

Intense Androgen-Deprivation Therapy With Abiraterone Acetate Plus Leuprolide Acetate in Patients With Localized High-Risk Prostate Cancer: Results of a Randomized Phase II Neoadjuvant Study

Mary-Ellen Taplin, Bruce Montgomery, Christopher J. Logothetis, Glenn J. Bubley, Jerome P. Richie, Bruce L. Dalkin, Martin G. Sanda, John W. Davis, Massimo Loda, Lawrence D. True, Patricia Troncoso, Huihui Ye, Rosina T. Lis, Brett T. Marck, Alvin M. Matsumoto, Steven P. Balk, Elahe A. Mostaghel, Trevor M. Penning, Peter S. Nelson, Wanling Xie, Zhenyang Jiang, Christopher M. Haqq, Daniel Tamae, NamPhuong Tran, Weimin Peng, Thian Kheoh, Arturo Molina, and Philip W. Kantoff

See accompanying editorial on page 3689

Author affiliations appear at the end of this article.

Published online ahead of print at www.jco.org on October 13, 2014.

Supported by Ortho Biotech Oncology Research and Development, unit of Cougar Biotechnology (now Janssen Research and Development); by Pacific Northwest Prostate Cancer Specialized Program of Research Excellence (SPORE) Grants No. P50CA097186, RCICA146849, and PC093509; by Dana-Farber Cancer Institute SPORE Grant No. 5 P50 CA090381-10; by National Cancer Institute Cancer Pharmacology Training Fellowship Grant No. R25 CA101871 (D.T.); by the Kohlberg Foundation; by a Challenge Award from the Prostate Cancer Foundation (P.S.N., S.P.B.); by Department of Defense Grants No. W81XWH-11-1-0295 and W81XWH07-1-0443 and National Institutes of Health (NIH) Grant No. P01 CA163227 (S.P.B.); by NIH Grant No. P01 CA163227 (S.P.B., P.S.N., E.A.M., T.M.P.); by the Department of Veterans' Affairs Puget Sound Health Care System (A.M.M.); and by Janssen Global Services, which funded writing assistance. The trial was conducted within the Prostate Cancer Clinical Trials Consortium.

Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical trial information: NCT00924469.

Corresponding author: Mary-Ellen Taplin, MD, Harvard Medical School, Lank Center for Genitourinary Oncology, Dana-Farber Cancer Institute, 450 Brookline Ave, Boston, MA 02215-5450; e-mail: mary_taplin@dfci.harvard.edu.

© 2014 by American Society of Clinical Oncology

0732-183X/14/3233w-3705w/\$20.00

DOI: 10.1200/JCO.2013.53.4578

ABSTRACT

Purpose

Cure rates for localized high-risk prostate cancers (PCa) and some intermediate-risk PCa are frequently suboptimal with local therapy. Outcomes are improved by concomitant androgen-deprivation therapy (ADT) with radiation therapy, but not by concomitant ADT with surgery. Luteinizing hormone–releasing hormone agonist (LHRHa; leuprolide acetate) does not reduce serum androgens as effectively as abiraterone acetate (AA), a prodrug of abiraterone, a CYP17 inhibitor that lowers serum testosterone (< 1 ng/dL) and improves survival in metastatic PCa. The possibility that greater androgen suppression in patients with localized high-risk PCa will result in improved clinical outcomes makes paramount the reassessment of neoadjuvant ADT with more robust androgen suppression.

Patients and Methods

A neoadjuvant randomized phase II trial of LHRHa with AA was conducted in patients with localized high-risk PCa (N = 58). For the first 12 weeks, patients were randomly assigned to LHRHa versus LHRHa plus AA. After a research prostate biopsy, all patients received 12 additional weeks of LHRHa plus AA followed by prostatectomy.

Results

The levels of intraprostatic androgens from 12-week prostate biopsies, including the primary end point (dihydrotestosterone/testosterone), were significantly lower (dehydroepiandrosterone, Δ^4 -androstene-3,17-dione, dihydrotestosterone, all $P < .001$; testosterone, $P < .05$) with LHRHa plus AA compared with LHRHa alone. Prostatectomy pathologic staging demonstrated a low incidence of complete responses and minimal residual disease, with residual T3- or lymph node–positive disease in the majority.

Conclusion

LHRHa plus AA treatment suppresses tissue androgens more effectively than LHRHa alone. Intensive intratumoral androgen suppression with LHRHa plus AA before prostatectomy for localized high-risk PCa may reduce tumor burden.

J Clin Oncol 32:3705-3715. © 2014 by American Society of Clinical Oncology

INTRODUCTION

Risk stratification for localized prostate cancer (PCa) is based on clinical stage, prostate-specific antigen (PSA), and Gleason score.¹ Patients with localized high-risk PCa experience the highest recurrence after prostatectomy, with PSA relapse rates of 40% to 65%. Patients with recurring disease after

local therapy experience the consequences associated with salvage local and systemic therapies and may eventually die as a result of advanced PCa.^{2,3} Innovative multimodality therapy for localized high-risk PCa is urgently needed.

Addition of androgen-deprivation therapy (ADT) to prostatectomy was evaluated in the late 1990s and early 2000s.⁴⁻⁸ ADT included luteinizing

hormone-releasing hormone agonists (LHRHa), alone or with anti-androgens, and the common duration was 3 months. In aggregate, despite reduction of surgical positive margin rates, there was no improvement in PSA failure, and this approach was abandoned. Few trials reported **pathologic complete response** (pCR) rates; however, in a trial of 3 versus 8 months of ADT in low- and intermediate-risk patients, a 9.3% pCR rate was reported in the 8-month treatment group, supporting longer duration of neoadjuvant ADT.⁹ In a contemporary trial of patients with localized PCa, treatment for 3 months with neoadjuvant LHRHa plus the **androgen receptor** (AR) antagonist bicalutamide, ketoconazole, and the 5- α -reductase inhibitor dutasteride produced two pCRs (6%).¹⁰

A plausible explanation for historic lack of efficacy of neoadjuvant LHRHa is incomplete tissue suppression of androgens. The importance of tissue androgens in PCa pathogenesis is well established. Although standard ADT lowers serum androgens by approximately 90%, tissue androgens are reduced by only 75%, with ongoing expression of some androgen response genes, including AR.¹¹ We recently showed that even with neoadjuvant LHRHa, bicalutamide, ketoconazole, and dutasteride, significant AR signaling remained.¹⁰ We thus designed this trial to evaluate the **pharmacodynamic** effect on prostate tissue of more intense androgen suppression.

Abiraterone acetate (AA) is a prodrug for abiraterone, a CYP450 **cytochrome P450 c17 (CYP17)** inhibitor, and provides more intensive ADT. All sources of androgens, including testicular, adrenal, prostate,

and, presumably, tumor androgens, are inhibited with AA,¹² with serum testosterone lowered to < 1 ng/dL.¹³ The growth of most castration-resistant PCa (CRPCs) may be maintained by persistent AR signaling through AR amplification/alteration and/or adaptive intratumoral androgen synthesis.¹⁴⁻¹⁷ AA treatment demonstrated improvements in survival and other response metrics in patients with metastatic CRPC, confirming the ongoing importance of AR signaling in CRPC.

Because in vivo effects of AA treatment on human prostate tissue have not been fully described, we investigated the impact of treatment using LHRHa combined with AA to determine whether intensive ADT would reduce intraprostatic androgens more than LHRHa alone after 12 weeks of therapy (primary end point, dihydrotestosterone [DHT] and testosterone levels) in the neoadjuvant setting of hormone-sensitive patients. Exploratory secondary end points included serum hormone and tissue pathology and **immunohistochemistry** (IHC) analysis.

PATIENTS AND METHODS

Patients

Intermediate- and high-risk patients had histologically confirmed localized PCa (\geq three positive biopsies) and \geq one of the following: PSA > 10 ng/mL, PSA velocity > 2 ng/mL per year (in preceding 12 months), and Gleason score \geq 7.

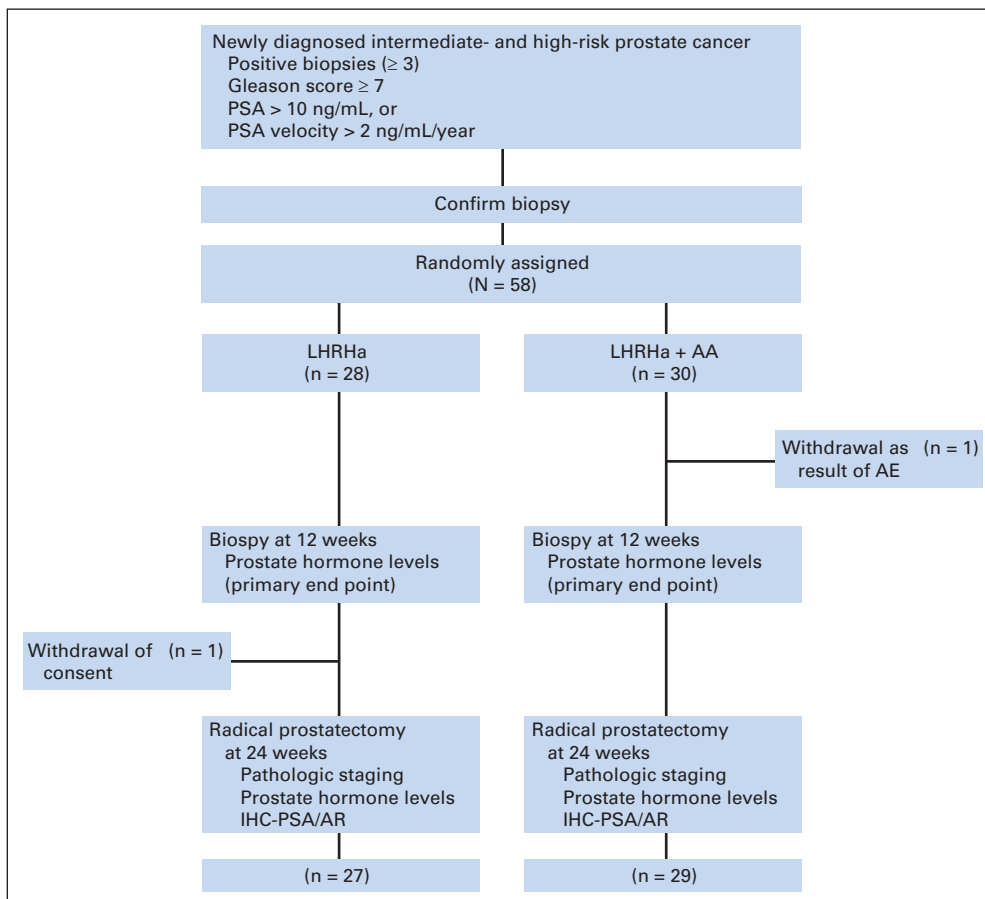


Fig 1. CONSORT diagram. Newly diagnosed intermediate- and high-risk patients with prostate cancer were randomly assigned to abiraterone acetate (AA) plus luteinizing hormone-releasing hormone agonist (LHRHa) plus prednisone versus LHRHa alone (at one-to-one ratio) for initial 12 weeks. After 12 weeks, patients in both groups received 12 weeks of AA, LHRHa, and prednisone followed by radical prostatectomy. Biopsy conducted at 12 weeks and postprostatectomy tissue sample at 24 weeks included analyses of prostate androgen levels and tissue pathologic response. Serum androgen levels were measured at baseline, week 12, and week 24. Prostate-specific antigen (PSA) levels were determined monthly. Patient who withdrew consent did not undergo prostatectomy; however, PSA data at week 24 were collected. AE, adverse effect; AR, androgen receptor; IHC, immunohistochemistry.

Study Design and Treatment

COU-AA-201 was a randomized open-label phase II study. Patients were randomly assigned at a one-to-one ratio to AA (1,000 mg/d), LHRHa (intramuscular; total of 22.5 mg over 12 weeks [7.5 mg every 4 weeks]) plus prednisone (5 mg/d) versus LHRHa for 12 weeks, with baseline risk stratification (high [Gleason score \geq 8 or PSA \geq 20 ng/mL] v intermediate [Gleason score 7 and PSA < 20 ng/mL]), followed by a research prostate biopsy for tissue hormone analysis. All patients received 12 additional weeks of LHRHa plus AA followed by radical prostatectomy (Fig 1). Thus, groups received 12 or 24 weeks of AA (LHRHa plus 12-week AA and LHRHa plus 24-week AA groups, respectively). Patients were enrolled from September 2009 through June 2011. Participating institutional review boards approved the study conducted per the Declaration of Helsinki, in accordance with the World Medicines Association and its amendments.

The primary end point was 12-week prostate tissue testosterone and DHT levels. Prespecified secondary end points included 12- and 24-week intraprostatic hormones, serum hormones, monthly serum PSA, and prostate pathologic assessment.

Pathology

Five pathologists performed blinded central review of selected slides for presence and extent of residual tumor and cellularity. Because ADT results in histologic changes, making tumor volume difficult to characterize accurately, residual cancer burden (RCB) was measured (ie, volume of tumor¹⁸ corrected by tumor cellularity). Tumor load was calculated using the largest cross-sectional dimension of tumor in reconstructed whole cross-sectional slices, with minimal residual disease (MRD) defined as \leq 0.5 cm, and using RCB, with tumor volume calculated by three-dimensional volume estimation based on the largest cross-sectional tumor dimension and number of cross sections involved by tumor, corrected for tumor cellularity, with MRD defined as $RCB \leq 0.25 \text{ cm}^3$ (tumor volume $\leq 0.5 \text{ cm}^3 \times$ tumor cellularity $\leq 50\%$).

IHC

One block was selected from each case, and 4- μm sections were cut onto charged slides. AR staining was assessed in the cytoplasm and nucleus of cancer cells and benign luminal epithelial cells. Cytoplasmic PSA staining was assessed in both cell types. Percentage of immunoreactive cells was categorized as none, < 10%, 10% to 50%, and > 50% of stained cells.

Statistical Analysis

The planned sample size of 29 patients per group (to assure 24 patients with evaluable core biopsies) provided 80% power to detect a difference in 12-week intraprostatic DHT levels between 1.92 ng/mL (reported post-medical castration DHT levels)¹¹ in the LHRHa plus 12-week AA group and 0.95 ng/mL in the LHRHa plus 24-week AA group, using two-sided *t* test with type I error of 10%. Given the exploratory nature of the study, no adjustment to multiple testing was made.

Intraprostatic and serum androgen levels at 12 weeks were compared between treatment groups using analysis of variance and analysis of covariance, respectively, based on log-transformed data, with two-sided *P* values of $\leq .10$ considered statistically significant. The stratification factor and baseline level were used as covariates for serum androgens, whereas only the stratification factor was used for intraprostatic androgens, because intraprostatic baseline data were not collected. For the LHRHa plus 12-week AA treatment group, intraprostatic androgens before and after 12 weeks of LHRHa plus AA were also compared by paired *t* test. The proportion of patients whose PSA levels achieved a nadir ≤ 0.2 ng/mL was compared using Fisher's exact test. PSA and AR IHC staining were compared between different cell types or locations by Wilcoxon signed rank test; their associations with tissue androgen levels were evaluated by Spearman's rank correlation. No imputation of missing data was made. Additional methods are provided in the Appendix (online only).

Table 1. Baseline Patient Demographic and Clinical Characteristics

Characteristic	LHRHa Plus 12-Week AA (n = 28)		LHRHa Plus 24-Week AA (n = 30)	
	No.	%	No.	%
Age, years				
Median	55.0		60.0	
Range	50-70		50-74	
High risk (Gleason score \geq 8 or PSA \geq 20 ng/mL)	21	75	22	73
Intermediate risk (Gleason score < 8 or PSA < 20 ng/mL)	7	25	8	27
Gleason score at baseline				
7	8	29	10	33
8	10	36	6	20
9	10	36	11	37
10	0	0	3	10
PSA at baseline, ng/mL				
Median	12.1		6.4	
Range	2.7-316.6		2.0-128.8	
< 10	12	43	20	67
10 to < 20	9	32	6	20
≥ 20	7	25	4	13
Elevated PSA velocity*	7	30	4	17
Stage T3 at initial diagnosis	8	29	6	20
Time from initial diagnosis to first dose, days				
Median	59		60	
Range	20-383		23-113	

Abbreviations: AA, abiraterone acetate; LHRHa, luteinizing hormone-releasing hormone agonist; PSA, prostate-specific antigen.
*Rise in PSA of > 2 ng/mL between any two time points within 12 months preceding initial diagnostic prostate biopsy.

RESULTS

Baseline Patient Characteristics

Patients (median age, 58.0 years) had intermediate- or high-risk PCa (Table 1). Sixty-nine percent of patients had Gleason score ≥ 8 . The remaining patients had Gleason score of 7 and PSA > 10 ng/mL or elevated PSA velocity. Baseline patient characteristics were well matched between treatment groups.

Intraprostatic Hormone Results

Median intraprostatic androgen levels (dehydroepiandrosterone [DHEA], Δ^4 -androstene-3,17-dione, testosterone, and DHT) in 12-week biopsy specimens were markedly reduced by LHRHa plus AA compared with LHRHa (eg, DHT: LHRHa, 1.307 pg/mg [90% CI, 0.17 to 18.76] v LHRHa plus AA, 0.180 pg/mg [90% CI, 0.08 to 114.63]; testosterone: LHRHa, 0.098 pg/mg [90% CI, 0.05 to 1.47] v LHRHa plus AA, 0.061 pg/mg [90% CI, 0.03 to 0.30]; Fig 2). The differences at week 12 in adjusted mean of log-transformed data between the two treatment groups were statistically significant (testosterone, *P* = .0216; all other intraprostatic androgens, *P* < .001). At 12 weeks, pregnenolone and progesterone (CYP17 proximal steroids) were significantly increased with LHRHa plus AA versus LHRHa alone (ie, no AA; *P* < .001; Fig 2). In both treatment groups, 24-week

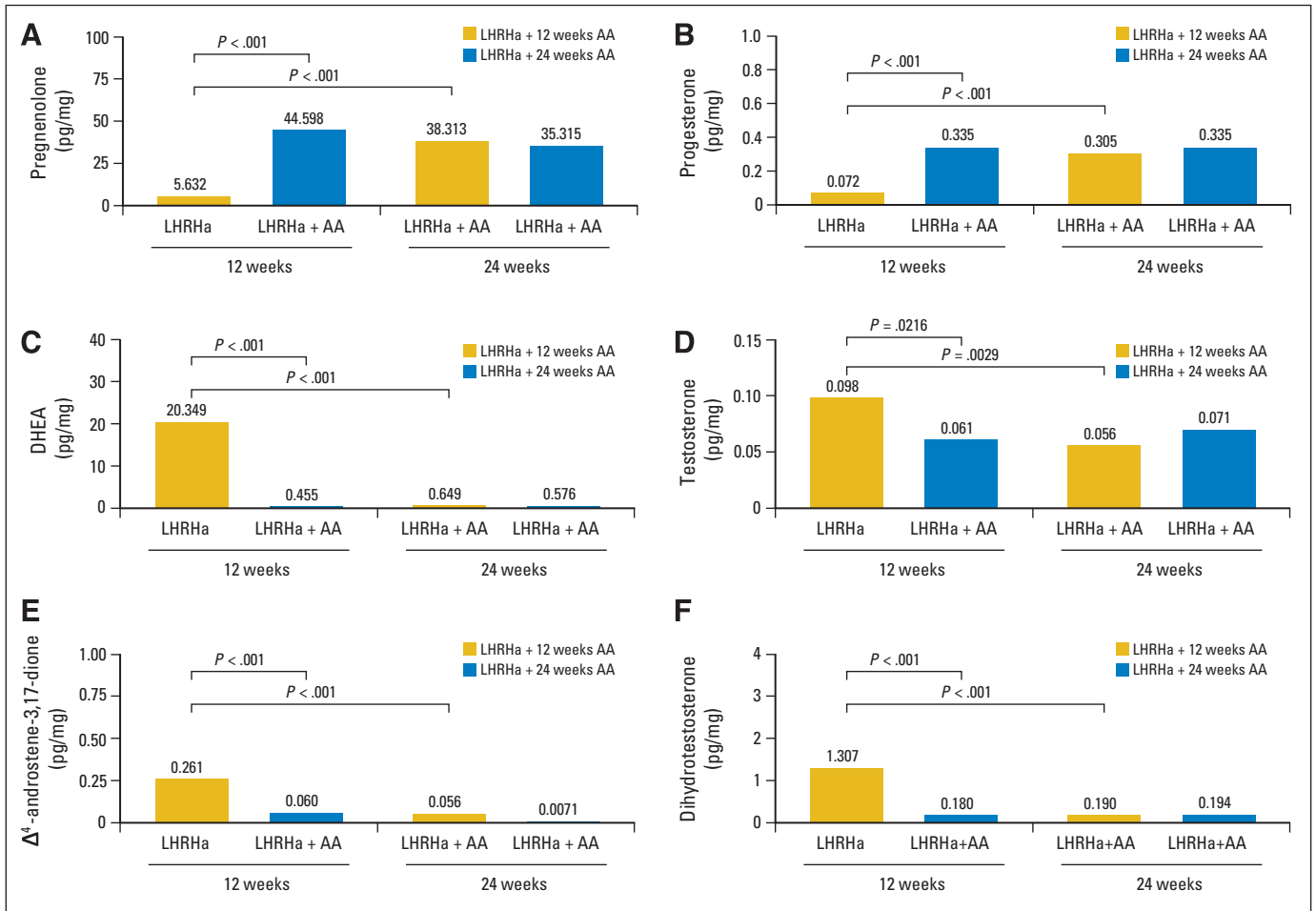


Fig 2. Abiraterone acetate (AA) reduces median intraprostatic tissue androgens via CYP17 inhibition. Compared with 12 weeks with luteinizing hormone–releasing hormone agonist (LHRHa) alone, CYP17 upstream androgens—(A) pregnenolone and (B) progesterone—were significantly higher in both LHRHa plus 12-week AA group and LHRHa plus 24-week AA group, whereas downstream androgens—(C) dehydroepiandrosterone (DHEA), (D) testosterone, (E) Δ⁴-androstene-3,17-dione, and (F) dihydrotestosterone (DHT)—were significantly lower in LHRHa plus 12-week AA group and LHRHa plus 24-week AA group. Lower limit of quantification for human prostate core tissue samples was 0.49 pg per sample for progesterone, Δ⁴-androstene-3,17-dione, DHT, and testosterone; 1.96 pg per sample for pregnenolone, and 0.98 pg per sample for DHEA.

testosterone and DHT levels were similar to those observed at week 12 in the LHRHa plus 24-week AA group.

PSA and Serum Hormone Results

At 12 weeks, median PSA was markedly lower with LHRHa plus AA (0.10 ng/mL) compared with LHRHa alone (1.06 ng/mL; Appendix Table A1, online only). By 24 weeks, median PSA levels in both the LHRHa plus 24-week AA and LHRHa plus 12-week AA groups were low.

Patients in both the LHRHa plus 12-week AA and LHRHa plus 24-week AA groups had markedly reduced mean serum androgen levels compared with baseline at both weeks 12 and 24 (Table 2). Serum levels of DHEA sulfate and DHEA glucuronide were reduced relatively less than other androgens, remaining at 10% of baseline levels at prostatectomy.

Pathology Results

The rates of pCR and MRD were greater in the LHRHa plus 24-week AA group (62% [90% CI, 45.1% to 77.1%] v 48% [90% CI, 31.3% to 65.3%]; Table 3), illustrated by greater density of this group

in waterfall plots of RCB dichotomized by MRD (Appendix Fig A1, online only; bottom left v top left panel). The log-transformed RCB correlated with ypT stage grouping between ypT ≤ 0.5 cm, 0.5 cm to ypT2, and ≥ ypT3, and residual tumor cellularity increased with ypT stage. The LHRHa plus 24-week AA group included more patients (seven v one) with residual tumor ≤ 0.5 cm; RCB was not lower in this cohort when residual cancer was > 0.5 cm.

Many patients had a significant volume of residual tumor, with ypT3 status in 48% and 59% and lymph node involvement in 24% and 11% of the LHRHa plus 24-week AA and LHRHa plus 12-week AA groups, respectively. One patient in the LHRHa plus 24-week AA group with MRD had lymph node metastases.

IHC

AR expression in prostatectomy specimens was highest in tumor nuclei versus tumor cytoplasm (P = .009) or benign nuclei (P < .001; Fig 3). Twenty-four percent of patients with residual tumor had > 10% PSA staining in tumor, supporting continued AR activity in some patients. PSA and AR IHC distributions were similar between treatment groups, except that the LHRHa plus 24-week AA group had

Table 2. Exposure to AA Significantly Reduces Serum Androgens

Serum Androgen (ng/dL)	LHRHa Plus 12-Week AA						LHRHa Plus 24-Week AA						P*
	Baseline (n = 28)		Week 12 (n = 28)		Week 24 (n = 27)		Baseline (n = 27)		Week 12 (n = 29)		Week 24 (n = 27)		
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	
Testosterone	429.4	0.5-1,183.7	17.3	0.5-61.1	5.3	0.5-64.5	425.1	141.3-765.0	6.7	0.5-14.2	15.5	0.5-330.4	< .001
DHT	28.6	2.5-129.9	13.3	2.5-54.3	17.2	2.5-349.8	40.1	2.5-205.5	12.5	2.5-134.6	6.1	2.5-30.6	.0176
DHEA	241.6	2.5-592.9	201.1	2.5-527.0	19.3	2.5-115.0	176.2	33.4-438.3	25.9	2.5-146.7	29.7	2.5-324.4	< .001
DHEA-glucuronide	1,901.5	351.9-10,480	1,508.2	2.5-5,593.7	515.5	2.5-2,284.1	1,522.1	380.4-7,432.6	415.1	2.5-1,872.3	292	2.5-1,225.2	< .001
DHEA-sulfate	230,810	24,191-519,046	200,846	5,855-547,036	22,379†	5,186-195,800	195,436	26,351-661,537	15,619‡	5,297-128,205	17,742†	5,391-167,957	< .001
Androsterone	11.3	0.5-61.6	5.4	0.5-56.8	0.6	0.5-1.9	9.4	0.5-35.0	1.0	0.5-7.6	0.8	0.5-4.4	< .001
Δ ⁴ -androstene-3,17-dione	76.5	2.5-254.2	51.6	2.5-137.5	7.4	2.5-37.4	59.4	24.5-133.2	9.2	2.5-35.1	7.9	2.5-70.6	< .001

Abbreviations: AA, abiraterone acetate; DHEA, dihydroepiandrosterone; DHT, dihydrotestosterone; LHRHa, luteinizing hormone-releasing hormone agonist.

*Mean comparison at 12 weeks based on log-transformed data, adjusted for baseline stratification factor (high v intermediate risk) and baseline androgen level.

†n = 26.

‡n = 28.

Table 3. Pathology Results

Variable	LHRHa Plus 12-Week AA (n = 27)		LHRHa Plus 24-Week AA (n = 29)	
	No.	%	No.	%
pCR	1	4	3	10
Largest CS dimension MRD (≤ 0.5 cm)	0	0	4	14
Total (pCR/largest CS dimension MRD)	1	4	7	24
RCB MRD (≤ 0.25 cm ³)	12	44	15	52
Total (pCR/RCB MRD)	13	48	18	62
> 0.5 cm to ypT2	10	37	8	28
\geq ypT3	16	59	14	48
Positive nodes	3	11	7	24
Positive margins	5	19	3	10

	LHRHa Plus 12-Week AA (n = 27)						LHRHa Plus 24-Week AA (n = 29)					
	≤ 0.5 cm (n = 1)		> 0.5 cm to ypT2 (n = 10)		\geq ypT3 (n = 16)		≤ 0.5 cm (n = 7)		> 0.5 cm to ypT2 (n = 8)		\geq ypT3 (n = 14)	
	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range
Tumor volume, cm ³	0	0-0	0.41	0.10-3.08	1.30	0.72-12.36	0	0-0.04	0.93	0.12-5.52	1.73	0.12-10.58
Tumor cellularity, %	0	0-0	10	1-50	30	1-60	3	0-60	15	3-27	39	9-75
Residual cancer burden, cm ³	0	0-0	0.02	0.01-1.48	0.29	0.01-6.80	0	0-0.02	0.12	0.01-1.49	0.74	0.05-5.90

Abbreviations: AA, abiraterone acetate; CS, cross sectional; LHRHa, luteinizing hormone-releasing hormone agonist; MRD, minimal residual disease; pCR, pathologic complete response; RCB, residual cancer burden.

higher expression of AR in tumor cytoplasm compared with the LHRHa plus 12-week AA group.

Safety Results

Six months of treatment with LHRHa plus AA with prednisone 5 mg daily was well tolerated, with a comparable incidence of adverse events (AEs) in the two treatment groups (Table 4). The most common AEs were hot flushes, increased AST and/or ALT, and fatigue. Grade 3 to 4 AEs and AEs leading to treatment discontinuation were comparable between groups. AEs of special interest, including mineralocorticoid-associated AEs, cardiac disorders, and liver function test abnormalities, were comparable between groups and not different than reported in phase III AA trials in which prednisone 5 mg twice daily was used.¹⁹⁻²¹ There were no increased complications from prostatectomy after neoadjuvant therapy.

DISCUSSION

To our knowledge, this trial is the first to assess the effects of LHRHa plus AA in hormone-naive localized PCa before prostatectomy. The primary objective was to test the hypothesis that addition of AA to LHRHa would reduce prostate tissue androgens compared with LHRHa alone at 12 weeks. The results demonstrate that as in serum, AA causes marked lowering of tissue androgens, with a rise in CYP17-upstream hormones, providing the first in vivo proof-of-principle demonstration of CYP17 inhibition on prostate tissue. Notably, as previously observed, changes in serum androgens do not accurately reflect the relative change in tissue androgen concentrations.²² Although addition of AA to LHRHa for 12 weeks decreased mean serum testosterone by 73% and DHT by 43%, AA decreased median intraprostatic levels of testosterone and DHT by 37% and 86%, respectively, emphasizing the importance of tissue-based measures to assess on-target efficacy.

Despite neoadjuvant therapy with LHRHa plus AA, tumor resistance was established early; responses were dichotomized as excellent (≤ 0.5 -cm tumor) or poor (\geq T3 or node positive). Fifty-four percent of patients (12- and 24-week AA) had residual cancer that was stage \geq ypT3a, and 18% had positive lymph nodes. A possible mechanism for LHRHa plus AA resistance is persistence of androgen metabolites within prostate tissue. Tissue levels of DHEA were higher than the other androgens, and interestingly, 24-week serum levels of DHEA-sulfate and DHEA-glucuronide remained at 10% of baseline levels. These levels of conjugated DHEA may provide a reservoir of androgen precursors that can be transported into tumor tissue for testosterone and DHT synthesis and contribute to LHRHa and AA resistance.^{23,24} Similarly, the higher levels of tissue testosterone and DHT at 12 weeks in the cohort receiving LHRHa without AA may reflect the impact of residual adrenal androgens, which could impede tumor apoptosis despite subsequent treatment with AA. The 12-week prostate biopsies also allowed investigation of intermediary tissue hormone levels as response biomarkers. However, there was no correlation between tissue androgens at 12 or 24 weeks with final pathologic assessments in either treatment arm (data not shown), suggesting resistance is mediated by factors in addition to AR ligand levels.

IHC analysis also provided insight into possible mechanisms of resistance. AR was present in the majority of tumor nuclei in both groups, and in the LHRHa plus 12-week AA group, nuclear AR was correlated with higher tissue androgens, suggesting an AR-related mechanism for LHRHa plus AA resistance. Immediate, intense ablation of tissue ligand may be critical for AR-regulated tumor death in some patients; however, persistent tumor was seen in the majority of patients, suggesting early emergence of resistance. However, it is also possible that PSA expression was low because of a global decrease in AR transcriptional activity but that these low levels of residual AR activity may still be important for tumor survival. Nuclear AR is a surrogate for AR transcription, and both groups had more nuclear

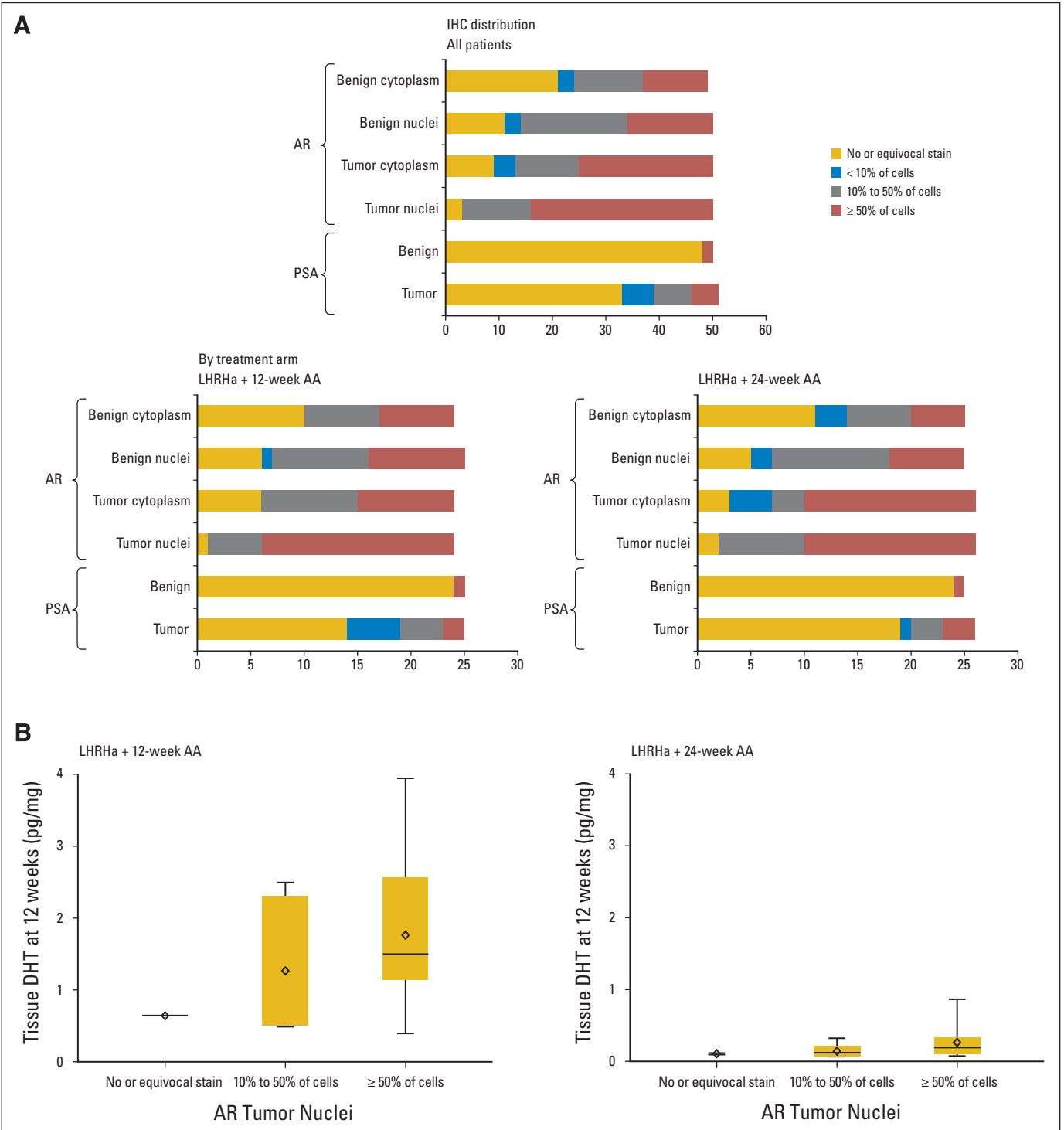


Fig 3. Androgen receptor (AR) and prostate-specific antigen (PSA) immunohistochemistry (IHC) on prostatectomy specimens. (A) AR and PSA IHC distributions in all patients and by treatment arm. (B) Box plots (mean, median, interquartile range, and range) of 12-week tissue dihydrotestosterone (DHT) levels by AR tumor nuclei expression levels. AA, abiraterone acetate.

than cytoplasmic AR in tumor cells compared with benign prostate cells at 24 weeks. The differential effect of abiraterone on the AR pathway in tumor versus benign cells suggests that tumor cells on AR suppression may be primed to circumvent androgen suppression, leading to tumor survival. These results suggest that either more intensive or longer ADT in combination with specific AR inhibition is

needed to kill tumor and/or suggest the presence of inherent non-androgen pathway mechanisms of resistance. Molecular interrogation is planned to help us further understand the tumor biology.

The trial was not powered to compare pathology outcomes between 12 and 24 weeks of AA treatment. A minority of participants, including some with Gleason score 8 to 10 and baseline T3 staging, had

small study or divergent biology of established nodal disease compared with the primary tumor. Larger trials are needed to establish the benefit of neoadjuvant therapy, but the 10% pCR rate is notable given the high-risk features of the cohort coupled with the detailed pathology analysis, both of which are lacking in the published literature.⁴⁻⁹ Surprisingly, pCRs have not been observed in neoadjuvant PCa trials of cytotoxic chemotherapeutics without ADT.^{26,27} Thus, we believe that ADT should be included in PCa neoadjuvant trials.

In PCa, the significance of the pathology response in the neoadjuvant setting has not been determined. In breast cancer, however, the pCR rate is considered clinically meaningful, and pertuzumab was approved in the neoadjuvant setting based on improvement in pCR.²⁸ To prove a benefit of neoadjuvant therapy in PCa, establishing clinically meaningful end points is paramount. To explore the pathology end point further, we went beyond classic pathology staging and measured residual tumor cellularity and calculated RCB and MRD. The waterfall plots of RCB presented by MRD ($\leq 0.25 \text{ cm}^3$ [tumor volume $\leq 0.5 \text{ cm}^3 \times$ tumor cellularity $\leq 50\%$]; Appendix Fig A1, online only) depict a higher density of patient cases with favorable response based on tumor volume and cellularity for 24 weeks of AA versus 12 weeks of AA. Consensus criteria for the measurement and reporting of pCR and MRD as performed here are not standardized. Central review provided a more detailed assessment compared with hospital pathology records, and on the basis of central review, there were fewer patients with ≤ 0.5 -cm tumor than reported previously.²⁹ This observation calls for standardized, expert pathologic characterization in neoadjuvant trials. Future studies are required to determine if these pathologic parameters, when assessed with freedom from PSA recurrence, salvage therapy, and metastasis, will represent a surrogate for cure, as has been observed for other malignancies.

The type and dose of corticosteroid with AA have effects on androgen synthesis pathway precursor hormones; our data are the first to our knowledge to provide insight into clinical prostate specimens. In the presurgery setting, a lower prednisone daily dose (5 mg) was chosen versus phase III CRPC AA trials (10 mg) to avoid potential corticosteroid toxicities.¹⁹⁻²¹ In the context of AA therapy without steroids, serum adrenocorticotropic hormone (ACTH) increases, with resultant increase in upstream precursors, including pregnenolone and progesterone as well as formation of 11-deoxycorticosterone, a potent mineralocorticoid, and potentially small increases in androgens downstream of CYP17 blockade. The addition of dexamethasone reverses the ACTH effect based on changed urinary hormone metabolites.³⁰ Our data demonstrate that clinical manifestations of mineralocorticoid excess were not different than those reported in phase III AA trials.¹⁹⁻²¹ More complete suppression of ACTH with alternative steroid dosing or inhibition of upstream steroid precursors with more intensive CYP17 inhibition may further lower tissue androgens and can be explored in future trials.

Prostatectomy alone is inadequate therapy for many patients with high-risk localized PCa, and neoadjuvant systemic therapy provides an opportunity to improve cure rates. Our data demonstrate that extremely low levels of prostate tissue androgens are achieved with LHRHa plus AA compared with LHRHa alone. pCRs and MRD were observed in a minority of patients; however, many had residual T3 or lymph node–positive staging at radical prostatectomy. The presence of nuclear AR and some residual tissue androgens suggests an AR-

related resistance mechanism, which is established early and which may potentially be abrogated with combination therapy. Progress will require identification of patients who will benefit from neoadjuvant therapy, validation of surrogates for cure, and establishment of an optimal treatment regimen.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: Christopher M. Haqq, Janssen Pharmaceuticals (C); NamPhuong Tran, Janssen Pharmaceuticals (C); Weimin Peng, Janssen Pharmaceuticals (C); Thian Kheoh, Janssen Pharmaceuticals (C); Arturo Molina, Janssen Pharmaceuticals (C) **Consultant or Advisory Role:** Mary-Ellen Taplin, Janssen Pharmaceuticals (C); Christopher J. Logothetis, Janssen Pharmaceuticals (C), Astellas Pharma (C), Medivation (C), Novartis (C), Bayer AG (C), Exelixis (C), Bristol-Myers Squibb (U), Pfizer (U), Karyopharm Therapeutics (U), sanofi-aventis (U); Steven P. Balk, Johnson & Johnson (C), Astellas Pharma (C); Trevor M. Penning, Tokai Pharmaceuticals (C), SAGE Therapeutics (C); Peter S. Nelson, Janssen Pharmaceuticals (C), Medivation (C); Philip W. Kantoff, Janssen Pharmaceuticals (C) **Stock Ownership:** Trevor M. Penning, Penzymes; Christopher M. Haqq, Johnson & Johnson; NamPhuong Tran, Johnson & Johnson; Thian Kheoh, Johnson & Johnson; Arturo Molina, Johnson & Johnson **Honoraria:** Christopher J. Logothetis, Janssen Pharmaceuticals, Astellas Pharma, Medivation, Novartis, Bayer AG, Exelixis; John W. Davis, Myriad Genetics, Intuitive Surgical; Elahe A. Mostaghel, Janssen Pharmaceuticals; Peter S. Nelson, Janssen Pharmaceuticals, Medivation **Research Funding:** Mary-Ellen Taplin, Janssen Pharmaceuticals; Bruce Montgomery, Janssen Pharmaceuticals; Christopher J. Logothetis, Janssen Pharmaceuticals, Astellas Pharma, Medivation, GlaxoSmithKline, Bristol-Myers Squibb, Novartis, Pfizer, sanofi-aventis, Bayer AG, Exelixis, Karyopharm Therapeutics; John W. Davis, Gen-Probe, Janssen Pharmaceuticals, Astellas Pharma, Medivation; Lawrence D. True, Janssen Pharmaceuticals; Steven P. Balk, Tokai Pharmaceuticals; Philip W. Kantoff, Janssen Pharmaceuticals **Expert Testimony:** None **Patents, Royalties, and Licenses:** None **Other Remuneration:** Peter S. Nelson, Janssen Pharmaceuticals

AUTHOR CONTRIBUTIONS

Conception and design: Mary-Ellen Taplin, Peter S. Nelson, Thian Kheoh, Arturo Molina, Philip W. Kantoff

Financial support: Arturo Molina

Administrative support: Arturo Molina, Philip W. Kantoff

Provision of study materials or patients: Glenn J. Bubley, Philip W. Kantoff

Collection and assembly of data: Mary-Ellen Taplin, Bruce Montgomery, Christopher J. Logothetis, Glenn J. Bubley, Jerome P. Richie, Bruce L. Dalkin, Martin G. Sanda, John W. Davis, Massimo Loda, Lawrence D. True, Patricia Troncoso, Huihui Ye, Rosina T. Lis, Brett T. Marck, Alvin M. Matsumoto, Steven P. Balk, Elahe A. Mostaghel, Trevor M. Penning, Peter S. Nelson, Zhenyang Jiang, Christopher M. Haqq,

Daniel Tamae, NamPhuong Tran, Weimin Peng, Thian Kheoh, Arturo Molina, Philip W. Kantoff

Data analysis and interpretation: Mary-Ellen Taplin, Lawrence D. True, Patricia Troncoso, Rosina T. Lis, Elahe A. Mostaghel, Trevor M.

Penning, Wanling Xie, Zhenyang Jiang, NamPhuong Tran, Weimin Peng, Thian Kheoh, Arturo Molina, Philip W. Kantoff

Manuscript writing: All authors

Final approval of manuscript: All authors

REFERENCES

- D'Amico AV, Whittington R, Malkowicz SB, et al: Biochemical outcome after radical prostatectomy, external beam radiation therapy, or interstitial radiation therapy for clinically localized prostate cancer. *JAMA* 280:969-974, 1998
- Albertsen PC, Hanley JA, Fine J: 20-year outcomes following conservative management of clinically localized prostate cancer. *JAMA* 293:2095-2101, 2005
- Albertsen PC: The face of high risk prostate cancer. *World J Urol* 26:205-210, 2008
- Aus G, Abrahamsson PA, Ahlgren G, et al: Hormonal treatment before radical prostatectomy: A 3-year followup. *J Urol* 159:2013-2016, 1998
- Fair WR, Cookson MS, Stroumbakis N, et al: The indications, rationale, and results of neoadjuvant androgen deprivation in the treatment of prostatic cancer: Memorial Sloan-Kettering Cancer Center results. *Urology* 49:46-55, 1997
- Klotz LH, Goldenberg SL, Jewett M, et al: CUOG randomized trial of neoadjuvant androgen ablation before radical prostatectomy: 36-month post-treatment PSA results—Canadian Urologic Oncology Group. *Urology* 53:757-763, 1999
- Meyer F, Moore L, Bairati I, et al: Neoadjuvant hormonal therapy before radical prostatectomy and risk of prostate specific antigen failure. *J Urol* 162: 2024-2028, 1999
- Soloway MS, Pareek K, Sharifi R, et al: Neoadjuvant androgen ablation before radical prostatectomy in cT2bNxm0 prostate cancer: 5-year results. *J Urol* 167:112-116, 2002
- Gleave ME, Goldenberg SL, Chin JL, et al: Randomized comparative study of 3 versus 8-month neoadjuvant hormonal therapy before radical prostatectomy: Biochemical and pathological effects. *J Urol* 166:500-506, 2001
- Mostaghel EA, Nelson PS, Lange P, et al: Targeted androgen pathway suppression in localized prostate cancer: A pilot study. *J Clin Oncol* 32:229-237, 2014
- Mostaghel EA, Page ST, Lin DW, et al: Intraprostatic androgens and androgen-regulated gene expression persist after testosterone suppression: Therapeutic implications for castration-resistant prostate cancer. *Cancer Res* 67:5033-5041, 2007
- O'Donnell A, Judson I, Dowsett M, et al: Hormonal impact of the 17 α -hydroxylase/C(17,20)-lyase inhibitor abiraterone acetate (CB7630) in patients with prostate cancer. *Br J Cancer* 90:2317-2325, 2004
- Attard G, Reid AH, Yap TA, et al: Phase I clinical trial of a selective inhibitor of CYP17, abiraterone acetate, confirms that castration-resistant prostate cancer commonly remains hormone driven. *J Clin Oncol* 26:4563-4571, 2008
- Bonkhoff H, Berges R: From pathogenesis to prevention of castration resistant prostate cancer. *Prostate* 70:100-112, 2010
- Montgomery RB, Mostaghel EA, Vessella R, et al: Maintenance of intratumoral androgens in metastatic prostate cancer: A mechanism for castration-resistant tumor growth. *Cancer Res* 68: 4447-4454, 2008
- Scher HI, Sawyers CL: Biology of progressive, castration-resistant prostate cancer: Directed therapies targeting the androgen-receptor signaling axis. *J Clin Oncol* 23:8253-8261, 2005
- Van Allen EM, Ryan CJ: Novel secondary hormonal therapy in advanced prostate cancer: An update. *Curr Opin Urol* 19:315-321, 2009
- Chen ME, Johnston D, Reyes AO, et al: A streamlined three-dimensional volume estimation method accurately classifies prostate tumors by volume. *Am J Surg Pathol* 27:1291-1301, 2003
- de Bono JS, Logothetis CJ, Molina A, et al: Abiraterone and increased survival in metastatic prostate cancer. *N Engl J Med* 364:1995-2005, 2011
- Fizazi K, Scher HI, Molina A, et al: Abiraterone acetate for treatment of metastatic castration-resistant prostate cancer: Final overall survival analysis of the COU-AA-301 randomised, double-blind, placebo-controlled phase 3 study. *Lancet Oncol* 13:983-992, 2012
- Ryan CJ, Smith MR, de Bono JS, et al: Abiraterone in metastatic prostate cancer without previous chemotherapy. *N Engl J Med* 368:138-148, 2013
- Page ST, Lin DW, Mostaghel EA, et al: Dihydrotestosterone administration does not increase intraprostatic androgen concentrations or alter prostate androgen action in healthy men: A randomized-controlled trial. *J Clin Endocrinol Metab* 96:430-437, 2011
- Wright JL, Kwon EM, Ostrander EA, et al: Expression of SLCO transport genes in castration-resistant prostate cancer and impact of genetic variation in SLCO1B3 and SLCO2B1 on prostate cancer outcomes. *Cancer Epidemiol Biomarkers Prev* 20:619-627, 2011
- Yang M, Xie W, Mostaghel E, et al: SLCO2B1 and SLCO1B3 may determine time to progression for patients receiving androgen deprivation therapy for prostate cancer. *J Clin Oncol* 29:2565-2573, 2011
- Reference deleted
- Febbo PG, Richie JP, George DJ, et al: Neoadjuvant docetaxel before radical prostatectomy in patients with high-risk localized prostate cancer. *Clin Cancer Res* 11:5233-5240, 2005
- Ross RW, Galsky MD, Febbo P, et al: Phase 2 study of neoadjuvant docetaxel plus bevacizumab in patients with high-risk localized prostate cancer: A Prostate Cancer Clinical Trials Consortium trial. *Cancer* 118:4777-4784, 2012
- Gianni L, Pienkowski T, Im YH, et al: Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): A randomised multicentre, open-label, phase 2 trial. *Lancet Oncol* 13: 25-32, 2012
- Taplin M-E, Montgomery RB, Logothetis C, et al: Effect of neoadjuvant abiraterone acetate (AA) plus leuprolide acetate (LHRHa) on PSA, pathological complete response (pCR), and near pCR in localized high-risk prostate cancer (LHRPC): Results of a randomized phase II study. *J Clin Oncol* 30: 282s, 2012 (suppl; abstr 4521)
- Attard G, Reid AH, Auchus RJ, et al: Clinical and biochemical consequences of CYP17A1 inhibition with abiraterone given with and without exogenous glucocorticoids in castrate men with advanced prostate cancer. *J Clin Endocrinol Metab* 97:507-516, 2012

Affiliations

Mary-Ellen Taplin, Massimo Loda, Rosina T. Lis, Wanling Xie, Zhenyang Jiang, and Philip W. Kantoff, Dana-Farber Cancer Institute, Harvard Medical School; Glenn J. Bubley, Huihui Ye, and Steven P. Balk, Beth Israel Deaconess Medical Center; Jerome P. Richie, Massimo Loda, and Rosina T. Lis, Brigham and Women's Hospital, Boston, MA; Bruce Montgomery, Bruce L. Dalkin, Lawrence D. True, and Alvin M. Matsumoto, University of Washington; Brett T. Marck and Alvin M. Matsumoto, Geriatric Research, Education and Clinical Center, Veterans' Affairs Puget Sound Health Care System; Elahe A. Mostaghel and Peter S. Nelson, Fred Hutchinson Cancer Research Center, Seattle, WA; Christopher J. Logothetis, John W. Davis, and Patricia Troncoso, University of Texas MD Anderson Cancer Center, Houston, TX; Martin G. Sanda, Emory University School of Medicine, Atlanta, GA; Massimo Loda, King's College London, London, United Kingdom; Trevor M. Penning and Daniel Tamae, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; and Christopher M. Haqq, NamPhuong Tran, Weimin Peng, Thian Kheoh, and Arturo Molina, Janssen Research and Development, Los Angeles, CA.

GLOSSARY TERMS

abiraterone acetate: selective inhibitor of androgen biosynthesis that potently blocks cytochrome P450c17 (CYP17).

androgen receptor: a DNA-binding and hormone-activated transcription factor important to the development and progression of prostate cancer. Its primary ligand is dihydrotestosterone. In later-stage (castration-resistant) prostate cancer, oncogenic alterations such as androgen receptor overexpression allow the androgen receptor to continue signaling despite undetectable, or castrate, levels of serum testosterone.

cytochrome P450 c17 (CYP17): a critical enzyme in testosterone synthesis with 17 α -hydroxylase and c17, 20 lyase activities, which are necessary for the conversion of pregnenolone to 17 α -hydroxypregnenolone and dehydroepiandrosterone and for the conversion of progesterone to 17 α -hydroxyprogesterone, respectively.

immunohistochemistry: the application of antigen-antibody interactions to histochemical techniques. Typically, a tissue section is mounted on a slide and incubated with antibodies (polyclonal or monoclonal) specific to the antigen (primary reaction). The antigen-antibody signal is then amplified using a second antibody conjugated to a complex of peroxidase-antiperoxidase, avidin-biotin-peroxidase, or avidin-biotin alkaline phosphatase. In the presence of substrate and chromogen, the enzyme forms a colored deposit at the sites of antibody-antigen binding. Immunofluorescence is an alternate approach to visualize antigens. In this technique, the primary antigen-antibody signal is amplified using a second antibody conjugated to a fluorochrome. On ultraviolet light absorption, the fluorochrome emits its own light at a longer wavelength (fluorescence), thus allowing localization of antibody-antigen complexes.

minimal residual disease (MRD): the low level of tumor cells (eg, after chemotherapy) that can only be detected with highly sensitive molecular methods (eg, polymerase chain reaction) or to molecularly defined relapse after long-term remission.

neoadjuvant therapy: the administration of chemotherapy prior to surgery. Induction chemotherapy is generally designed to decrease the size of the tumor prior to resection and to increase the rate of complete (R0) resections.

pathologic complete response: the absence of any residual tumor cells in a histologic evaluation of a tumor specimen.

pharmacodynamics: the study of the biochemical and physiological effects of a drug on the body.

prostate-specific antigen (PSA): a protein produced by cells of the prostate gland. The blood level of prostate-specific antigen (PSA) is used as a tumor marker for men who may be suspected of having prostate cancer. Most physicians consider 0 to 4.0 ng/mL to be the normal range. Levels of 4 to 10 and 10 to 20 ng/mL are considered slightly and moderately elevated, respectively. PSA levels have to be complemented with other tests to make a firm diagnosis of prostate cancer.

residual cancer burden (RCB): an index to estimate the extent of residual invasive cancer in the breast and regional lymph nodes after neoadjuvant chemotherapy. RCB combines parameters derived from the review of routine pathology materials: two-dimensional extent of residual primary tumor, proportion of this primary tumor area that contains cancer cells, proportion of the residual primary cancer that is in situ, the number of involved regional lymph nodes, and the diameter of the largest nodal metastasis.

Acknowledgment

We thank Elizabeth M. Genega, MD, who was involved in tissue sample collection at Beth Israel Deaconess Medical Center, and Ira Mills, PhD, PAREXEL, who provided writing assistance.

Presented in part at the 48th Annual Meeting of the American Society of Clinical Oncology, Chicago, IL, May 31-June 4, 2012; American Association of Genitourinary Surgeons Annual Meeting, April 3-6, 2013, New Orleans, LA; and American Urological Association Annual Meeting, May 4-8, 2013, San Diego, CA.

Appendix

Patients

Additional eligibility criteria beyond those described in the main article included Eastern Cooperative Oncology Group performance status of 0 to 1 and hemoglobin and chemical laboratory values that met predefined criteria. Exclusion criteria included prior androgen-deprivation therapy for prostate cancer, including luteinizing hormone–releasing hormone agonists/antagonists, orchiectomy, antiandrogens, ketoconazole, or estrogens (5α -reductase inhibitors allowed) and radiotherapy, chemotherapy, or immunotherapy for prostate cancer. Patients were not allowed the following agents during administration of study treatment: 5α -reductase inhibitors, nonsteroidal or steroidal antiandrogens, androgen receptor (AR) partial agonists, spironolactone, ketoconazole, chemotherapy, immunotherapy, estrogens, PC-SPES, PC-HOPE, or saw palmetto. All patients provided written consent to participate in the study.

Study Design and Treatment

The study was conducted at four academic centers: Dana-Farber Cancer Institute (Boston, MA; $n = 30$), MD Anderson Cancer Center (Houston, TX; $n = 13$), University of Washington (Seattle, WA; $n = 10$), and Beth Israel Deaconess Medical Center (Boston, MA; $n = 5$). Participants were evaluated every 4 weeks during the 24 weeks of androgen-deprivation therapy, with physical examination, vital signs, and laboratory assessment, including prostate-specific antigen, serum androgens (testosterone, dihydrotestosterone [DHT], Δ^4 -androstene-3,17-dione, dehydroepiandrosterone [DHEA], DHEA-sulfate, DHEA-glucuronide, androsterone), alkaline phosphatase, ALT, and AST. Research blood samples were obtained at baseline, week 12, and week 24 for hormone analysis.

Biopsies were performed according to standard methods in urology offices. One core from each area of collected tissue was snap frozen immediately in dry ice/ethanol bath for tissue androgen measurements. A second core from each area was embedded in optimal cutting temperature compound (Tissue-Tek; Pelco International, Redding, CA), snap frozen in Cryomolds with Tissue-Tek O.C.T. Compound (Sakura Finetek, Torrance, CA), and immersed in isopentane precooled to a near-slush state in liquid nitrogen for histology and immunohistochemistry.

Both open and robotic prostatectomies were allowed per the treating urologist's discretion. One patient underwent cystoprostatectomy. All prostatectomy specimens were handled per consensus guidelines (Samaratunga H et al: *Mod Pathol* 24:6-15, 2011). The surface (surgical margin) was inked, tissue from cancer-containing regions were snap frozen, and tissue blocks from transverse sections were fixed in buffered formalin and embedded in paraffin for subsequent histologic characterization. Research prostate biopsies were obtained in the operating room with a standard 18-gauge biopsy gun, before any tissue ischemia. Robot-assisted surgeons used a 3-mm suprapubic port to gain access to the fully vascularized prostate during the dissection.

Study Assessments

Prostate specimens were submitted entirely, and all formalin-fixed paraffin-embedded and frozen section slides were submitted for evaluation. For measurement of intraprostatic ketoandrogens, two frozen needle biopsy tissue cores were weighed, added to 60°C water containing deuterated internal standards, heated at 60°C for 10 minutes, homogenized using a Precellys tissue homogenizer (Bertin, Rockville, MD), supernatant extracted twice with hexane: ethyl acetate (80:20 v/v), organic layer dried in a SpeedVac (Thermo Scientific, Waltham, MA), derivatized with 0.025-M hydroxylamine hydrochloride for 24 hours at room temperature to form oximes, and quantified using electrospray ionization tandem mass spectrometry. Serum ketoandrogens (unconjugated and those released by digestion with glucuronidase and sulfatase) were derivatized as Girard T oximes and quantified using a stable isotope dilution liquid chromatography electrospray ionization–selected reaction monitoring mass spectrometry method at three time points on entry into the trial and at 12 and 24 weeks (Tamae D et al: *J Steroid Biochem Mol Biol* 138:281-289, 2013).

Measured intraprostatic hormones included DHEA, testosterone, Δ^4 -androstene-3,17-dione, and DHT. Serum hormones included testosterone, DHT, DHEA, DHEA-glucuronide, DHEA-sulfate, androsterone, and Δ^4 -androstene-3,17-dione.

Immunohistochemistry

Slides were evaluated by two study pathologists (M.L., R.T.L.). Slides were stained with anti-AR (M3562, clone AR441, lot No. 10070477; Dako North America, Carpinteria, CA) and with anti–prostate-specific antigen (M0750, clone ER-PR8, lot No. 36,017; Dako). Antigen retrieval was performed in citrate buffer using a microwave set on high for 5 minutes and was repeated three times. After antigen retrieval, slides were transferred to an automated staining platform (BioGenex i6000; BioGenex, Fremont, CA). Slides were rinsed in a phosphate-buffered saline-T wash for 15 minutes, incubated in a commercial peroxidase blocking solution (Dako) for 30 minutes, and then incubated with protein block (Dako) for 20 minutes. The slides

were then incubated with the primary antisera to AR (ratio, 1:50; 1 hour) and to PSA (ratio, 1:250; 1 hour). For antibody visualization, a peroxidase-based detection kit was used (Envision; Dako), following the manufacturer’s protocol. The slides were counterstained with hematoxylin (BioGenex), dehydrated with alcohol and xylene, and cover slipped.

Safety Assessments

Safety assessments included adverse events (AEs), serious AEs, AEs of special interest, including hypokalemia, hypertension, fluid retention/edema, and liver function tests, and AEs that led to discontinuation of study treatment. Assessments of perioperative AEs/serious AEs were collected by questionnaire during a 1-month postoperative visit.

Table A1. PSA Levels

PSA (ng/mL)	LHRHa Plus 12-Week AA (n = 28)		LHRHa Plus 24-Week AA (n = 30)*		P†
	Median	Range	Median	Range	
Baseline	12.1	2.69-316.57	6.365	2.00-128.82	
Week 4	4.34	0.53-179.21	0.65	0.10-7.61	
Week 8	1.35	0.26-132.97	0.17	0.04-1.17	
Week 12	1.06	0.10-109.29	0.10	0.02-0.50	
Week 16	0.20	0.04-27.53	0.09	0.01-0.59	
Week 20	0.09	0.01-11.95	0.06	0.01-0.84	
Week 24	0.06	0.00-8.32	0.04	0.01-2.02	
≤ 0.2 at 12 weeks					< .001
No.	1 of 28		26 of 29		
%	4		90		
≤ 0.2 at 24 weeks					.42
No.	23 of 28		26 of 28		
%	82		93		

Abbreviations: AA, abiraterone acetate; LHRHa, luteinizing hormone–releasing hormone agonist; PSA, prostate-specific antigen.

*PSA data (n = 29).

†Mean comparison at 12 weeks based on log-transformed data, adjusted for baseline stratification factor (high v intermediate risk) and baseline androgen level.

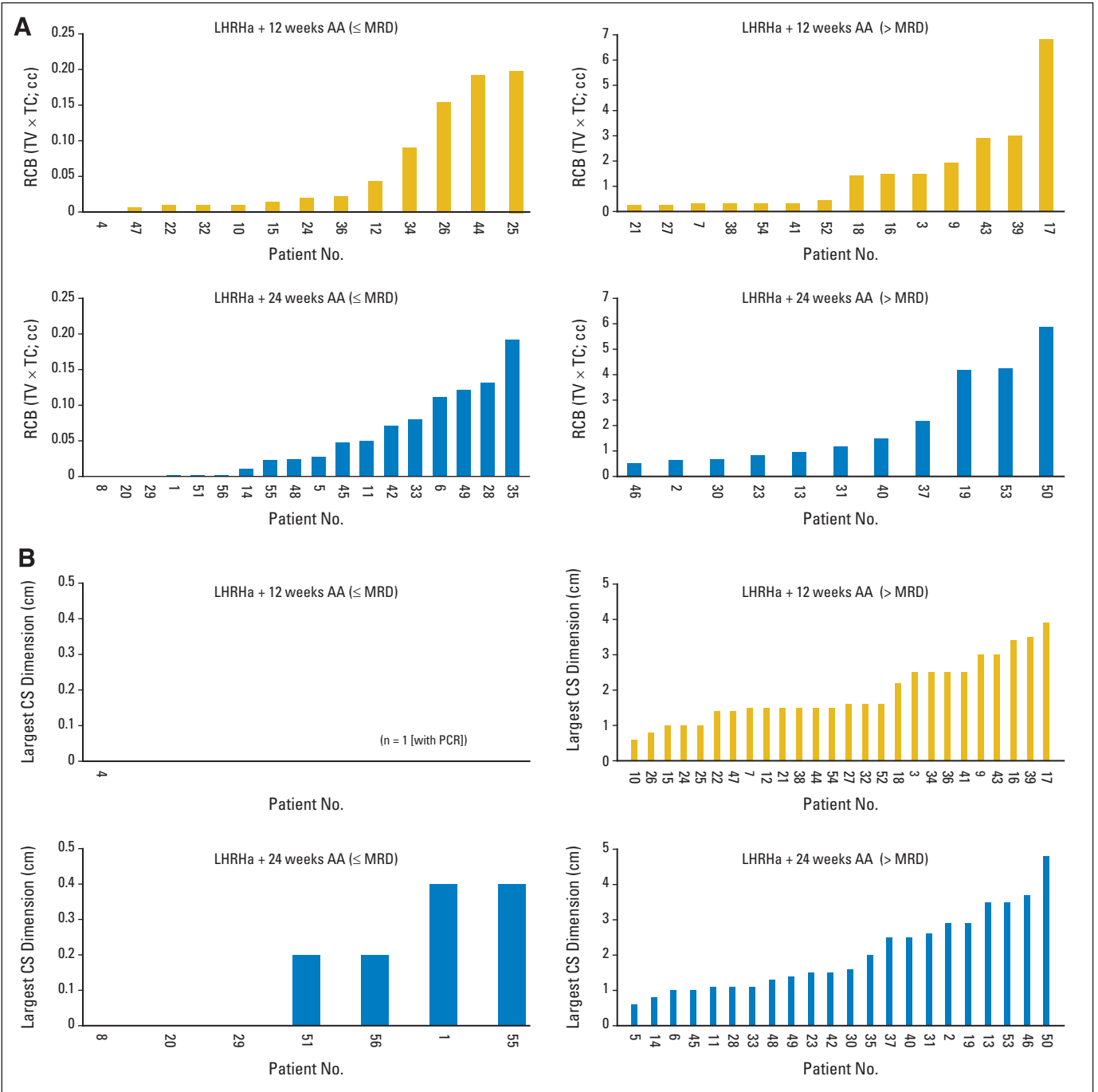


Fig A1. Waterfall plots of tumor load in individual patients. (A) Residual cancer burden (RCB) dichotomized by minimal residual disease (MRD; $\leq 0.25 \text{ cm}^3$). (B) Largest cross-sectional (CS) dimension by MRD ($\leq 0.5 \text{ cm}$). AA, abiraterone acetate; LHRHa, luteinizing hormone–releasing hormone agonist; pCR, pathologic complete response; TC, tumor cellularity; TV, tumor volume.