

Adrenal androgen levels as predictors of outcome in castration-resistant prostate cancer patients treated with combined androgen blockade using flutamide as a second-line anti-androgen

著者	Narimoto Kazutaka, Mizokami Atsushi, Izumi Kouji, Mihara Shinya, Sawada Kiyoshi, Sugata Toshiaki, Shimamura Masayoshi, Miyazaki Kimiomi, Nishino Akio, Namiki Mikio
journal or publication title	International Journal of Urology
volume	17
number	4
page range	337-345
year	2010-04-01
URL	http://hdl.handle.net/2297/23948

doi: 10.1111/j.1442-2042.2010.02473.x

Adrenal androgen levels as predictors of outcome in castration-resistant prostate cancer patients treated with combined androgen blockade using flutamide as second-line hormonal therapy

Kazutaka Narimoto¹, Atsushi Mizokami¹, Kouji Izumi¹, Shinya Mihara², Kiyoshi Sawada², Toshiaki Sugata³, Masayoshi Shimamura⁴, Kimiomi Miyazaki⁵, Akio Nishino⁶, and Mikio Namiki

1 Department of Integrative Cancer Therapy and Urology Kanazawa University
Graduate School of Medical Science

Department of Integrative Cancer Therapy and Urology, Kanazawa University Graduate
School of Medical Science, 13-1 Takaramachi, Kanazawa, Ishikawa 920-8641, Japan

Telephone: +81-76-265-2393; Fax:+81-76-222-6726;

2 Municipal Tsuruga Hospital

3 FukuiKen Saiseikai Hospital

4Ishikawa Prefectural Central Hospital

5 Kanazawa Arimatsu Hospital

6 Komatsu Municipal Hospital

Corresponding Author: Atsushi Mizokami,

Department of Integrative Cancer Therapy and Urology, Kanazawa University Graduate

School of Medical Science, 13-1 Takaramachi, Kanazawa, Ishikawa 920-8641, Japan

Telephone: +81-76-265-2393; Fax:+81-76-222-6726;

E-mail: mizokami@med.kanazawa-u.ac.jp

Shortened title: Androstenediol as outcome predictor of alternative antiandrogen therapy
using flutamide

Abstract

Objectives

We analyzed clinical effects and mechanism of flutamide as a second-line antiandrogen for combined androgen blockade (CAB) in patients with castration-resistant prostate cancer (CRPC) treated with bicalutamide as the first-line antiandrogen.

Methods

Our study population consisted of 16 patients with CRPC who were treated with flutamide (375 mg daily) as second-line hormonal therapy. Five androgens dehydroepiandrosterone (DHEA), androstenedione, androstenediol, testosterone, and dihydrotestosterone (DHT) were measured for investigating the relationship between plasma androgens and outcome following treatment. Furthermore we measured the adrenal androgen levels in medium of adrenal cancer cell line.

Results

Second-line hormonal therapy using flutamide resulted in a reduction of the PSA level in 14 (87.5%) of 16 patients. Greater than 50% decline in PSA was observed in 8 (50%) of the 16 patients. Median responsiveness duration was 6.25 months. PSA elevation of baseline androstenediol level was a predictive factor of PSA responsiveness. The lower DHEA group improved duration of responsiveness to flutamide. In vitro, 3 μ M

flutamide suppressed DHEA, androstenedione, and androstenediol synthesis compared with bicalutamide in medium of adrenal cancer cell line.

Conclusions

Our data demonstrated that flutamide suppressed the adrenal androgens compared with bicalutamide. We could predict the responsiveness and response duration of flutamide in patients with higher baseline androstenediol level and lower DHEA level. These data suggest that metabolites from adrenal androgens contribute to the progression of prostate cancer and that flutamide inhibits androgen synthesis.

Key word: adrenal androgen, prostate cancer, second-line hormonal therapy.

Introduction

In 2009, prostate cancer was the most commonly diagnosed malignancy in the USA.¹ Androgen-deprivation therapy is the standard treatment for men with advanced prostate cancer. Akaza *et al.* reported that treatment with bicalutamide (80 mg) in combination with an LHRH agonist is superior to LH-RH agonist monotherapy in terms of antitumor response at 12 weeks² In Japan, this combined androgen blockade (CAB) has become the most widely used form of hormonal therapy for advanced prostate cancer. While almost all patients with prostate cancer show a response to first-line hormonal therapy, up to one third of patients eventually experience biochemical recurrence. The median duration of responsiveness to CAB has been reported to be approximately 16 to 18 months.³ Relapse or progression after hormonal therapy is initially detected by an increasing PSA level, which typically predicts the worsening of clinical disease by 3 to 12 months.⁴ Although many urologists provide strategies such as radiation therapy or chemotherapy as the next stage of treatment for the progressive disease, these therapies have the adverse effects and do not show satisfactory efficacy.

In 1997, Scher *et al.* reported that 38.5% of patients with advanced prostate cancer who relapsed after first-line hormonal therapy responded to second-line hormonal therapy with flutamide.⁵ In Japan, it has recently been reported that patients

with castration-resistant prostate cancer (CRPC) responded to second-line hormonal therapy using flutamide.⁶⁻⁹ Previous reports showed a PSA decrease exceeding 50% in about half of all patients and prolonged clinical benefit with few adverse effects. Whitaker reported that not all antiandrogens work *via* the same mechanism and suggested that an informed sequential treatment regime may benefit prostate cancer patients.¹⁰ It remains unclear why flutamide is effective after first-line hormonal therapy using bicalutamide although both antiandrogens are nonsteroidal and bind the same ligand-binding domain. Flutamide may circumvent the mechanisms of failure after bicalutamide treatment or act through different means.

Although CAB using bicalutamide reduces serum testosterone levels by >95%, CAB does not reduce adrenal androgens, such as androstenedione, androstenediol, and dehydroepiandrosterone (DHEA).^{11, 12} The persistence of adrenal androgens has clinical significance, as these androgens have been shown to activate androgen receptors *in vitro*.¹³⁻¹⁵ Although several studies have demonstrated the efficacy of flutamide as second-line hormonal therapy after using bicalutamide, little is known about the precise mechanisms of the effects of this agent. Ayub reported that flutamide is different from bicalutamide in decreasing adrenal androgen by suppressing CYP17.¹⁶

It is, therefore, of interest to determine how flutamide affects adrenal

androgens and whether the levels of adrenal androgens may be of value for predicting prognosis in patients with CRPC. We evaluated the PSA responsiveness rate and androgen levels in patients with flutamide as second-line hormonal therapy. Furthermore we examined the effect of antiandrogens on adrenal androgens secretion in adrenal cancer cell line (NCI-H295R).

Patients and Methods

Patients

The eligibility criterion was histologically confirmed adenocarcinoma of the prostate with progressive disease. All patients were initially treated with CAB consisting of either surgical or medical castration using LHRH agonist plus antiandrogen (80 mg bicalutamide daily) as first-line hormonal therapy. Progression was defined as a PSA of $> 0.2\text{ng/mL}$, which had increased from baseline on at least three successive occasions at least 4 weeks apart. Patients were excluded if they had received prior radical prostatectomy, chemotherapy, immunotherapy, experimental therapy, or radiation therapy. All participants gave their informed consent to participation in this study in accordance with our institutional guidelines.

Procedures

When first-line hormonal therapy was judged to have failed, bicalutamide was discontinued to exclude antiandrogen withdrawal (AW). After confirmation of increasing serum PSA, subsequent second-line hormonal therapy using 375 mg of flutamide daily combined with either surgical or medical castration was started. In this series, clinical progression was not considered, and treatment failure was defined as increased serum PSA level on three successive occasions, and the response duration was regarded as the time from the start of second-line hormonal therapy to failure.

Good responders were patients in whom the PSA level decreased by more than 50% with second-line hormonal therapy, while nonresponders were those in whom PSA level continued to rise after commencement of second-line hormonal therapy, and others were defined as moderate responders. Patient plasma samples were isolated at 0, 4, and 12 weeks after commencement of therapy, and frozen until analysis at a commercial laboratory to determine androstenedione, androstenediol, DHEA, testosterone, and DHT levels by liquid chromatography tandem mass spectrometry (LC-MS/MS) at a commercial company (Teikoku Hormone MFG. Co., Kanagawa Japan). We divided the patients in two groups according to the median hormonal level and compared the PSA progression-free survival between the two groups.

Data analyses

Statistical analyses were performed using commercial software (Prism; Graphpad Software). Statistic analyses of differences in responsiveness to therapy were evaluated by the X^2 test or log rank test or two-ANOVA with $P < 0.05$ considered to indicate significance in all tests.

The effect of flutamide and bicalutamide on adrenal androgens secretion in vitro

The NCI-H295 (CRL-2128, ATCC, Rockville, MD, USA) cell line derived from a human adrenocortical carcinoma that expressed a multitude of steroidogenic cytochrome P450s and a standard model for the study of human adrenocortical steroidogenesis was purchased from American Type Culture Collection (ATCC, Manassas VA USA). NCI-H295R cells were maintained in Dulbecco's modified Eagle's medium/F12 (DMEM/F12, Invitrogen, Renfrew, UK) containing 2% Ultroser SF (Biosepra, France), 5 g/ml insulin, 5 g/ml transferrin and 5 ng/ml sodium selenite (as 1% ITS, Invitrogen). To see the effect of antiandrogens, the cells (3×10^5 cells per well) were treated in the above medium with the appropriate addition of flutamide or bicalutamide (0, 0.3, 1, and 3 μM) for 48h. After treatment, we collected the cultivated medium and measured adrenal androgens (DHEA, Androstenediol, Androstenedione) in

it using LC-MS/MS.

.

Results

Sixteen patients (median age, 76 years; range, 65 to 88 years) were recruited into this study between 26 May 2005 and 17 December 2008. All patients were refractory to first-line hormonal therapy (LH-RH agonist and bicalutamide). The median baseline of serum PSA was 122 ng/mL (range, 0.243 to 5904 ng/mL). The median nadir PSA after first-line CAB using bicalutamide was 0.700 ng/mL (range, 0.013 to 13.9 ng/mL). The median time to progression of the first-line hormonal therapy was 15 months (range, 3 to 90 months). Antiandrogen withdrawal (AW) was observed in three men (18.8%) and the median period of this phenomenon was 4.5 months (range, 1 to 9.5 months) (Table 1).

Second-line hormonal therapy using flutamide resulted in a reduction of the PSA level in 14 of the 15 patients (87.5%) (Fig. 1). Greater than 50% declines in PSA from the start of treatment were observed in 8 (50%) of 16 patients with CRPC. Twelve (75%) of 16, 4 (25%) of 16, and 2 (12.5%) of 16 patients had > 30%, > 75%, and > 90% declines in PSA 12 weeks after flutamide treatment, respectively. The median nadir PSA during second-line CAB was 1.063 ng/mL (range, 0.008 to 1805 ng/mL) and the median

duration of PSA responsiveness was 6.25 months (range, 1 to 24 months) after the start of second-line CAB therapy (Table 2). There were no severe side effects associated with second-line hormonal therapy using flutamide.

We subsequently analyzed several clinical factors associated with PSA responsiveness after second-line CAB using flutamide. As shown in Table 3, age, pretreatment PSA level, nadir PSA after first-line CAB, time to progression after first-line hormonal therapy, and AW PSA levels at the start of second-line hormonal therapy were not associated with PSA responsiveness to second-line therapy.

To investigate the effects of flutamide on adrenal androgens, we examined the concentration of serum adrenal androgens of the patients by LC-MS/MS. Since LC-MS/MS can examine androgen concentration more accurately than previous assays, we used this assay system in the present study. Concentrations of all adrenal androgens (DHEA, androstenedione, androstenediol, testosterone, and DHT) decreased from the start of second-line CAB (Fig. 2). To investigate the correlation between PSA responsiveness and decreases in androgen levels, we classified the patients according to the degree of PSA decrease induced by flutamide. Baseline of androstenediol of good responders, defined as patients in whom PSA level showed a decrease of $\geq 50\%$ by flutamide, was higher than that of patients defined as moderate responders (Fig. 3 A).

The relationships of all other baseline adrenal androgen levels to 50% PSA decline were not significant.

Furthermore, we demonstrated stratified data on changes in androstenediol level after introduction of second-line CAB. (Fig 3 B) Although androstenediol levels of the good responders were higher than that of the moderate responders at base level, there were no differences between good responders and moderate responders in decline rate of PSA level by flutamide.

We divided all patients into two groups (high androgen level and lower androgen levels) according to the median baseline androgen level to evaluate the association between the time to progression and baseline androgens. There were no differences in the association between time to progression and androstenediol, androstenedione, testosterone, or DHT level (data not shown). When we divided into higher and lower DHEA groups according to baseline DHEA level, a significant difference in time to progression was observed between the two groups. The median DHEA levels in the higher and lower DHEA groups were 1214.2 ng/mL (826.9 to 1713.7 ng/mL) and 736.2 ng/mL (289.7 to 810.0 ng/mL), respectively. As shown in Fig. 4, the time to progression of the patients with lower baseline level of DHEA was significantly longer than that in those with higher level of DHEA (0 – 24.0 months,

median 9.75 months vs. 0 – 9.0 months, median 6.00 months, $P = 0.04$).

In order to investigate the effect of antiandrogens on adrenal androgen synthesis in adrenal gland in vitro, we examined the concentration of adrenal androgens (DHEA, androstenedione, and androstenediol) after treatment with antiandrogens in adrenal cancer cell line NCI-H295R. As shown in Fig. 5, 3 μ M flutamide (the concentration that we can achieve clinically) suppressed all adrenal androgens compared with bicalutamide. This result indicated that flutamide inhibited CYP17 activity more than bicalutamide because the enzyme converted from these three androgens into testosterone

Discussion

The results of the present study indicated that second-line hormonal therapy using flutamide decreased adrenal androgens and was effective for bicalutamide-refractory prostate cancer. Although several studies have reported the effectiveness of second-line hormonal therapy, it was not clear why secondary therapy using flutamide is effective. Three hypotheses were proposed regarding the mechanism of the effectiveness of second-line hormonal therapy using flutamide: 1) differences in location of androgen

receptor mutation; 2) differences in affinity for androgen receptors; 3) differences in inhibitory effects of androgen synthesis. Hara and Happala reported that the mechanism of bicalutamide resistance was mutation of codon 741 in a bicalutamide-resistant cell line that they established *in vitro*.^{17, 18} On the other hand, Furutani and Kemppainen reported that flutamide was superior to bicalutamide in its inhibitory effect on androgen receptor transcriptional activity.^{19, 20} Recent studies also indicated the efficacy of ketoconazole and abiraterone, which inhibit cytochrome P (CYP) 17:17 α -hydroxylase, in suppressing castration-resistant prostate cancer.^{21, 22} CYP17 is a key enzyme related to androgen biosynthesis from cholesterol to DHEA. These reports indicated that adrenal androgens still play an important role in prostate cancer progression after castration. Ayub *et al.* reported that flutamide decreased the serum level of testosterone, DHT, and DHEA-S by inhibiting the activity of CYP17.¹⁶ We focused on the inhibitory effects of flutamide on androgen biosynthesis and investigated the prognostic factors in second-line hormonal therapy using flutamide. All adrenal androgens were inhibited in almost all cases by changing from bicalutamide to flutamide, suggesting that the difference in the inhibitory effect of androgen synthesis shows one of the mechanisms of the decrease of PSA by flutamide. Furthermore we had been found to suppress the adrenal androgen (DHEA, androstenedione and androstenediol) in NCI-H295R by

flutamide that we can achieve clinically. The results of this study demonstrated that the inhibition of adrenal androgens by flutamide was associated with suppression of prostate cancer in vitro.

The purpose of this study was to predict the effect of second-line hormonal therapy using flutamide. In terms of the characteristics of the enrolled patients, there were no significant differences in the distribution of age, pretreatment PSA, Nadir PSA after first line CAB, Time to progression after first-line CAB, antiandrogen withdrawal, PSA at start of second-line CAB. Although Kojima found that second-line responders tended to have lower PSA levels at the start of second-line CAB,⁶ we could not prove their results in the present study. Miyake and Nishimura also reported that PSA levels at the start of second-line CAB had no significant effect on the responsiveness to second-line CAB.^{7, 8} These results demonstrated that patient's characteristics were not associated with the effect of second-line hormonal therapy.

The present study showed that the patients who experienced a 50% decline in PSA in responsiveness to flutamide had a significantly higher baseline level of androstenediol compared with those who did not respond. Androstenediol is the precursor of testosterone in the adrenal steroid synthesis pathway, and this value may be of physiological importance. Furthermore, androstenediol may itself bind to the mutated

AR, or be converted to DHT, the androgen that binds with the highest avidity to and activates AR. Mizokami *et al.* reported that androstenediol is present in prostate cancer tissue after androgen deprivation therapy and activates androgen receptor in androgen-sensitive LNCaP cells more effectively than DHEA and androstenedione.¹²

Titus *et al.* and Stanbrough *et al.* showed that tumors progressing despite androgen deprivation therapy may contain measurable concentrations of adrenal androgens as well as express the enzymes responsible for conversion of adrenal androgens to testosterone.^{23, 24} A higher androstenediol level may identify patients more likely to benefit from flutamide. Although androstenediol was good predictive factor, it was not useful for transition of therapy effect because it was a little in serum. We supposed that we could use androstenediol levels as predictive factor, but could not use as response maker.

We showed that time to progression of second-line CAB responders with lower DHEA levels at the start of second-line CAB was extended relative to that of second-line CAB responders with higher DHEA levels. It is possible that the patients with low serum DHEA levels have low ability to produce DHEA from the adrenal gland. Because the prostate cancer still maintains androgenic sensitivity after the first line CAB, low-level androgen should be able to control growth of the prostate cancer. Therefore, since the androgenic source after the hormonal therapy is adrenal androgen DHEA, a group of the low DHEA level out of the serum will have good PFS.

Montgomery reported that castration-resistant metastases displayed alterations in

genes encoding steroidogenic enzymes compared with primary prostate tumors, and intracrine steroidogenesis may permit tumors to circumvent low levels of circulating androgens.²⁵ Stanbrough also demonstrated that the expression of genes associated with the conversion of adrenal androgens to testosterone may adapt to androgen deprivation.²³ However, if there is little supply of DHEA to CRPC, cancer progression may be delayed due to the inability to produce sufficient testosterone or DHT.

Although flutamide is known to act as an androgen receptor antagonist, the present study showed that flutamide inhibits biosynthesis of androgens, such as testosterone, DHEA, androstenediol, androstenedione, and DHT, and prevents the progression of CRPC. We hope to be able to use adrenal androgen as a predictor of the efficacy of second-line hormonal therapy.

Conclusions

Our study indicated that second-line hormonal therapy using flutamide resulted in improvement of clinical outcome in 50% of patients who relapsed after CAB using bicalutamide. Moreover, these data suggest that the baseline levels of androstenediol and DHEA are predictive factors of clinical outcome in patients with castration-resistant prostate cancer. We intend to further clarify those patients that are most likely to benefit

from flutamide and to target the mechanism of eventual resistance to flutamide therapy.

It will be necessary to collect data from more patients and examine the adrenal androgen levels to accurately investigate the prognostic factors after alternative antiandrogen therapy.

For Peer Review

References

1. Jemal, A., Siegel, R., Ward, E. *et al.*: Cancer statistics, 2009. *CA Cancer J Clin* 2009; **59**: 225-249
2. Usami, M., Akaza, H., Arai, Y. *et al.*: Bicalutamide 80 mg combined with a luteinizing hormone-releasing hormone agonist (LHRH-A) versus LHRH-A monotherapy in advanced prostate cancer: findings from a phase III randomized, double-blind, multicenter trial in Japanese patients. *Prostate Cancer Prostatic Dis* 2007; **10**: 194–201
3. Pienta, K. J., Bradley, D.: Mechanisms underlying the development of androgen-independent prostate cancer. *Clin Cancer Res* 2006; **12**: 1665–71
4. Newling, D. W., Denis, L., Vermeulen, K.: Orchiectomy versus goserelin and flutamide in the treatment of newly diagnosed metastatic prostate cancer. Analysis of the criteria of evaluation used in the European Organization for Research and Treatment of Cancer—Genitourinary Group Study 30853. *Cancer*

1993; **72**: 3793–8

5. Scher, H. I., Liebertz, C., Kelly, W. K. *et al.*: Bicalutamide for advanced prostate cancer: the natural versus treated history of disease. *J Clin Oncol* 1997; **15**: 2928–38

6. Kojima, S., Suzuki, H., Akakura, K. *et al.*: Alternative antiandrogens to treat prostate cancer relapse after initial hormone therapy. *J Urol* 2004; **171**: 679–83

7. Miyake, H., Hara, I., Eto, H.: Clinical outcome of maximum androgen blockade using flutamide as second-line hormonal therapy for hormone-refractory prostate cancer. *BJU Int* 2005; **96**: 791–5

8. Nishimura, K., Arichi, N., Tokugawa, S. *et al.*: Effects of flutamide as a second-line agent for maximum androgen blockade of hormone refractory prostate cancer. *Int J Urol* 2007; **14**: 264–7

9. Okihara, K., Ukimura, O., Kanemitsu, N. *et al.*: Clinical efficacy of

alternative antiandrogen therapy in Japanese men with relapsed prostate cancer after first-line hormonal therapy. *Int J Urol* 2007; **14**: 128–32

10. Whitaker, H. C., Hanrahan, S., Totty, N. *et al.*: Androgen receptor is targeted to distinct subcellular compartments in response to different therapeutic antiandrogens. *Clin Cancer Res* 2004; **10**: 7392–401

11. Belanger, A., Labrie, F., Dupont, A. *et al.*: Endocrine effects of combined treatment with an LHRH agonist in association with flutamide in metastatic prostatic carcinoma. *Clin Invest Med* 1988; **11**: 321–6

12. Mizokami, A., Koh, E., Fujita, H. *et al.*: The adrenal androgen androstenediol is present in prostate cancer tissue after androgen deprivation therapy and activates mutated androgen receptor. *Cancer Res* 2004; **64**: 765–71

13. Culig, Z., Stober, J., Gast, A. *et al.*: Activation of two mutant androgen receptors from human prostatic carcinoma by adrenal androgens and metabolic derivatives of testosterone. *Cancer Detect Prev* 1996; **20**: 68–75

14. Labrie, F., Belanger, A., Simard, J. et al.: Combination therapy for prostate cancer. Endocrine and biologic basis of its choice as new standard first-line therapy. *Cancer* 1993; **71**: 1059-67
15. Tan, J., Sharief, Y., Hamil, K. G. et al.: Dehydroepiandrosterone activates mutant androgen receptors expressed in the androgen-dependent human prostate cancer xenograft CWR22 and LNCaP cells. *Mol Endocrinol* 1997; **11**: 450-9
16. Ayub, M., Levell, M. J.: Suppression of plasma androgens by the antiandrogen flutamide in prostatic cancer patients treated with Zoladex, a GnRH analogue. *Clin Endocrinol (Oxf)* 1990; **32**: 329-39
17. Hara, T., Miyazaki, J., Araki, H. et al.: Novel mutations of androgen receptor: a possible mechanism of bicalutamide withdrawal syndrome. *Cancer Res* 2003; **63**: 149-53

18. Haapala, K., Hyytinen, E. R., Roiha, M. *et al.*: Androgen receptor alterations in prostate cancer relapsed during a combined androgen blockade by orchiectomy and bicalutamide. *Lab Invest* 2001; **81**: 1647–51
19. Furutani, T., Watanabe, T., Tanimoto, K. *et al.*: Stabilization of androgen receptor protein is induced by agonist, not by antagonists. *Biochem Biophys Res Commun* 2002; **294**: 779–84
20. Kemppainen, J. A., Wilson, E. M.: Agonist and antagonist activities of hydroxyflutamide and Casodex relate to androgen receptor stabilization. *Urology* 1996; **48**: 157–63
21. Ryan, C. J., Halabi, S., Ou, S. S. *et al.*: Adrenal androgen levels as predictors of outcome in prostate cancer patients treated with ketoconazole plus antiandrogen withdrawal: results from a cancer and leukemia group B study. *Clin Cancer Res* 2007; **13**: 2030–7
22. Attard, G., Reid, A. H., A'Hern, R. *et al.*: Selective Inhibition of

CYP17 With Abiraterone Acetate Is Highly Active in the Treatment of Castration-Resistant Prostate Cancer. *J Clin Oncol* 2009; in press

23. Stanbrough, M., Bubley, G. J., Ross, K. *et al.*: Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. *Cancer Res* 2006; **66**: 2815–25

24. Titus, M. A., Schell, M. J., Lih, F. B. *et al.*: Testosterone and dihydrotestosterone tissue levels in recurrent prostate cancer. *Clin Cancer Res* 2005; **11**: 4653–7

25. Montgomery, R. B., Mostaghel, E. A., Vessella, R. *et al.*: Maintenance of intratumoral androgens in metastatic prostate cancer: a mechanism for castration-resistant tumor growth. *Cancer Res* 2008; **68**: 4447–54

Legends

Fig. 1 Changes in PSA with flutamide. The waterfall plot shows the percentage change of PSA from baseline 12 weeks after flutamide treatment. The dashed line indicates 50% PSA decrease from baseline.

Fig. 2 Time course of changes in serum levels of various androgens after flutamide treatment. Columns show various serum androgens (DHEA, androstenediol, androstenedione, testosterone, and DHT) levels measured by LC-MS/MS analysis at baseline and 4 and 12 weeks after flutamide treatment. The data show the means \pm SD of serum androgen levels in all patients. Concentrations of all adrenal androgens decreased from the start of second-line CAB.

Fig. 3 Serum levels of various androgens in the good and intermediate responder groups. (A) Serum levels of various androgens in the good and intermediate responder groups after introduction of second-line CAB. (B) A chronologic change of androstenediol after the second line CAB using flutamide. The data show the means \pm SD of serum androgen levels of each group.

Fig. 4 Time to progression in responders. The patients who responded to flutamide ($n = 14$) were divided into two groups by baseline DHEA level. The Kaplan-Meier plot shows the time to PSA progression on flutamide treatment. The two lines show the lower DHEA level group (red line) and higher DHEA level group (blue line). The time to progression of the patients with lower baseline level of DHEA was significantly longer than that in those with higher level of DHEA.

Fig 5 The effect of bicalutamide and flutamide on the adrenal androgen synthesis in adrenal cancer cell line. Forty-eight h after treatment with the indicated concentrations of bicalutamide or flutamide, the cultivated medium was collected and the concentration of DHEA, androstenediol, and androstenedione were measured by LC-MS/MS analysis. Columns and bars represent the means \pm SD ($n=3$). * $P<0.05$.

Table 1 Patient Characteristics

Patient No	Age (years)	GS at Diagnosis	PSA at diagnosis (ng/mL)	Time to Progression after firstline therapy (months)	nadir PSA during firstline therapy (ng/mL)	Clinical Stage	EOD grade	Baseline PSA (ng/mL)	Baseline T (pg/mL)	Baseline DHT (pg/mL)	Baseline DHEA (pg/mL)	Baseline Adione (pg/mL)	Baseline Adiol (pg/mL)	Confirmed PSA decline (%)	Duration of PSA decline (months)
1	80	N/A	17.4	13	1.1	D2	1	2.7	115.1	17.8	675.8	313.4	20.2	No	0
2	67	4+3	101.9	15	0.2	D2	1	1.96	144.2	20.5	1494.2	562.6	71.9	47	6.5
3	77	4+5	7416.5	10	2.8	D2	4	5904	53.4	14.5	1130.9	280.3	67.0	26	1.75
4	69	3+5	40.66	23	0.06	D2	1	0.83	100.0	19.4	720.4	504.4	36.2	34	4.25
5	84	4+5	268.7	3	2.172	D2	1	11.278	130.3	35.2	1297.6	347.6	85.9	36	6
6	81	3+4	91.6	23	0.2	B2	0	0.243	70.1	16.3	752.1	117.0	93.8	71	26
7	71	N/A	29.1	60	0.1	D2	1	2.299	85.6	4.4	765.6	303.7	101.0	82	9.75
8	78	3+5	115.2	28	1.23	B1	0	5.12	55.9	2.7	930.5	234.6	53.7	67	9
9	88	4+5	1215	11	13.854	D2	4	70.557	74.9	12.8	289.7	214.0	32.8	34	3
10	74	5+4	84.4	10	0.961	D2	2	135.967	43.7	12.0	810.0	374.6	113.7	99	7
11	76	4+5	149.5	15	0.6	D2	1	5	77.3	7.0	777.0	206.4	46.8	86	17
12	72	4+5	221.5	66	0.2	C	0	1.589	89.6	22.1	1713.7	259.3	212.9	50	8
13	66	4+5	1813	64	0.1	D2	4	1.04	112.02	38.11	1460.69	509.37	191.51	63	8
14	76	N/A	18	90	0.8	N/A	N/A	4.37	71.42	7.00	424.78	253.14	39.68	12	8
15	65	4+5	1600	10	0.932	D2	4	7.58	124.98	8.14	863.78	552.78	78.38	99	6.5
16	75	4+3	122	14	0.013	D1	0	9.68	40.62	11.04	826.90	104.92	128.98	No	0

GS: Gleason score, T: testosterone, Adione: androstenedione, Adiol: androstenediol

Table2 Treatment outcomes			range
Response to first-line CAB			
	CR	15	
	PR	1	
Median : after first-line CAB			
	nadir PSA (ng/mL)	0.7	(0.013-5904)
	time to progression (months)	15	(3-90)
antiandrogen withdrawal phenomenon			
	Yes	3	
	No	13	
	Median peiod of antiandrogen withdrawal phenomenon (months)	4.5	(1-9.5)
Response to second-line CAB			
	good responder	8(50%)	
	moderate responder	6(42.9%)	
	none responder	2(14.3%)	
Median : after second-line CAB			
	PSA at start of second-line hormonal therapy (ng/mL)	4.6835	(0.243-5904)
	nadir PSA (ng/mL)	1.063	(0.387-1805)
	time to progression (months)	6.25	(0-24)

Table 3 Association between factors and response to second-line CAB therapy

		pts	response pts (%)	p-value
Age (years)	75 or more	9	3 (33%)	0.310
	under 75	7	5 (71%)	
Pretreatment PSA level (ng/ml)	150 or more	6	3 (50%)	1.000
	under 150	10	5 (50%)	
Nadir PSA after firstline CAB (ng/ml)	1 or more	5	1 (20%)	0.282
	under 1	11	7 (64%)	
Time to progression after first-line CAB (months)	15 or more	9	6 (67%)	0.310
	under 15	7	2 (28.6%)	
Antiandrogen withdrawal	Yes	3	2 (67%)	1.000
	No	13	6 (46.2%)	
PSA at start of second-line CAB (ng/ml)	2 or more	11	5 (45.5%)	1.000
	under 2	5	3 (60%)	

CAB:combined androgen blockade

Figure 1 Narimoto et al.

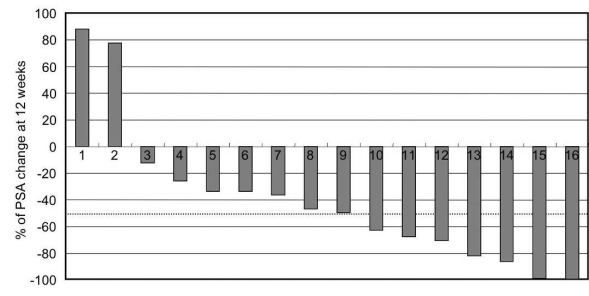


Fig. 1 Changes in PSA with flutamide. The waterfall plot shows the percentage change of PSA from baseline 12 weeks after flutamide treatment. The dashed line indicates 50% PSA decrease from baseline.
210x280mm (600 x 600 DPI)

Figure 2 Narimoto et al.

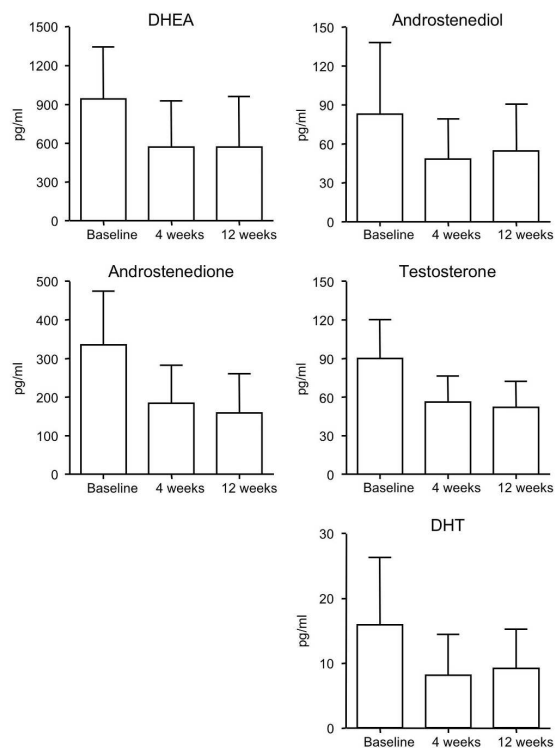


Fig. 2 Time course of changes in serum levels of various androgens after flutamide treatment. Columns show various serum androgens (DHEA, androstenediol, androstenedione, testosterone, and DHT) levels measured by LC-MS/MS analysis at baseline and 4 and 12 weeks after flutamide treatment. The data show the means \pm SD of serum androgen levels in all patients. Concentrations of all adrenal androgens decreased from the start of second-line CAB.
210x280mm (600 x 600 DPI)

Figure 3 Narimoto et al.

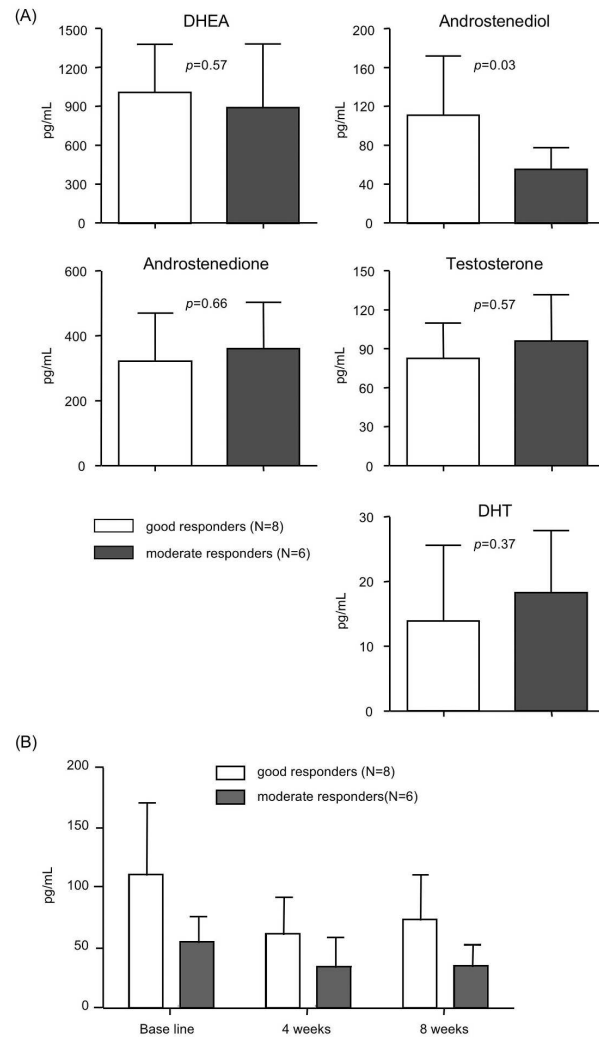


Fig. 3 Serum levels of various androgens in the good and intermediate responder groups. (A) Serum levels of various androgens in the good and intermediate responder groups, after introduction of second-line CAB. (B) A chronologic change of androstenediol after the second line CAB using flutamide. The data show the means \pm SD of serum androgen levels of each group. 210x280mm (600 x 600 DPI)

Figure 4 Narimoto et al.

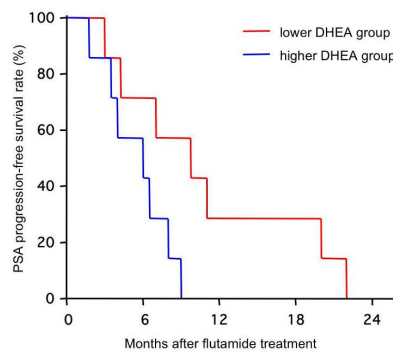


Fig. 4 Time to progression in responders. The patients who responded to flutamide ($n = 14$) were divided into two groups by baseline DHEA level. The Kaplan-Meier plot shows the time to PSA progression on flutamide treatment. The two lines show the lower DHEA level group (red line) and higher DHEA level group (blue line). The time to progression of the patients with lower baseline level of DHEA was significantly longer than that in those with higher level of DHEA.
210x280mm (600 x 600 DPI)

Figure 5 Narimoto et al.

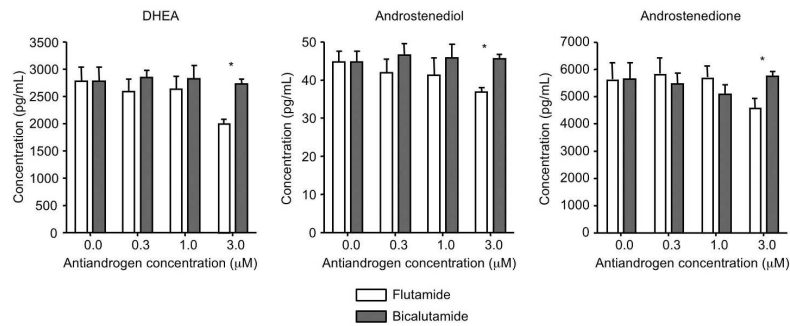


Fig 5 The effect of bicalutamide and flutamide on the adrenal androgen synthesis in adrenal cancer cell line. Forty-eight h after treatment with the indicated concentrations of bicalutamide or flutamide, the cultivated medium was collected and the concentration of DHEA, androstenediol, and androstenedione were measured by LC-MS/MS analysis. Columns and bars represent the means \pm SD (n=3). * P<0.05.
210x280mm (600 x 600 DPI)