Supplementary material

Web extra material—online supplement

This Supplementary material has been provided by the authors to give readers additional information about the work.

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Methods

Study design

An institutional review board (IRB)-approved study was conducted at MD Anderson Cancer Center, Houston, Texas, USA, according to the Declaration of Helsinki and International Conference on Harmonisation's Guidelines for Good Clinical Practice (ClinicalTrials.gov: NCT01088529).

Eastern Cooperative Oncology Group performance status of ≤1 was required. Patients provided written informed consent.

We used computer-generated randomisation 2:1 to oral abiraterone acetate (AA; 1000 mg/d) and prednisone (P; 5 mg/d) for 3 mo plus intramuscular luteinising hormone-releasing hormone agonist (LHRHa; monthly or 3-monthly leuprolide acetate; AAP + LHRHa) or LHRHa only (LHRHa). We used this schema to limit the number of patients in the LHRHa arm, since it was not found beneficial.

The primary endpoint was pathologic ≤ ypT2N0. The secondary endpoints included positive surgical margins, undetectable prostate-specific antigen (PSA; ≤0.1 ng/ml), and preoperative safety. Exploratory endpoints included quantitative tumour volume measures and associations with biochemical relapse. Following prostatectomy, we measured PSA at 8 wk and q3 mo for ≥5 yr.

Severity of adverse events (AEs) was reported according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0.

Pathology

Two independent and blinded pathologists evaluated radical prostatectomy specimens and biomarker expression. Exploratory measures to evaluate residual tumour included tumour volume, tumour cell density, and tumour epithelium volume. We calculated tumour volume with three-dimensional estimation. Tumour cell density is the percentage of carcinoma cells in the largest tumour surface area and tumour epithelium volume (TEV) is tumour cell density multiplied by tumour volume.

Biomarker analysis

Biomarker analysis (Supplementary Fig. 2) included biomarkers evaluated by immunohistochemistry (Supplementary Table 1) and RNA (data not shown). We acquired

images of biomarkers using Olympus imaging system (Olympus Corporation, Center Valley, PA, USA). We recorded involvement as percentage tumour cells exhibiting detectable staining, intensity, and subcellular distribution.

We estimated proliferation by nuclear antigen Ki-67 and defined glucocorticoid receptor overexpression as ≥10% involvement of 3+ intensity.

Intraprostatic hormone analysis

We evaluated cortisol concentrations with an analysis of tissue by liquid chromatography–tandem mass spectrometry. We measured steroid panel from snap-frozen intraprostatic core biopsy samples, and carried out assessment in set pairs of unconjugated and 5-alpha reduced androgens as internal controls.

Statistical analysis

The sample size of 66 study patients (44 and 22 patients in the AAP + LHRHa arm and the LHRHa arm, respectively) provided 80% power to detect the difference of proportions of ≤ypT2N0 disease between arms, using a two-sided chi-square test at a significance level of 0.1. All patients in the intent-to-treat population were evaluated in the primary analysis. Patients who received at least one dose of study drug were assessed for safety.

We determined Spearman's correlation between residual tumours with biomarkers in each treatment arm and in overall population, and evaluated the association of measures of residual tumour with biomarkers by multivariate linear regression analysis of treatment, the biomarker, and interaction of treatment and the biomarker. We used the Kaplan-Meier method and the log-rank test for time-to-event analysis.

The median of normalised values for biomarkers in a group was used to determine the composite score for each patient by first calculating the standardised score for each biomarker's imputed and $\log_2$-transformed value across all patients using (biomarker value – mean across all patients)/(standard deviation across all patients), and then summarising the

standardised score of all genes in each group by the median value to generate a biomarker group composite score. Differences between biomarker levels were compared using the Wilcoxon ranked test.

The trial was monitored according to the sponsor's current Standard Operating Procedure for the Monitoring of Clinical Trials.

Role of the funding source

Employees of Janssen Research & Development participated in data monitoring and collection, data analysis, and writing of the report. The study sponsor provided grants to trial site, but had no involvement in the conduct of the trial. Analyses conducted by Janssen in support of this manuscript were funded by Janssen Global Services. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit.

Results

Thirty-four men in the AAP + LHRHa arm (80%) and all patients in the LHRHa arm received the total 3 mo of treatment.

Sixty-six patients were enrolled, and one withdrew consent prior to treatment.

Pathology stage was positively associated with lymph node status ($p < 0.0001$; Supplementary Table 3).

By all three exploratory quantitative tumour measures, residual tumour was significantly lower in the AAP + LHRHa arm than in the LHRHa arm (Fig. 1).

Median tumour volumes were 0.5 cc (range, 0.0–6.7 cc) and 2.6 (range, 0.1–6.3 cc) for the AAP + LHRHa and LHRHa arms, respectively ($p = 0.0026$). Median tumour cell density was 35% (range, 1–75%) for the AAP + LHRHa arm and 70% (range, 50–85%) for the LHRHa arm ($p < 0.0001$). Median tumour TEV was 0.1 cc (range, 0.0–4.8 cc) for the AAP + LHRHa arm and 1.6 cc (range, 0.0–4.6 cc) for the LHRHa arm ($p = 0.0001$).

Five patients (11%) in the AAP + LHRHa arm had treatment-emergent AEs (TEAEs) leading to treatment discontinuation. No patients had TEAEs grade ≥4.

One 74-yr-old patient in the AAP + LHRHa arm had a perioperative pulmonary embolism unrelated to study drugs, which resolved later. One LHRHa patient had pelvic bleeding. There was no increase in perioperative complications in either arm (data not shown). Fifty-Eight of 65 operated patients underwent laparoscopic robotic-assisted radical prostatectomy. Median blood loss in radical prostatectomies was 250 ml (range, 25–2000) in both treatment arms.

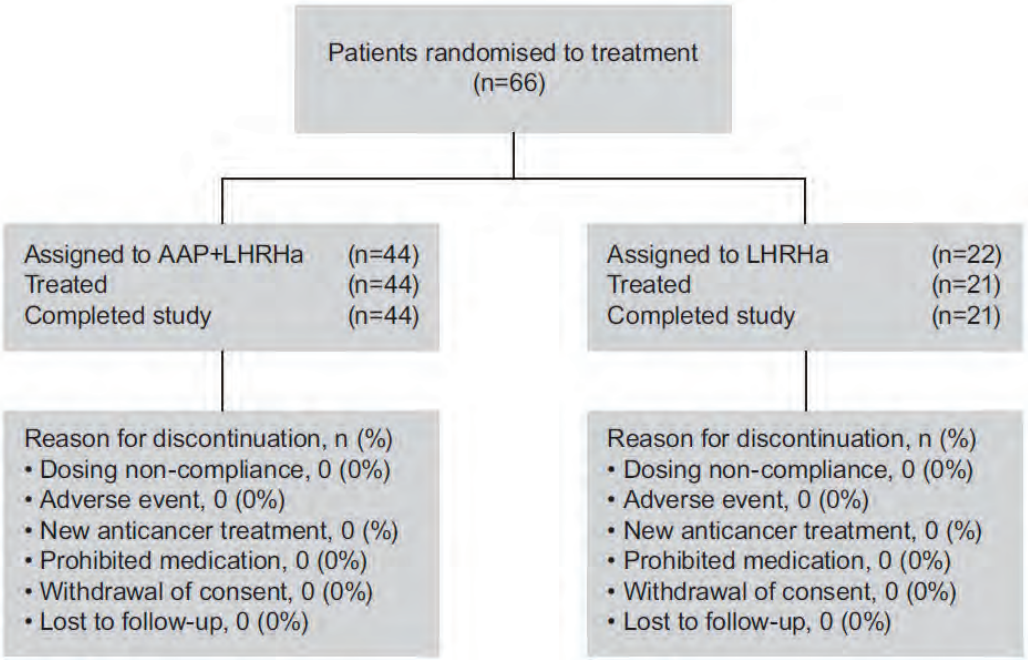
With regard to subsequent treatments, one patient in each treatment arm was given adjuvant radiation as per physician decision (ypT3bN0 stage).

Five patients (11%) in the AAP + LHRHa arm discontinued study treatment due to increased transaminase levels (grade 3 alanine aminotransferase increase). AA was interrupted for four patients because of gastrointestinal disorders (n = 2) and hypertension (n = 2).

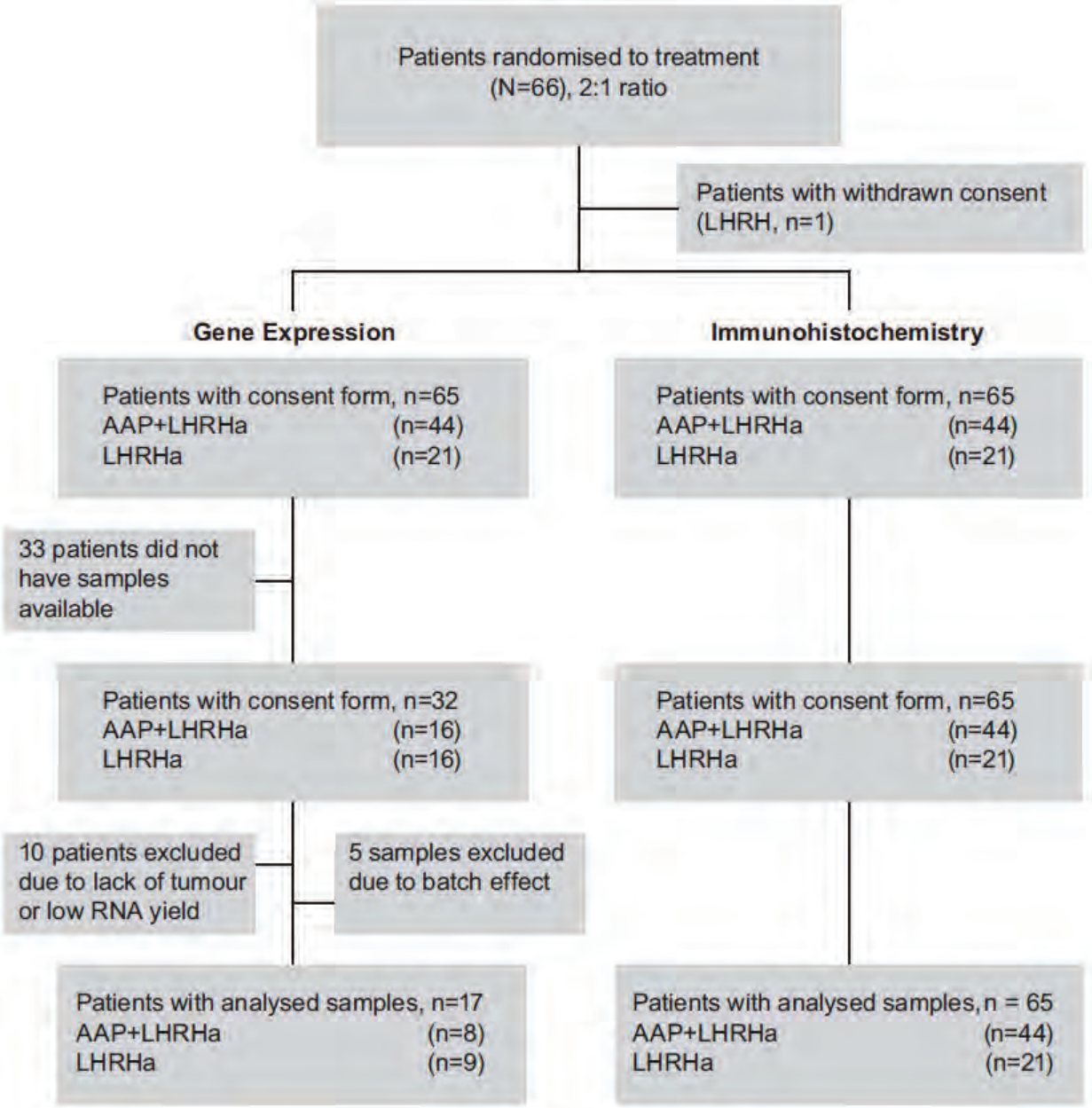
Since the association of PSA recurrence with TEV was more significant than the association between recurrence and pathology stage (p = 0.01; Table 3), we employed TEV for further analysis.

Protein findings presented aligned with the RNA analysis (data not shown).

Supplementary Fig. 1 – Study design. Five patients in the AAP + LHRHa arms discontinued study treatment. One patient in the LHRHa arm did not undergo radical prostatectomy. AAP = abiraterone acetate plus prednisone; LHRHa = leuprolide acetate.



Supplementary Fig. 2 – Study design of biomarker analysis. AAP = abiraterone acetate plus prednisone; LHRH = luteinising hormone-releasing hormone; LHRHa = luteinising hormone-releasing hormone agonist.



Supplementary Table 1 – Predefined immunohistochemistry biomarkers

Antibody	Dilution	Company	Catalogue no.
AR	1/50	DAKO	M3562
ARv7	1/75	Abcam	ab198394
AR-C19	1/500	Santa Cruz	SC-815
CYP17A1	1/300	Novus Biologicals	NB100-2842
Phospho-cMET	1/175	Novus Biologicals	NBP1-40436
Phospho-SRC	1/50	Cell Signaling	2101
PSA	1/2	Ventana Medical Systems	760-4271
CD56	1/50	DAKO	M7304
Chromogranin A	1/200	DAKO	M0869
GR	1/300	BD Biosciences	61226
Ki67	1/50	DAKO	M7240
ERG	1/50	Biocare Medical	CM421C
NKX 3.1	1/500	Athena Enzyme System	0314-16286
Retinoblastoma	1/50	Calbiochem	OP66
P53	1/1000	DAKO	M7001
ATM	1/300	GeneTex	GTX70103

Supplementary Table 2 – Treatment-emergent adverse events (TEAEs) in safety population

	AAP + LHRHa (*n* = 44)			LHRHa (*n* = 21)		
No. of patients with TEAEs (%) [a]	44 (100)			21 (100)		
No. of patients with grade 3 TEAEs (%) [a]	17 (38·6)			5 (23·8)		
No. of patients with TESAEs (%) [a]	2 (4·5)			2 (9·5)		
No. of patients with TEAEs leading to treatment discontinuation (%) [a,b]	5 (11·4)			0		
Most frequent TEAEs, *n* (%) [c]						
	Grade 1/2	Grade 3§	Total	Grade 1/2	Grade 3 [d]	Total
Hot flush	37 (84)	0	37 (84)	18 (86)	0	18 (86)
Anaemia	23 (52)	0	23 (52)	8 (38)	0	8 (38)
Fatigue	18 (41)	1 (2)	19(43)	8(38)	2(10)	10 (48)
ALT increase	15 (34)	7 (16)	22 (50)	7 (33)	0	7 (33)
AST increase	16 (36)	1 (2)	17 (39)	7 (33)	0	7 (33)
Hypertension	2 (5)	8 (18)	10 (23)	1 (5)	2 (10)	3 (14)
Hypercholesterolaemia	9 (21)	0	9 (21)	5 (24)	0	5 (24)
Hyperglycaemia	9 (21)	0	9 (21)	3 (14)	0	3 (14)
Hyperbilirubinemia	8 (18)	0	8 (18)	1 (5)	0	1 (5)
Insomnia	3 (7)	0	3 (7)	5 (24)	1 (5)	6 (29)

AA = abiraterone acetate; ALT = alanine aminotransferase; AST = aspartate aminotransferase; LHRHa = luteinising hormone-releasing hormone agonist; P = prednisone; TESAE = treatment-emergent serious adverse event.

[a] Adverse events with toxicity grade 5 are not included.

[b] Discontinuation of study medication includes discontinuation of any of the study drugs (AA, P, or LHRHa).

[c] TEAE in ≥15% of patients in either treatment group.

[d] No grade 4 TEAEs were observed.

Supplementary Table 3 – Patient and tumour characteristics of intent-to-treat population

	Intent-to-treat		
	AAP + LHRHa	LHRHa	Total
N	44	22	66
Age (yr), median (range)	62 (46–73)	60 (42–75)	61 (42–75)
Days from initial diagnosis to first dose, median (range)	49 (1–889)	42 (14–512)	48 (1–889)
PSA pretreatment (ng/ml), median (range)	9.9 (1.6–69.0)	12.4 (4.8–118.8)	11.4 (1.6–118.8)
ECOG PS, *n* (%)			
0	39 (88.6)	15 (68.2)	54 (81.8)
1	5 (11.4)	7 (31.8)	12 (18.2)
Clinical stage, *n* (%)			
T1	9 (20.5)	3 (13.6)	12 (18.2)
T2	32 (72.7)	17 (77.3)	49 (74.2)
T3	3 (6.8)	1 (4.5)	4 (6.1)
Gleason score at initial diagnosis, *n* (%)			
7 (3 + 4)	0	3 (13.6)	3 (4.5)
7 (4 + 3)	4 (9.1)	3 (13.6)	7 (10.6)
8	21 (47.7)	10 (45.5)	31 (47.0)
≥9	19 (43.2)	6 (27.3)	25 (37.9)

AAP = abiraterone acetate plus prednisone; ECOG PS = Eastern Cooperative Oncology Group performance status; LHRHa = luteinising hormone-releasing hormone agonist; PSA = prostate-specific antigen.

Supplementary Table 4 – Correlation of tumour extent and lymph node status

Pathological stage		Lymph node status		*p* value [a]
		N0	N1 or N2	
ypT2	*N*	0.28	0.4	<0.0001
	%	65.9	14.3	
ypT3 or T4	*N*	0.14	0.19	
	%	34.1	85.7	<0.0001

Correlation percentage represents the correlation ratio on how pathological stage and lymph node status are related. The higher the correlation percentage, the more closely related the variables.

[a] *p* value is based on the Fisher exact test.

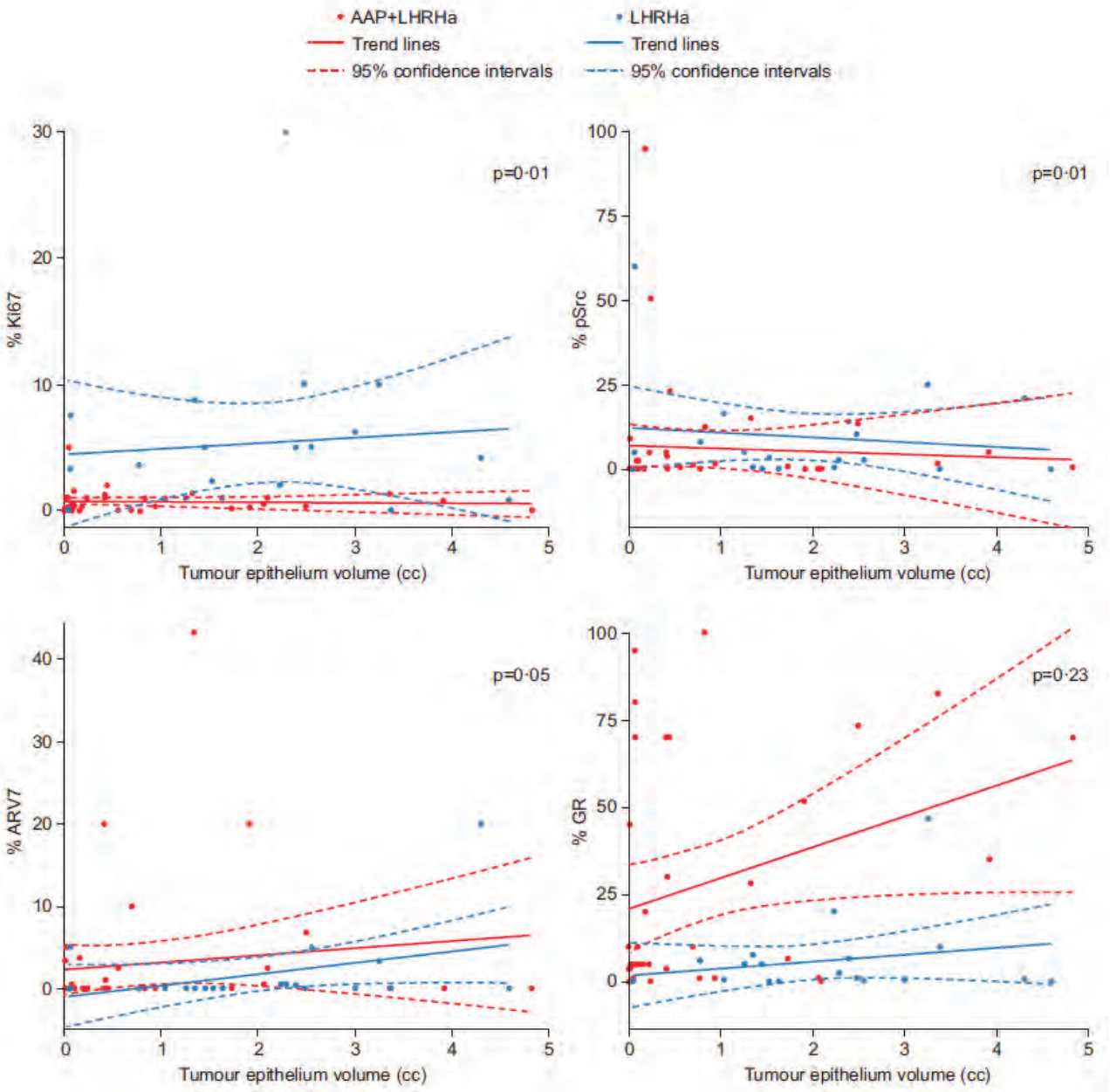
Supplementary Table 5 – Summary of cell density by treatment

	AAP + LHRHa	LHRHa	Total	*p* value [a]
N	44	21	65	
Tumour volume (cc)				
Median (range)	0.5 (0.0–6.7)	2.6 (0.1–6.3)	1.0 (0.0–6.7)	0.0026
Tumour cell density (%)				
Median (range)	35.0 (1.0–75.0)	70.0 (50.0–85.0)	55.0 (1.0–85.0)	<0.0001
Tumour epithelium volume (cc)				
Median (range)	0.1 (0.0–4.8)	1.6 (0.0–4.6)	0.5 (0.0–4.8)	0.0001

AAP = abiraterone acetate plus prednisone; LHRHa = luteinising hormone-releasing hormone agonist.

[a] *p* values are based on the Wilcoxon rank sum test for continuous cellularity data.

Supplementary Fig. 3 – Association of the protein expression of markers of interest by study arm and tumour epithelial volume in the entire study population. AAP = = abiraterone acetate plus prednisone; ARV7 = androgen receptor splice variant; GR = glucocorticoid receptor; LHRHa = luteinising hormone-releasing hormone agonist.



Supplementary Table 6 – Spearman correlation between estimated tumour epithelium volume and immunohistochemistry markers (weighted mean)

	AAP + LHRHa			LHRHa			Total		
	Correlation	*p* value	*n*	Correlation	*p* value	*n*	Correlation	*p* value	*n*
PSA	0.24939	0.1158	41	–0.31001	0.1835	20	0.29676	0.0202	61
nARv7	0.27439	0.0825	41	0.33227	0.1523	20	0.22631	0.0795	61
GR	0.37629	0.0182	39	0.14740	0.5352	20	0.15737	0.2339	59
p-Src	0.49280	0.0011	41	–0.00266	0.9911	20	0.40567	0.0012	61

AAP = abiraterone acetate plus prednisone; GR = glucocorticoid receptor; LHRHa = luteinising hormone-releasing hormone agonist; nARv7 = nuclear expression of androgen receptor splice variant 7; PSA = prostate-specific antigen.

Supplementary Table 7 – Comparison of markers of interest between treatment arms

Table 2 markers	Treatment arm	1st quartile	Median	Mean	3rd quartile	SD	*p* value
AR-N	LHRHa	44.25	68	65.31	90.92	28.5	0.05
	AAP + LHRHa	10	50	46.39	72.5	35.1	
PSA	LHRHa	41.46	74.55	66.26	93	29.6	0.008
	AAP + LHRHa	1	20	31.51	55	32.7	
nARV7	LHRHa	0	0	1.733	0.5	4.6	0.99
	AAP + LHRHa	0	0	2.902	1	7.9	
GR	LHRHa	0	0.8333	5.625	6.167	10.8	0.008
	AAP + LHRHa	2.333	6.5	27.89	60.83	33.5	
pSRC	LHRHa	0.375	4.167	9.455	14.25	14.1	0.02
	AAP + LHRHa	0	0	6.061	3.75	16.8	
Ki67	LHRHa	1	3.883	5.337	6.562	6.6	0.008
	AA + LHRHa	0	0.4167	0.6952	1	0.89	

AAP = abiraterone acetate plus prednisone; GR = glucocorticoid receptor; LHRHa = luteinising hormone-releasing hormone agonist; nARv7 = nuclear expression of androgen receptor splice variant 7; PSA = prostate-specific antigen; SD = standard deviation.