63–37.54) months for the cohort with AAP-naïve mCRPC and 4.87 (1.28–23.2) months for those with post-AAP mCRPC. A low frequency of AR mutations was detected in the overall patient population (Table 1). *AR* F877L and *AR* T878A mutations were found in more than one patient, and these are the focus of this report.

Table 1. Summary of overall androgen receptor mutation status

AR point mutation[b]	Associated drug resistance	Baseline[a] *N* = 93 *n* (%)	Progression 'acquired' *N* = 82 *n* (%)	Total baseline and progression 'acquired' *N* = 93 *n* (%)
F877L[c]	Enzalutamide [14, 18, 19] Apalutamide [14, 18]	2 (2.2)	3 (3.7)	5 (5.4)
T878A[d]	Abiraterone [15, 16]	3 (3.2)	1 (1.2)	4 (4.3)
W742C[e]	Bicalutamide [17]	1 (1.1)	0	1 (1.1)
V716T	Flutamide [17]	0	1 (1.2)	1 (1.1)
H875Y	Flutamide [20] Abiraterone [13]	1 (1.1)	1 (1.2)	2 (2.2)

[a]Four nmCRPC patients were excluded from the efficacy analysis as they were later determined to have metastases on their screening scans.
[b]*AR* M896T and *AR* M896V were not detected.
[c]Three possible nucleotide changes (T → C, C → A and C → G).
[d]Two possible amino acid changes (T → A and T → S).
[e]Two possible amino acid changes (W → C and W → L).
AR, androgen receptor.

Table 2. Androgen receptor F877L and T878A mutation status[a] in individual patients treated with apalutamide in the nmCRPC, AAP-naïve and post-AAP cohorts

Cohort	Patient ID#	*AR* mutation[b]	Mutation fraction at baseline[c]	Cycle at which mutation fraction at progression detected	Mutation fraction at progression[d,e]	12-Week PSA change[f]	Treatment duration (months)[g]
nmCRPC	1	F877L	(0.02%)	8	(0.3%)	−92.2%	6.9
AAP-naïve	2	F877L	–	22	(0.721%)	−77.7%	24.9
	3	F877L	(0.032%)	11	(0.41%)	−66.9%	11.0
	4	F877L	–	9	(0.18%)	−97.3%	8.0
Post-AAP	5	F877L	–	4	(0.04%)	+55.9%	3.4
	6	T878A	(0.84%)	4	(5.46%)	+112.7%	2.8
	7	T878A	(0.07%)	14	–	−62.7%	12
	8	T878A	(1.96%)	6	(0.4%)	−90.1%	4.8
	9	T878A	–	10	(0.02%)	−80.8%	23.2

[a]A plasma sample was deemed positive for a given mutation if the percentage of mutant beads was above the cutoff (0.02%).
[b]No F877L/T878A double mutants were detected.
[c]Number of mutation positive patients at baseline (F877L, $n = 2/93$; T878A, $n = 3/93$).
[d]Number of mutation positive patients at progression (F877L, $n = 5/82$; T878A, $n = 3/82$).
[e]Disease progression on apalutamide was defined as evidence of both PSA progression (≥25% and >2 ng/ml above PSA nadir confirmed ≥3 weeks later or >2 ng/ml above baseline PSA after 12 weeks) and radiographic progression (soft tissue metastases by modified Response Evaluation Criteria In Solid Tumors 1.0) seen on computed tomography/magnetic resonance imaging scans and/or bone metastases by ^{99m}Tc-methylene diphosphate bone scans by Prostate Cancer Working Group 2 criteria, and clinically by the occurrence of a skeletal-related event, pain progression, or worsening of disease-related symptoms requiring new systemic anti-prostate cancer therapy.
[f]Median 12-week PSA change in F877L mutation negative patients ($n = 86$) was −79.8% (range, −99.9 to +175). Median 12-week PSA change in T878A mutation negative patients ($n = 87$) was −81.2% (range, −99.9 to +175).
[g]Median treatment duration in F877L mutation-negative patients ($n = 92$) was 19.6 months (range, 0.03–37.8). Median treatment duration in T878A mutation-negative patients ($n = 93$) was 18.4 months (range, 0.03–37.8).
–, undetected. AAP, abiraterone acetate plus prednisone; AR, androgen receptor; mCRPC, metastatic castration-resistant prostate cancer; PSA, prostate-specific antigen.

AR F877L

Two of the 93 (2.2%) patients harbored the *AR* F877L mutation at baseline at a mutation frequency of <0.05%, and both were subsequently found to have a PSA decline in response to apalutamide (Table 2; Figure 1A and B). One of these patients was in the nmCRPC cohort (12-week PSA change, –92.2%; treatment duration, 6.9 months) and the other was in the AAP-naïve cohort (12-week PSA change, –66.9%; treatment duration,

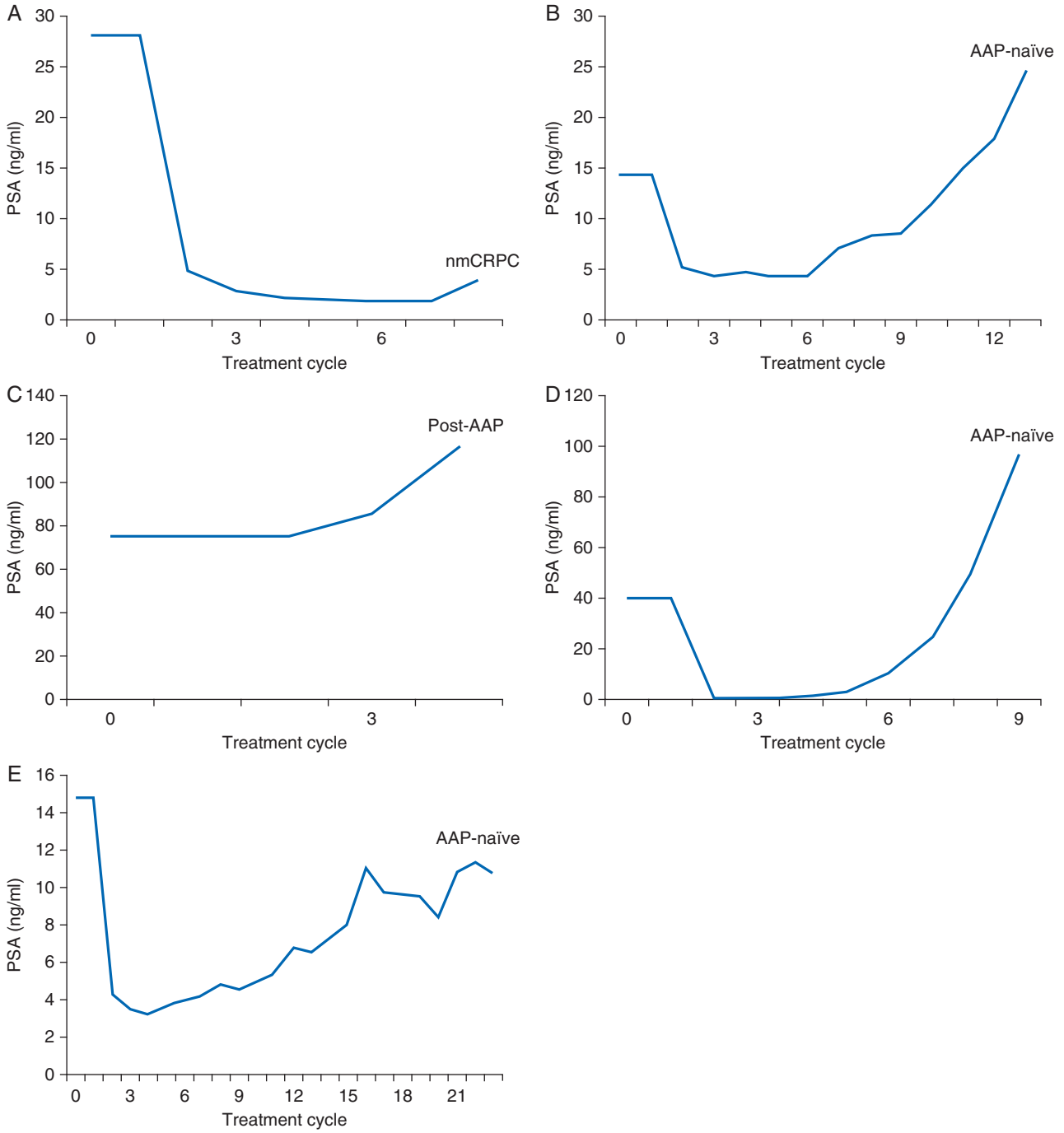

Figure 1. PSA changes in patients with androgen receptor F877L mutations detected at baseline [(A) Pt ID#1, (B) Pt ID#3] and at progression on apalutamide [(C) Pt ID#5, (D) Pt ID#4, (E) Pt ID#2]. AA, abiraterone acetate; PSA, prostate-specific antigen.

11.0 months). Both patients had detectable *AR* F877L and an increase in the mutation frequency at the time of progression.

Three additional patients [3/82 (3.7%)] were found to have the mutation at progression that had not been detected at baseline (Table 2); the PSA trajectory is shown in Figure 1C–E. The single patient in the post-AAP cohort who acquired *AR* F877L demonstrated no PSA decline (12-week PSA change, +55.9%; treatment duration, 3.4 months) and had a relatively low mutation frequency of 0.04% (Table 2; Figure 1C). The other 2 patients with acquired *AR* F877L were both in the AAP-naïve cohort with 12-week PSA changes of –97.3% and –77.7%, treatment durations of 8.0 and 24.9 months, respectively, and mutation frequencies of 0.18% and 0.72%, respectively (Table 2; Figure 1D and E, respectively).

AR T878A

Three of 93 (3.2%) patients had the *AR* T878A mutation at baseline (Table 2); all had previously received at least 6 months of abiraterone and demonstrated similar baseline characteristics.

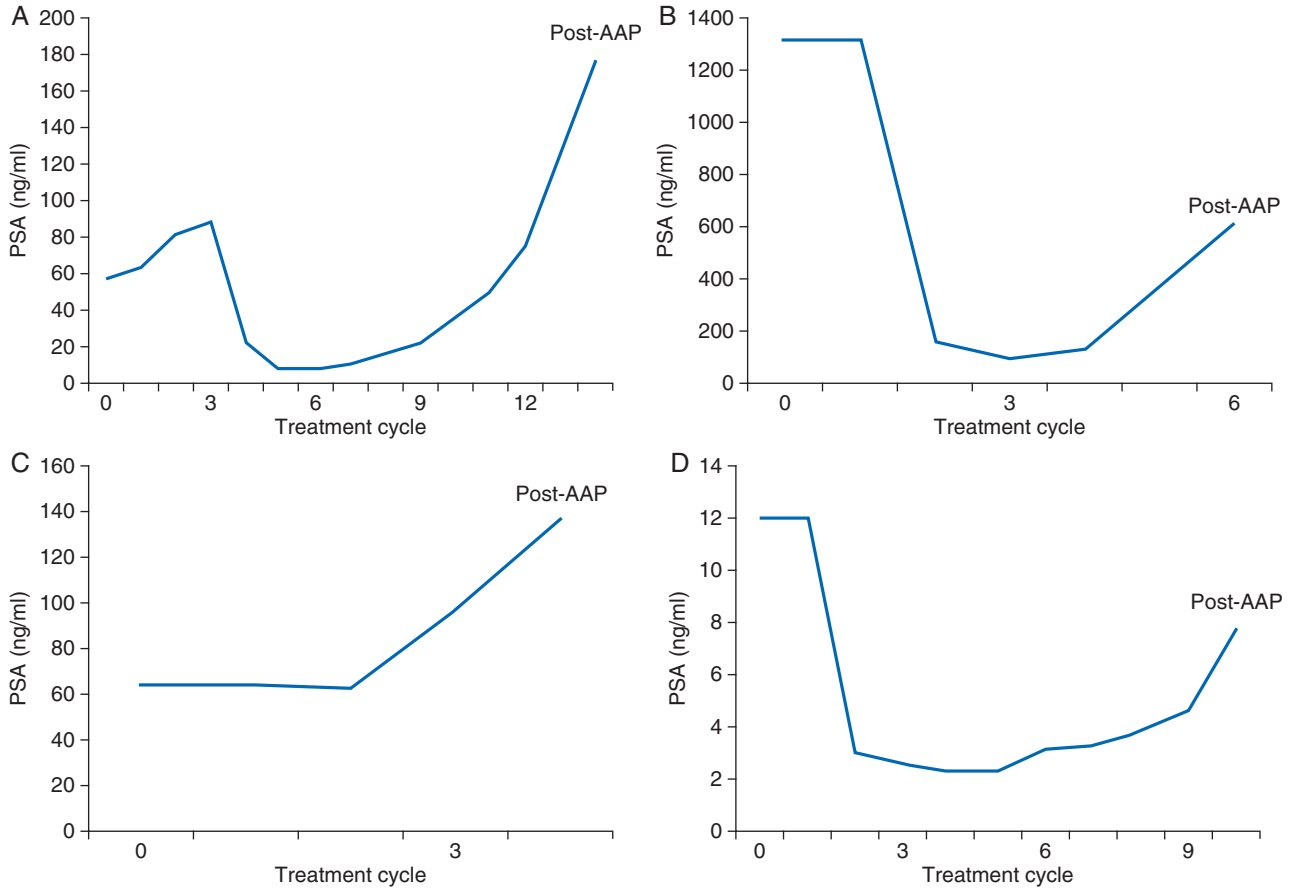

Figure 2. PSA changes in patients with androgen receptor T878A mutations detected at baseline [(A) Pt ID#7, (B) Pt ID#8, (C) Pt ID#6] and at progression on apalutamide [(D) Pt ID#9]. AA, abiraterone acetate; PSA, prostate-specific antigen. Baseline characteristics for these patients are shown in Table 3.

Two had a PSA decline while on treatment with apalutamide, including 1 who had lost the mutation by the time of progression on apalutamide (Figure 2A; Table 3) (12-week PSA change, −62.7%; treatment duration, 12.0 months), and a second who had a decreased mutation fraction from 1.96% at baseline to 0.4% at progression (Figure 2B; Table 3) (12-week PSA change, −90.1%; treatment duration, 4.8 months). The third patient had an increased mutation fraction from 0.84% at baseline to 5.46% at progression and had no PSA decline (12-week PSA change, +112.7%; treatment duration, 2.8 months) (Figure 2C; Table 3). The PSA kinetics increased for these patients after *AR* T878A detection at progression (Figure 2A–D). One post-AAP patient acquired the *AR* T878 mutation at progression at a relatively low frequency of 0.02%. This patient had a PSA decline in response to apalutamide (12-week PSA change, –80.8%; treatment duration, 23.2 months) (Figure 2D; Table 3).

Table 3. Baseline demographics and disease characteristics of post-AAP patients with T878A mutations at baseline corresponding to patients shown in Figure 2 (per Figure 2A–C) and progression on apalutamide (per Figure 2D)

Patient	A (Pt ID#7)	B (Pt ID#8)	C (Pt ID#6)	D (Pt ID#9)
Age	83	64	74	58
Race	White	White	White	White
Baseline PSA (ng/ml)	58.4	1315.2	64.1	12.0
ECOG PS	1	1	1	0
Gleason score	4+5	4+3	4+3	N/A

AAP, abiraterone acetate plus prednisone; ECOG PS, Eastern Cooperative Oncology Group performance status; PSA, prostate-specific antigen.

Discussion

The survival benefits seen with agents that target the AR-signaling pathway have transformed the management of mCRPC. Nevertheless, one-third of patients do not respond to second-generation AR-targeted therapies, and the majority of those who initially respond, will acquire resistance to these agents. The optimal treatment of these patients, and how best to sequence available life-prolonging therapies, have not been established due to the inability to identify patients most likely to respond (or not respond) to specific AR-targeted drugs. This demonstrates the need for predictive molecular biomarkers to better inform treatment selection [11, 12, 24, 29]. Here, we report results of ctDNA sequencing using the BEAMing assay on samples from a phase II study of apalutamide in 3 distinct cohorts

(nm, metastatic AAP-naïve, and metastatic post-AAP). The assay was selected because of its increased sensitivity versus an AR exon 8 sequencing approach used by others [13].

Overall, we tested 5 mutations derived from 11 possible amino acid alterations in 5 codons (supplementary Table S1, available at *Annals of Oncology* online) for which the assay was designed, including: F877L ($n=5$), T878A ($n=4$), W742C ($n=1$), V716M ($n=1$) and H875Y ($n=2$). The most common (occurring in more than one subject) were *AR* F877L and *AR* T878A, LBD mutations associated in laboratory models and in the clinic with resistance to enzalutamide and apalutamide (*AR* F877L) [14, 18, 19] and abiraterone (*AR* T878A) [15, 16].

The frequency of *AR* F877L mutations (i.e. copies of mutant AR per genomic equivalent) increased in the mCRPC cohort after exposure to apalutamide, suggesting the possibility of preexisting clones that underwent positive selection with treatment. The 2 patients with the *AR* F877L mutations at baseline had a 12-week PSA decline of >50% after treatment with apalutamide. Notably, both had a relatively low frequency of the mutation at baseline (<0.05%) that increased at the time of progression. Another AAP-naïve mCRPC patient remained on study for 24.9 months and acquired the *AR* F877L mutation at progression (mutation frequency, 0.72%), suggesting a possible mechanism for secondary resistance. *AR* F877L was not detected in any post-AAP mCRPC patients at baseline. One post-AAP patient acquired the *AR* F877L mutation at progression on apalutamide; however, this patient had a low frequency of the mutation (0.04%) and was only on study for 3.4 months with a rising PSA, potentially suggesting a method of resistance other than development of *AR* F877L in the setting of prior AAP exposure.

All patients in our study who harbored the *AR* T878A mutation were in the mCRPC post-AAP cohort, consistent with the results of a recent analysis showing that AR T878A was associated with resistance to abiraterone [13] and consistent with prevalence reported in prior studies [13, 16], whereas *AR* T878A was never detected in patients with nmCRPC or in those in the AAP-naïve mCRPC cohort. One of the patients who lost the *AR* T878A mutation at progression initially had a PSA elevation but subsequently experienced a robust PSA decline and was on treatment for 12 months until treatment discontinuation due to PSA, radiographic and clinical progression. The decrease or loss of the *AR* T878A mutation observed in 2 of the 3 post-AAP patients who received treatment with apalutamide suggests 3 possibilities: apalutamide may have selectively inhibited the clone with this mutation and restored sensitivity to AR-directed treatment; discontinuation of abiraterone may have removed the evolutionary selection pressure that encouraged this AR mutation to emerge during abiraterone treatment; or discontinuation may have removed selective advantage of progesterones with the availability of endogenous steroids.

Blood samples were collected from 93 patients at baseline and from 82 patients at progression using a BEAMing assay designed to detect 11 selected AR-LBD mutations. These mutations were found at a relatively low incidence and frequency. Potential limitations of the analysis include the use of only one assay (limited to one assay per study sample availability) predesigned to detect 11 AR-LBD mutations already known to be associated with resistance to AR signaling-directed therapies. There may be other as of yet not well defined AR-LBD mutations that contribute to resistance. For example, the clinical significance of emergence of *AR* L702H in patients treated with exogenous glucocorticoids was not known when this study was designed [30]. Larger, prospective studies using assays that can detect mutations as well as other alterations in the receptor such as the AR splice variants [12, 31] to more completely address the question of the role of AR-LBD mutations in both *de novo* and acquired resistance would require a different type of blood sample. Given the high sensitivity of the BEAMing assay, it is likely that the *AR* F877L and *AR* T878A mutations are not major contributors to *de novo* or acquired resistance with apalutamide. It is also possible that the presence of *AR* F877L and *AR* T878A mutations in apalutamide-treated patients is an epiphenomenon associated with clonal selection pressures rather than being a driver of apalutamide resistance. Notably, however, preclinical data strongly suggest that these AR mutations confer resistance to AR-targeting agents. Ultimately, an integrated analysis of tumor-specific mRNA and DNA would be required to study the full complement of AR aberrations in men receiving novel hormonal therapies.

Conclusions

Although *AR* F877L has previously been associated with resistance to apalutamide and enzalutamide, patients with CRPC who were treated with apalutamide in our study had a low rate of *de novo* acquisition of the *AR* F877L mutation [3 of 82 patients (4%)] even using the sensitive BEAMing method. Not surprisingly, in patients without prior exposure to second-generation AR antagonists, *AR* F877L was detected at a low frequency at baseline [2 of 93 (2%)], and the presence of these mutations did not preclude PSA declines with apalutamide. The increased frequency of the mutation at the time of progression does suggest that *AR* F877L mutation may contribute to apalutamide resistance, although the frequency of these mutations in patients progressing on apalutamide in this study was low.

Second-line therapy with apalutamide in 2 post-AAP patients resulted in either a decrease or a loss of the preexisting *AR* T878A mutation while on therapy. Given the low frequency of the *AR* F877L and *AR* T878A mutations, they are unlikely to play a dominant role in the mechanism of primary or acquired resistance to apalutamide in CRPC patients.

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