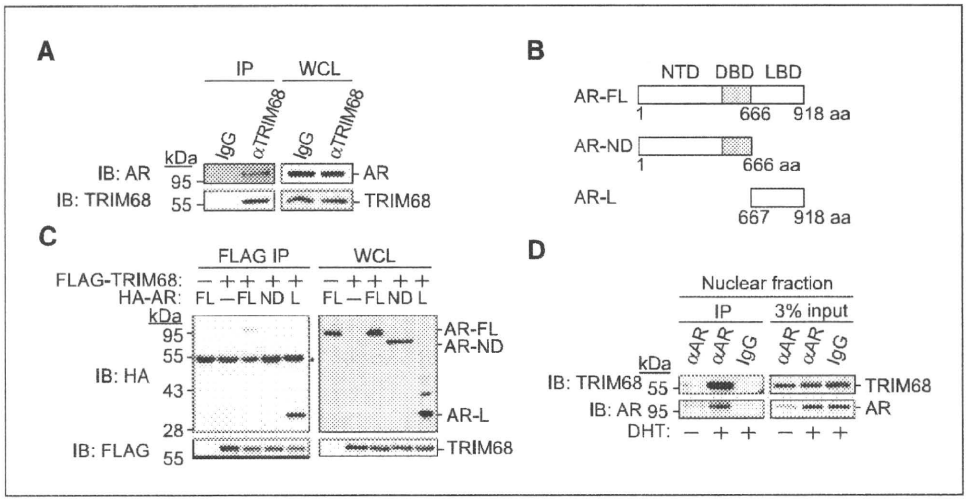


Figure 2. Interaction between TRIM68 and AR. *A*, interaction between endogenous TRIM68 and AR in prostate cancer cells. LNCaP cells were lysed and subjected to immunoprecipitation with anti-TRIM68 antibody followed by immunoblotting with anti-AR and anti-TRIM68 antibodies. Irrelevant IgG was used as a control. Cell lysates (WCL) were also subjected to immunoblotting to confirm the expression levels of endogenous TRIM68 and AR. *B*, schematic representation of the domain structures of AR derivatives. Deletion mutants of AR were constructed. AR-FL has full-length AR. AR-ND has an amino-terminal domain plus DNA-binding/hinge domain of AR. AR-L has a ligand-binding domain of AR. *NTD*, amino-terminal domain; *DBD*, DNA-binding/hinge domain; *LBD*, ligand-binding domain. *C*, ligand-binding domain of AR is responsible for the interaction with TRIM68. Expression vectors encoding HA-tagged AR derivatives (AR-FL, AR-ND, and AR-L) and FLAG-tagged TRIM68 were transfected into HEK293T cells as indicated. After 48 h, cells were lysed and subjected to immunoprecipitation with anti-FLAG antibody followed by immunoblotting with anti-HA and anti-FLAG antibodies. Whole-cell lysates were also subjected to immunoblotting to confirm the expression of AR derivatives and TRIM68. *, IgG heavy chain. *D*, interaction of TRIM68 with AR in the nucleus is enhanced by dihydrotestosterone (*DHT*) treatment. LNCaP cells were incubated in 10% charcoal–treated FBS medium for 48 h and then treated with or without 10 nmol/L dihydrotestosterone for 24 h. Subcellular fractions were separated biochemically, and then nuclear fractions were immunoprecipitated with anti-AR antibody followed by immunoblotting with anti-TRIM68 and anti-AR antibodies. Irrelevant IgG was used as a control. A portion of the nuclear extracts corresponding to 3% of the input for immunoprecipitation was also subjected to immunoblotting to confirm the expression levels of endogenous TRIM68 and AR in the nucleus.

Next, to confirm subcellular colocalization of TRIM68 and AR in the nucleus, we performed an immunoprecipitation assay using nuclear fractions of LNCaP cells treated or not treated with dihydrotestosterone. The immunoprecipitation assay showed that the nuclear interaction of TRIM68 with AR was enhanced by dihydrotestosterone treatment, suggesting that TRIM68 serves as a coregulator for androgen-dependent transcription (Fig. 2*D*).

TRIM68 enhances AR-mediated transcriptional activity. Having shown coprecipitation and colocalization of TRIM68 and AR, we next examined whether TRIM68 functionally affects AR-mediated transcription. To examine the effect of TRIM68 on AR-mediated transcriptional activity, we performed a luciferase reporter assay using an MMTV promoter-driven luciferase construct (MMTV-Luc). A TRIM68 expression vector and MMTV-Luc were transfected into LNCaP and CWR22Rv1 cells, and luciferase assays were then performed with and without dihydrotestosterone. The luciferase assays showed that TRIM68 enhances androgen-dependent AR-mediated transcriptional activity in a dose-dependent manner, whereas TRIM68ΔRING mutant, which lacks ubiquitin ligase activity, showed a dominant negative effect, suggesting that TRIM68 acts as a positive regulator for AR signaling and that ubiquitin ligase activity of TRIM68 is indispensable for AR transactivation (Fig. 3*A*).

To confirm the physiologic role of TRIM68 in AR-mediated transcription, we used RNAi to knockdown endogenous TRIM68 in LNCaP cells. Two different short interference RNAs (siRNA) targeting TRIM68 were introduced into LNCaP cells by using a retroviral infection system. RNAi treatment resulted in significant silencing of TRIM68 at protein level in LNCaP cells (Fig. 3*B*, *left*). To examine the effect of the depletion of TRIM68 on AR-mediated transcription, we performed a relative luciferase assay for AR signal in LNCaP cells transfected with TRIM68 siRNA. The relative luciferase activities of cells transfected with TRIM68 siRNA were decreased compared with those of cells transfected with the control siRNA (Fig. 3*B*, *right*).

To determine whether the effect of TRIM68 is specific to AR-mediated transcription, we transfected expression vectors encoding TRIM68 and wild-type AR, glucocorticoid receptor (GR) or ER into the androgen-independent prostate cancer cell line PC3 and performed luciferase reporter assays using each reporter system for AR, GR, and ER. Luciferase assays showed that TRIM68 markedly enhances AR-mediated transcription in PC3 cells transfected with AR, whereas TRIM68 enhances GR-mediated transcription to a lesser degree and has no effect on ER-mediated transcription (Fig. 3*C*). These findings indicate that TRIM68 is a comparatively specific coactivator for AR.

It has been reported that ubiquitin modification of substrates and the sequential degradation by the proteasome is involved in transcription activity of AR (25). To determine whether proteasome activity is required for TRIM68-mediated transcriptional activity of AR, we performed a luciferase assay for AR transactivation in the presence or absence of a proteasome inhibitor, MG132. TRIM68 enhanced AR transactivation in the absence of MG132, whereas the effect of TRIM68 was dramatically suppressed in the presence of MG132, indicating that the proteasome activity is required for the effect of TRIM68 on AR transcriptional activity (Fig. 3*D*).

TRIM68 cooperates with TIP60 and p300 to enhance AR-mediated transcriptional activity. The activity of AR is regulated by several posttranslational modifications. Previous studies have shown that AR and coregulators are regulated by histone acetyltransferases, such as TIP60 and p300, to enhance AR transcriptional activity (9, 26). Therefore, we hypothesized that TRIM68 physically or functionally interacts with TIP60. To determine whether TRIM68 physically interacts with TIP60, expression

vectors encoding FLAG-tagged TRIM68 and HA-tagged TIP60 were transfected into HEK293T cells. The cell lysates were subjected to immunoprecipitation with anti-HA antibody, and then immunoblot analysis was performed using anti-FLAG antibody. An *in vivo* binding assay showed that TRIM68 specifically interacts with TIP60 (Fig. 4*A*). To further determine whether TRIM68 functionally interacts with TIP60, we performed an AR transactivation assay. LNCaP cells were cotransfected with expression vectors encoding

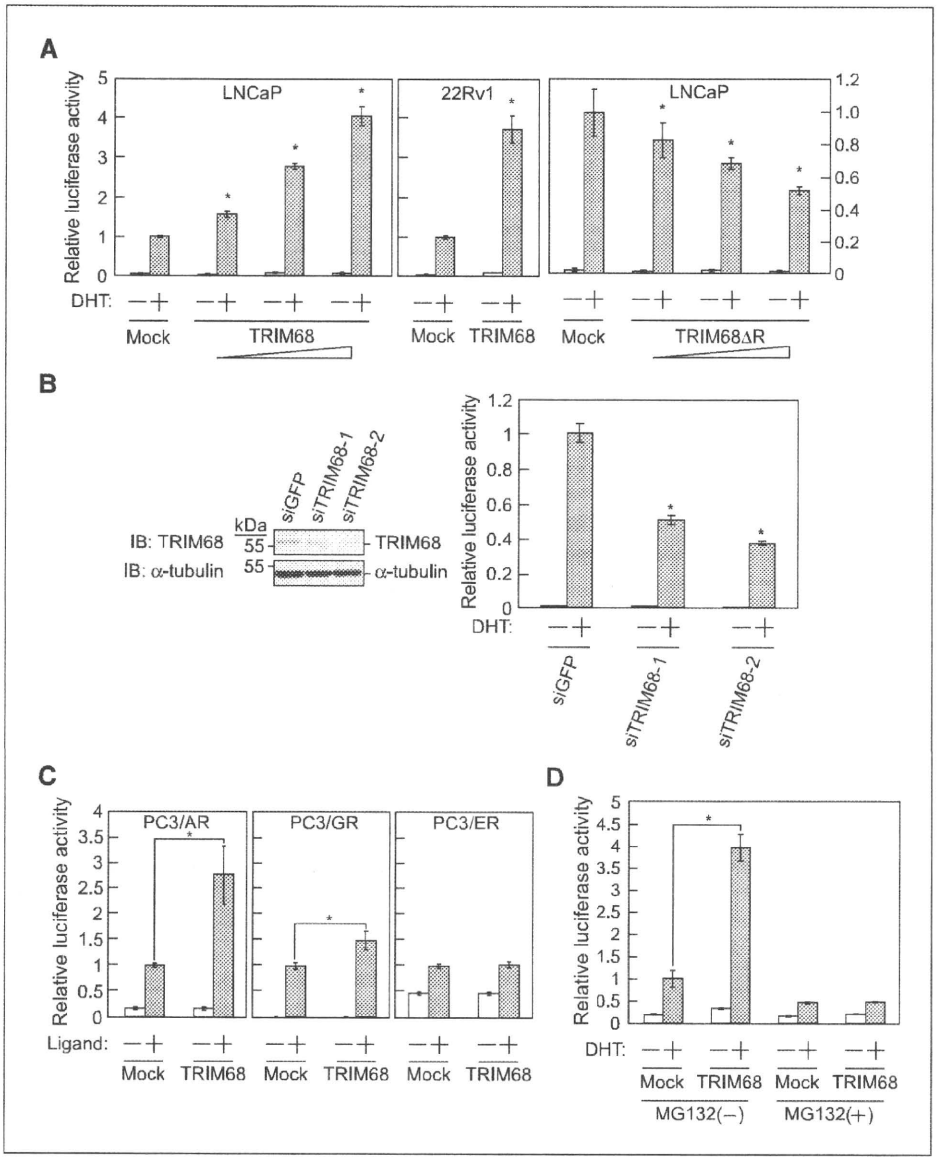


Figure 3. TRIM68 enhances AR-mediated transcriptional activity. *A*, TRIM68 enhances AR-mediated transcriptional activity in a dose-dependent manner. MMTV luciferase reporter vector (MMTV-Luc) and various amounts of TRIM68 expression vector (wild type or ΔR) were transfected into LNCaP (*left and right*) and CWR22Rv1 (*middle*) cells. Transfected cells were incubated in 10% charcoal–treated FBS medium for 48 h and then treated with or without 10 nmol/L dihydrotestosterone for 24 h. The cells were then harvested and assayed for luciferase activity. Relative luciferase activities of cells that had been transfected with an empty vector and then treated with dihydrotestosterone were defined as 1. *Columns*, mean of values from three independent experiments; *bars*, SD. *, statistically significant based on attaining *P*'s of <0.05 (unpaired Student's *t* test). *B*, knockdown of TRIM68 causes attenuation of AR-mediated transcriptional activity. Two different siRNAs targeting TRIM68 (siTRIM68-1 or siTRIM68-2) or targeting GFP (siGFP, used as a control) were introduced into LNCaP cells by a retrovirus expression system. Knocked-down LNCaP cell lines with siTRIM68 were analyzed by immunoblotting using anti-TRIM68 and anti–α-tubulin (an internal control) antibodies (*left*). Knocked-down LNCaP cell lines with siTRIM68 were transfected with MMTV-Luc reporter vector. Cells were incubated in 10% charcoal–treated FBS medium for 48 h and then treated with or without 10 nmol/L dihydrotestosterone for 24 h. The cells were then harvested and assayed for luciferase activity. Relative luciferase activities of cells that had been transfected with siGFP expression vector and then treated with dihydrotestosterone were defined as 1. *Columns*, mean of values from three independent experiments; *bars*, SD (*right*). *C*, effects of TRIM68 on the transcriptional activities of AR, GR, and ER. PC3 cells were transiently transfected with steroid receptor expression vectors (AR, GR, or ER), reporter vectors (MMTV-Luc for AR and GR, ERE-Luc for ER) and TRIM68 expression vector. Transfected cells were incubated in 10% charcoal–treated FBS medium for 48 h and then treated with or without a cognate ligand (10 nmol/L dihydrotestosterone, 10 nmol/L dexamethasone, or 10 nmol/L 17β-estradiol) for 24 h. The cells were then harvested and assayed for luciferase activity. Relative luciferase activities of cells that had been transfected with an empty vector and then treated with dihydrotestosterone, dexamethasone or 17β-estradiol were defined as 1. *Columns*, mean of values from three independent experiments; *bars*, SD. *D*, proteasome activity is required for the effect of TRIM68 on AR-mediated transactivation. MMTV-Luc and TRIM68 expression vectors were transfected into LNCaP cells. Transfected cells were incubated in 10% charcoal–treated FBS medium for 48 h, treated with 10 μmol/L MG132 for 30 min, and then treated with 10 nmol/L dihydrotestosterone for 8 h. The cells were then harvested and assayed to detect luciferase activity. Relative luciferase activities of cells that had been transfected with an empty vector and then treated with dihydrotestosterone in the absence of MG132 were defined as 1. *Columns*, mean of values from three independent experiments; *bars*, SD.

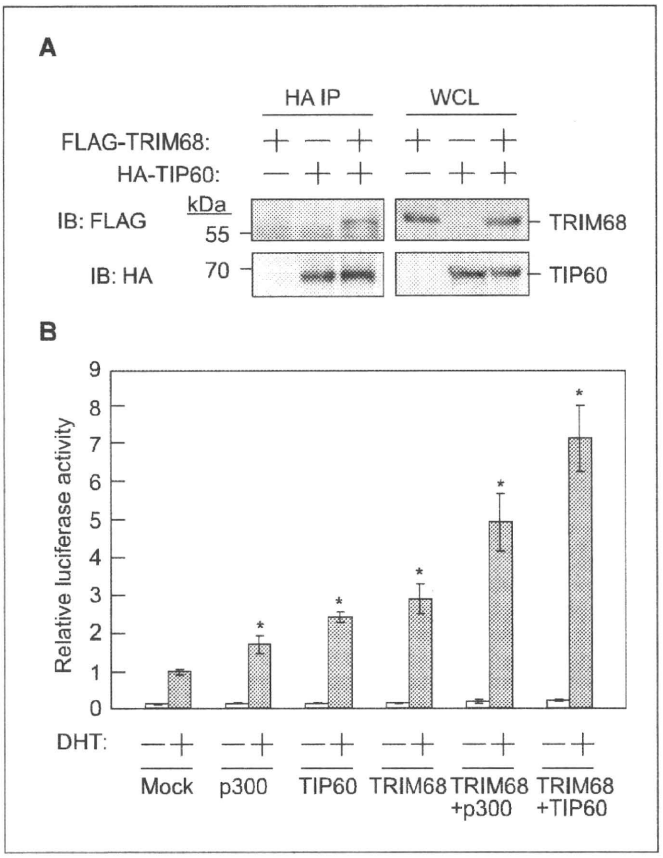


Figure 4. TRIM68 cooperates with TIP60 and p300 to enhance AR-mediated transactivation. *A*, physical interaction between TRIM68 and TIP60. Expression vectors encoding FLAG-tagged TRIM68 and HA-tagged TIP60 were transfected into HEK293T cells as indicated. After 48 h, cells were lysed and subjected to immunoprecipitation with anti-HA antibody followed by immunoblotting with anti-FLAG and anti-HA antibodies. Whole-cell lysates were also subjected to immunoblotting to confirm the expression of TRIM68 and TIP60 (*right*). *B*, TRIM68 cooperates with TIP60 or p300 to enhance AR-mediated transactivation. LNCaP cells were cotransfected with expression vectors encoding TIP60, p300, and TRIM68 with MMTV-Luc as a reporter. Transfected cells were incubated in 10% charcoal–treated FBS medium for 48 h and then treated with or without 10 nmol/L dihydrotestosterone for 24 h. The cells were then harvested and assayed for luciferase activity. Relative luciferase activities of cells that had been transfected with an empty vector and then treated with dihydrotestosterone were defined as 1. *Columns*, mean of values from three independent experiments; *bars*, SD. *, statistically significant based on attaining *P*'s of <0.05 (unpaired Student's *t* test).

TIP60 and/or TRIM68 with MMTV-Luc as a reporter and treated with dihydrotestosterone, and then luciferase assays were performed. TIP60 and TRIM68 individually enhanced AR-mediated transactivation with dihydrotestosterone treatment, and the combination of TIP60 and TRIM68 markedly enhanced AR-mediated transcriptional activity (Fig. 4*B*). Furthermore, the combination of another coactivator, p300, and TRIM68 also enhanced AR-mediated transcriptional activity (Fig. 4*B*). These findings suggest that TRIM68 cooperates with coactivators, including TIP60 and p300, for AR-mediated transactivation.

Effects of TRIM68 on the expression and secretion of PSA. PSA is a secretory glycoprotein that acts as a serine protease and exists exclusively in prostate epithelial cells (27). Serum PSA level is usually increased in patients with prostate cancer (28). Because the *PSA* gene contains an androgen-responsive element (ARE) and its transcriptional level is regulated by AR, we hypothesized that TRIM68 also affects the expression of PSA. LNCaP cells, which express AR and secrete PSA, were used for the PSA expression and secretion assays. LNCaP cells stably expressing TRIM68 or TRIM68ΔRING by a retroviral expression system were treated with or without dihydrotestosterone, and then cell lysates were subjected to immunoblot analysis with anti-PSA antibody (Fig. 5*A*). Furthermore, cell culture supernatants were collected and assayed for PSA concentration by ELISA analysis (Fig. 5*B*). TRIM68 increased both the expression and secretion of PSA by treatment with dihydrotestosterone, whereas TRIM68ΔRING did not. To confirm the relationship between TRIM68 and PSA production, we used LNCaP cells transfected with TRIM68 siRNA and analyzed the expression and secretion levels of PSA. Knockdown of TRIM68 decreased both the expression and secretion of PSA in LNCaP cells (Fig. 5*C* and *D*). These findings suggest that TRIM68 contributes to the expression and secretion of PSA in prostate cancer cells.

TRIM68 is overexpressed in human prostate cancer. Given that TRIM68 modulates AR-mediated transcription, we hypothesized that TRIM68 affects androgen-dependent cell growth. An MTS cell proliferation assay was performed to examine the effects of TRIM68 on cell growth. Knockdown of TRIM68 significantly inhibited the growth of LNCaP cells, whereas overexpression of TRIM68 slightly increased the growth of LNCaP cells (Fig. 6*A*). These results suggest that TRIM68 has a significant effect on the androgen-dependent growth of LNCaP cells.

To examine the effect of TRIM68 on oncogenic phenotype in prostate cancer cells, we performed an anchorage-independent colony formation assay in soft agar. Overexpression of TRIM68 marginally increased colony-forming rate, whereas knockdown of TRIM68 significantly inhibited colony formation of LNCaP cells, indicating that TRIM68 has a significant effect on the oncogenic properties of prostate cancer cells (Fig. 6*B*). These findings may indicate that TRIM68 is required for oncogenic properties of prostate cancer cells but is not sufficient to enhance oncogenic phenotypes (Fig. 6*A* and *B*).

Considering the involvement of TRIM68 in prostate cancer cell proliferation and PSA production, we hypothesized that TRIM68 is aberrantly expressed in human prostate cancers. TRIM68 mRNA levels of 35 cases of human prostate cancer and adjacent normal tissue, which were surgically resected by radical prostatectomy in patients with primary prostate cancer, were quantified by real-time quantitative RT-PCR. TBP was selected as an internal control to normalize the expression levels, because there are no known retropseudogenes for it and TBP is not differentially expressed in tumor and normal prostate tissues (29, 30). Relative mRNA levels of TRIM68 were significantly increased in the majority of human prostate cancers compared with the levels in normal prostate tissues (Mann-Whitney *U* test, $P < 0.05$; Fig. 6*C*). These results indicate that TRIM68 gene expression is up-regulated in human prostate cancer.

Next, to examine the protein expression levels of TRIM68 in human prostate cancers, prostate cancer tissues and adjacent normal tissues simultaneously obtained from radical prostatectomy in patients with primary prostate cancer were analyzed by immunohistochemistry using anti-TRIM68 antibody. TRIM68 staining in tissues was detected mainly in the nucleus of epithelial cells, as observed in LNCaP cells treated with dihydrotestosterone. TRIM68-expressing cells were more abundant in cancer tissues than in normal tissues (Fig. 6*D*, *a–f*). TRIM68 expression in the benign sample group was low or absent (mean staining score, 91). In cancer samples, on the other hand, TRIM68 exhibited mainly a moderate or high level of expression (mean staining score, 220), indicating that TRIM68 immunoreactivity was significantly higher

in cancer tissues than in benign tissues (Fig. 6*D*, *g*). These findings indicate that TRIM68 is overexpressed in human prostate cancer and may serve as a significant marker protein for prostate cancer.

Discussion

Recent advances have indicated that AR-mediated transactivation is regulated by posttranslational modification, including phosphorylation, acetylation, and ubiquitination. Ubiquitination involves degradation of AR and coregulators, and ubiquitination also has a nonproteolytic role in transcription (10). E3 ubiquitin ligases should play an important role in the regulation of AR-mediated transcriptional activity. However, only a few E3 ligases for AR, coactivators, or corepressors have been identified.

Recently, AR NH_2 terminal–interacting protein, also known as p53-induced protein with a RING-H2 domain (PIRH2), has been reported to interact with histone deacetylace 1 and promote its degradation (31). In addition, E6-AP, a HECT type ubiquitin ligase, has been shown to enhance the hormone-dependent transcriptional activity of AR (32). The coactivating function may result from the ability of E6-AP to target nuclear receptor corepressor (NcoR) for degradation (33). However, PIRH2 and E6-AP do not display tissue-specific expression in prostate, and the effects are not restricted to the regulation of AR activity.

The present study is the first study to provide evidence that TRIM68, which is preferentially expressed in prostate cancer cells, is a novel AR-interacting protein and acts as a coactivator of AR depending on its ubiquitin ligase activity. TRIM68 possesses E3 ubiquitin ligase activity in collaboration with E2, including Ubc4 and UbcH5. TRIM68 physically associates with AR and enhances the transcriptional potential of AR. Interaction of TRIM68 with AR in the nucleus was further enhanced by dihydrotestosterone treatment, indicating that TRIM68 behaves as a coregulator that assembles into an AR-associated transcription factor complex. Overexpression of TRIM68 enhanced AR-mediated transactivation in various prostate cancer cell lines, whereas knockdown of TRIM68 gene expression using RNAi caused suppression of AR-mediated transactivation. These findings indicate that TRIM68 is an intrinsic cofactor for AR activation in prostate cancer cells.

It has been reported that regulation of AR activity by ubiquitination is divided into two roles: a proteolytic role of AR and its coregulators linked to proteasome machinery and a nonproteolytic role without proteasome machinery (34, 35). We showed that the proteasome activity is also required for the effect of TRIM68 on AR-mediated transactivation. Therefore, coactivating function of TRIM68 may be involved in the proteolytic role for AR or AR-associated proteins. However, TRIM68 could not directly ubiquitinate AR (data not shown). Hence, TRIM68 may ubiquitinate one of the corepressors for AR-mediated transcription.

Cyclical recruitment of transcriptional coregulators is now an established phenomenon that is intrinsic to transcriptional activation by steroid receptors (36). However, few molecules that

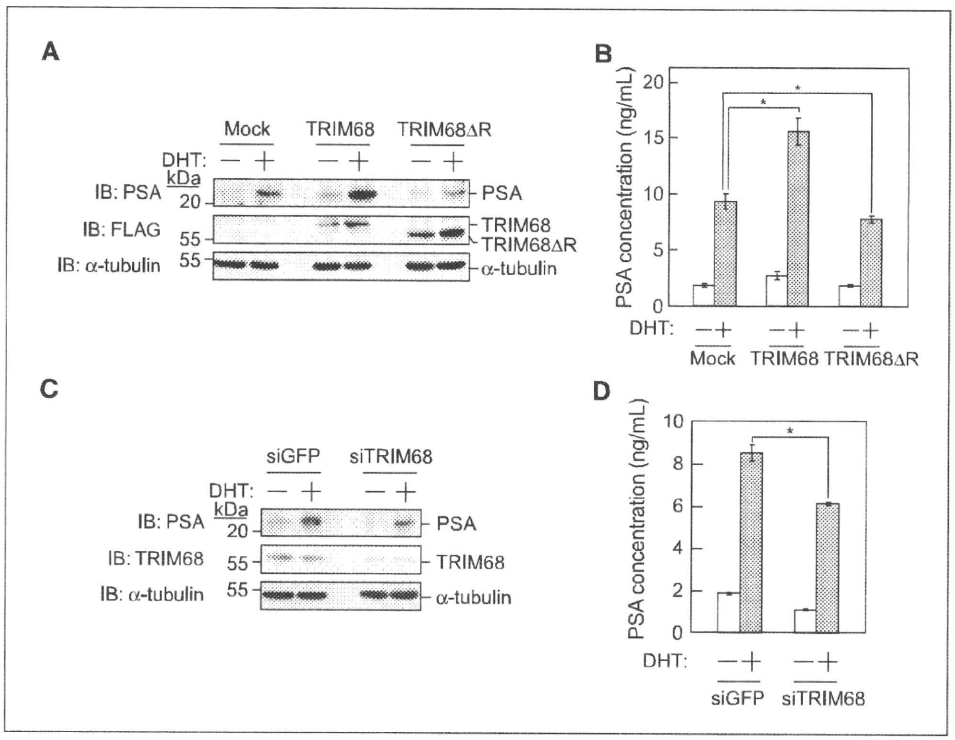


Figure 5. Effects of TRIM68 on PSA expression and secretion. *A*, TRIM68 increases PSA expression in prostate cancer cells. LNCaP cells stably expressing FLAG-tagged TRIM68 or TRIM68ΔR by a retroviral expression system were incubated in 10% charcoal–treated FBS medium for 48 h, washed, and treated with or without 10 nmol/L dihydrotestosterone for 24 h. Cells were lysed and subjected to immunoblotting with anti-PSA, anti-FLAG, and anti–α-tubulin antibodies. *B*, TRIM68 increases PSA secretion in prostate cancer cells. Cell culture media in *A* were collected and assayed for quantifying PSA concentration using ELISA analysis. *Columns*, mean of values from three independent experiments; *bars*, SD. *, statistically significant based on attaining *P*'s of <0.05 (unpaired Student's *t* test). *C*, knockdown of TRIM68 attenuates PSA expression in prostate cancer cells. Stably knocked-down LNCaP cell lines with siTRIM68 or siGFP were incubated in 10% charcoal–treated FBS medium for 48 h, washed, and treated with or without 10 nmol/L dihydrotestosterone for 24 h. Cells were lysed and subjected to immunoblotting with anti-PSA, anti-TRIM68, and anti–α-tubulin antibodies. *D*, knockdown of TRIM68 decreases PSA secretion in prostate cancer cells. Cell culture media in *C* were collected and assayed for quantifying PSA concentration using ELISA analysis. *Columns*, mean of values from three independent experiments; *bars*, SD. *, statistically significant based on attaining *P*'s of <0.05 (unpaired Student's *t* test).

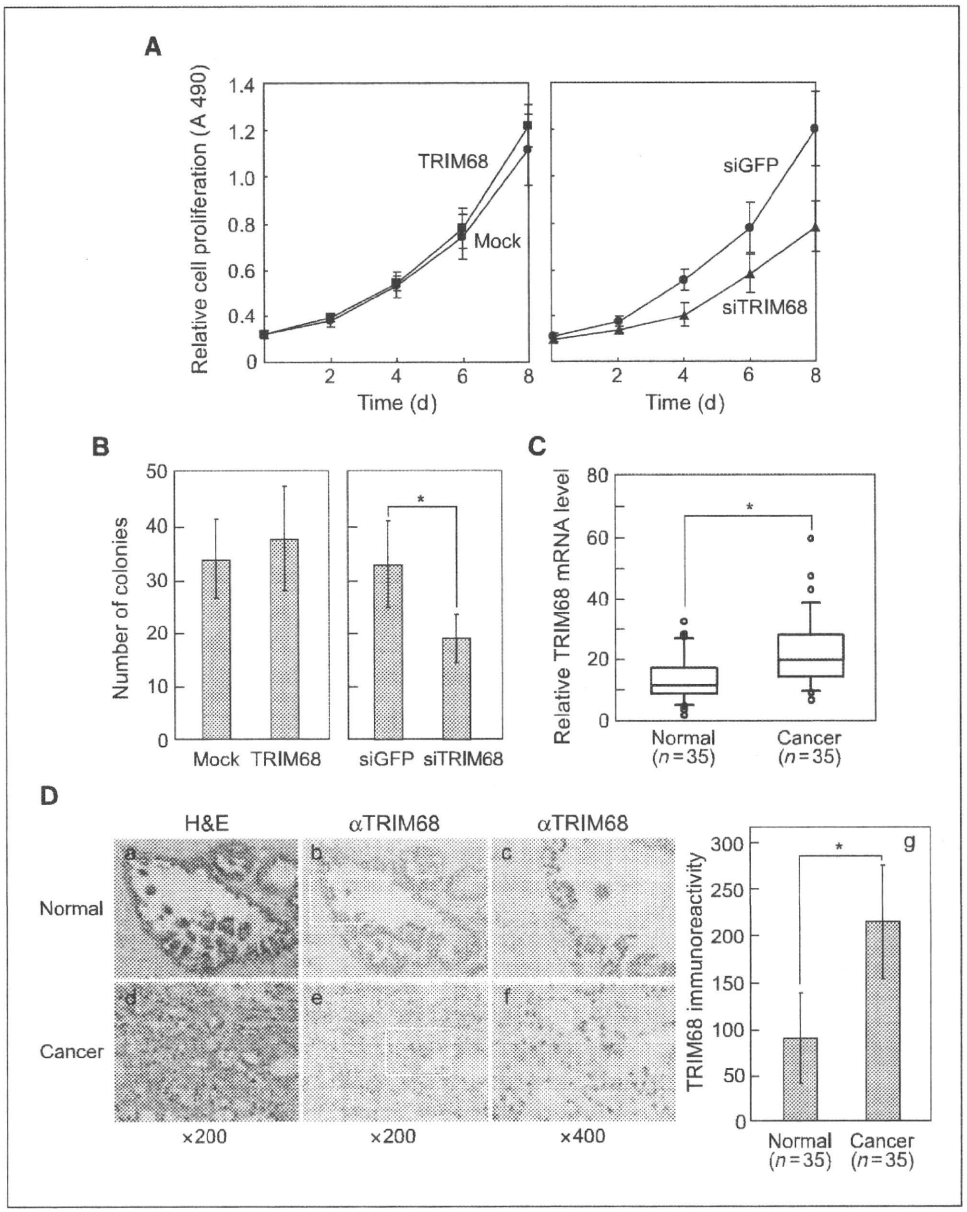


Figure 6. TRIM68 is overexpressed in human prostate cancer. *A*, TRIM68 affects prostate cancer cell growth. LNCaP cell lines stably expressing TRIM68, an empty vector (mock), siTRIM68, or siGFP were incubated in 10% charcoal–treated FBS medium for 48 h and then plated in 96-well plates (5,000 per well). Cells were treated with 10 nmol/L dihydrotestosterone and refed with fresh medium containing dihydrotestosterone every 2 d. Relative cell number was assayed at various times using the MTS assay. Absorbances at 490 nm versus time for each treatment were plotted. *Points,* mean of six replicates; *bars,* SD. *B*, anchorage-independent colony formation assay. Equal numbers of LNCaP cells stably expressing TRIM68, TRIM68 siRNA, or their respective controls were plated in 0.4% soft agar and cultured for 3 wk, and then colonies were counted microscopically. *Columns,* mean of values from three independent experiments; *bars,* SD. *, statistically significant based on attaining *P*'s of <0.05 (unpaired Student's *t* test). *C*, TRIM68 gene expression is up-regulated in human prostate cancer. TRIM68 mRNA levels were compared in human prostate cancers and adjacent normal tissues of 35 cases by real-time quantitative RT-PCR. Samples were surgically resected by radical prostatectomy in patients with primary prostate cancer. The expression level of TRIM68 mRNA was normalized to that of TBP mRNA and shown as relative expression level. The boxes within the plots represent the 25th to 75th percentiles. The horizontal line in the boxes indicates median value. White circles indicate outlier values outside of the 10th and 90th percentiles. *, statistically significant based on attaining *P*'s of <0.05 (Mann-Whitney *U* test). *D*, immunohistochemistry of human prostate tissues with anti-TRIM68 antibody. Samples were surgically resected by radical prostatectomy in patients with primary prostate cancer. Prostate cancer tissues (*d–f*) and adjacent normal tissues (*a–c*) were stained with H&E (*a* and *d*) or with anti-TRIM68 antibody (*b, c, e,* and *f*). *c* and *f* are higher magnification views of the rectangles in *b* and *e*, respectively. Magnifications, 200× (*a, b, d,* and *e*) and 400× (*c* and *f*). TRIM68 immunoreactivities were compared in human prostate cancers and adjacent normal tissues of 35 cases by immunohistochemistry (*g*). The immunointensity of samples was categorized as negative (score of 0), weak (score of 1), medium (score of 2), or strong (score of 3), and the final score was obtained by multiplying the percentage of positive cells by the intensity score. *Columns,* mean of values; *bars,* SD. *, statistically significant based on attaining *P*'s of <0.05 (Mann-Whitney *U* test).

regulate the recruitment and activation of coregulators have been identified. We showed that TRIM68 is associated with TIP60 and p300, which act as coactivators of AR, and cooperates in enhancing AR-mediated transcriptional activity. This raises the possibility that TRIM68 assembles into an AR complex with coactivators, such as TIP60 and p300, and ubiquitinate corepressors, leading to exchange of corepressors for coactivators after ligand binding.

The *PSA* gene is known to contain an *ARE*, and its transcriptional level is regulated by AR and coregulators, including TIP60 (37). We showed that TRIM68 increases both expression and

secretion of PSA in LNCaP cells, whereas knockdown of TRIM68 decreases both expression and secretion of PSA. Furthermore, we showed that knockdown of TRIM68 significantly attenuates prostate cancer cell growth. These findings imply that TRIM68 does play an important role in AR transcriptional complexes that regulate gene expression, including expression of the *PSA* gene, or in cell proliferation.

We showed that TRIM68 is predominantly expressed in LNCaP cells among various human cell lines, including sex hormone–related prostate and breast cancer cell lines. Thus, we speculate that effects of TRIM68 are restricted in prostate cancer cells and are particularly involved in AR-mediated transcription. Furthermore, we showed by immunohistochemistry and real-time quantitative RT-PCR that TRIM68 expression is significantly up-regulated in the majority of primary human prostate cancers compared with its expression in adjacent normal prostate tissues. As observed in LNCaP cells with dihydrotestosterone treatment, TRIM68 staining was dominantly detected by immunohistochemistry in the nuclear compartment in human prostate cancer tissues compared with the staining in adjacent normal prostate tissues. These results indicate the possibility that TRIM68 is a potential regulator for prostate carcinogenesis and cancer development.

It has been reported that autoantibodies to TRIM68 are frequently found in the sera of patients with Sjögren's syndrome (19). However, the clinical significance of these autoantibodies in human cancers has not been investigated. Considering the overexpression of TRIM68 in prostate cancers, autoantibodies to TRIM68 may be found in the sera of patients with prostate cancer and could be used as an additional diagnostic tool for prostate cancer.

We showed that TRIM68 is a positive modulator of transcriptional activity of AR. We suggest that ubiquitination activity of TRIM68 regulates the functions of AR or corepressors critically involved in proliferation, differentiation, or oncogenesis of prostate epithelial cells. Thus, it is probably important to identify physiologic substrates of TRIM68 and pharmacologic inhibitors of AR-associated ubiquitin ligases, including TRIM68 for establishing novel therapeutic tools for advanced hormone refractory and metastatic prostate cancer.

Acknowledgments

Received 10/31/2007; revised 1/21/2008; accepted 2/11/2008.

Grant support: Ministry of Education, Culture, Sports, Science and Technology grants 18013001 and 18390079, Kato Memorial Bioscience Foundation, and Sagawa Foundation for Promotion of Cancer Research (S. Hatakeyama).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank T. Kamura, G. Sobue, T. Ikura, and T. Kitamura for the plasmids and cell lines and Y. Soida for helping with the preparation of the manuscript.

References

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2007. CA Cancer J Clin 2007;57:43–66.
2. Liao DJ, Dickson RB. Roles of androgens in the development, growth, and carcinogenesis of the mammary gland. J Steroid Biochem Mol Biol 2002;80:175–89.
3. Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. Nat Rev Cancer 2001; 1:34–45.
4. Chen CD, Welsbie DS, Tran C, et al. Molecular determinants of resistance to antiandrogen therapy. Nat Med 2004;10:33–9.
5. McKenna NJ, O'Malley BW. Combinatorial control of gene expression by nuclear receptors and coregulators. Cell 2002;108:465–74.
6. Glass CK, Rosenfeld MG. The coregulator exchange in transcriptional functions of nuclear receptors. Genes Dev 2000;14:121–41.
7. Schulz WA, Hatina J. Epigenetics of prostate cancer: beyond DNA methylation. J Cell Mol Med 2006;10: 100–25.
8. Gioeli D, Ficarro SB, Kwiek JJ, et al. Androgen receptor phosphorylation. Regulation and identification of the phosphorylation sites. J Biol Chem 2002;277:29304–14.
9. Fu M, Wang C, Reutens AT, et al. p300 and p300/cAMP-response element-binding protein-associated factor acetylate the androgen receptor at sites governing hormone-dependent transactivation. J Biol Chem 2000; 275:20853–60.
10. Faus H, Haendler B. Post-translational modifications of steroid receptors. Biomed Pharmacother 2006; 60:520–8.
11. Hershko A, Ciechanover A. The ubiquitin system. Annu Rev Biochem 1998;67:425–79.
12. Scheffner M, Nuber U, Huibregtse JM. Protein ubiquitination involving an E1-2-E3 enzyme ubiquitin thioester cascade. Nature 1995;373:81–3.
13. Wolf DH, Hilt W. The proteasome: a proteolytic nanomachine of cell regulation and waste disposal. Biochim Biophys Acta 2004;1695:19–31.
14. Huibregtse JM, Scheffner M, Beaudenon S, Howley PM. A family of proteins structurally and functionally related to the E6-AP ubiquitin-protein ligase. Proc Natl Acad Sci U S A 1995;92:2563–7.
15. Joazeiro CA, Weissman AM. RING finger proteins: mediators of ubiquitin ligase activity. Cell 2000;102: 549–52.
16. Hatakeyama S, Yada M, Matsumoto M, Ishida N, Nakayama KI. U box proteins as a new family of ubiquitin-protein ligases. J Biol Chem 2001;276:33111–20.
17. Reymond A, Meroni G, Fantozzi A, et al. The tripartite motif family identifies cell compartments. EMBO J 2001;20:2140–51.
18. Meroni G, Diez-Roux G. TRIM/RBCC, a novel class of 'single protein RING finger' E3 ubiquitin ligases. BioEssays 2005;27:1147–57.
19. Billaut-Mulot O, Cocude C, Kolesnitchenko V, et al. SS-56, a novel cellular target of autoantibody responses in Sjogren syndrome and systemic lupus erythematosus. J Clin Invest 2001;108:861–9.
20. Frank MB. Characterization of DNA binding properties and sequence specificity of the human 52 kDa Ro/SS-A (Ro52) zinc finger protein. Biochem Biophys Res Commun 1999;259:665–70.
21. Kong HJ, Anderson DE, Lee CH, et al. Cutting edge: autoantigen Ro52 is an IFN inducible E3 ligase that ubiquitinates IRF-8 and enhances cytokine expression in macrophages. J Immunol 2007;179:26–30.
22. Espinosa A, Zhou W, Ek M, et al. The Sjogren's syndrome-associated autoantigen Ro52 is an E3 ligase that regulates proliferation and cell death. J Immunol 2006;176:6277–85.
23. Morita S, Kojima T, Kitamura T. Plat-E: an efficient and stable system for transient packaging of retroviruses. Gene Ther 2000;7:1063–6.
24. Kamura T, Hara T, Matsumoto M, et al. Cytoplasmic ubiquitin ligase KPC regulates proteolysis of p27(Kip1) at G1 phase. Nat. Cell Biol 2004;6:1229–35.
25. Dennis AP, O'Malley BW. Rush hour at the promoter: how the ubiquitin-proteasome pathway polices the traffic flow of nuclear receptor-dependent transcription. J Steroid Biochem Mol Biol 2005;93:139–51.
26. Gaughan L, Logan IR, Cook S, Neal DE, Robson CN. Tip60 and histone deacetylase 1 regulate androgen receptor activity through changes to the acetylation status of the receptor. J Biol Chem 2002;277: 25904–13.
27. Wang MC, Valenzuela LA, Murphy GP, Chu TM. Purification of a human prostate specific antigen. Investig Urol 1979;17:159–63.
28. Wang MC, Papsidero LD, Kuriyama M, et al. Prostate antigen: a new potential marker for prostatic cancer. Prostate 1981;2:89–96.
29. Bieche I, Laurendeau I, Tozlu S, et al. Quantitation of MYC gene expression in sporadic breast tumors with a real-time reverse transcription-PCR assay. Cancer Res 1999;59:2759–65.
30. Schmidt U, Fuessel S, Koch R, et al. Quantitative multi-gene expression profiling of primary prostate cancer. Prostate 2006;66:1521–34.
31. Logan IR, Gaughan L, McCracken SR, et al. Human PIRH2 enhances androgen receptor signaling through inhibition of histone deacetylase 1 and is overexpressed in prostate cancer. Mol Cell Biol 2006;26:6502–10.
32. Nawaz Z, Lonard DM, Smith CL, et al. The Angelman syndrome-associated protein, E6-AP, is a coactivator for the nuclear hormone receptor superfamily. Mol Cell Biol 1999;19:1182–9.
33. Zhang J, Guenther MG, Carthew RW, Lazar MA. Proteasomal regulation of nuclear receptor corepressor-mediated repression. Genes Dev 1998;12:1775–80.
34. Lin HK, Wang L, Hu YC, Altuwaijri S, Chang C. Phosphorylation-dependent ubiquitylation and degradation of androgen receptor by Akt require Mdm2 E3 ligase. EMBO J 2002;21:4037–48.
35. Burgdorf S, Leister P, Scheidtmann KH. TSG101 interacts with apoptosis-antagonizing transcription factor and enhances androgen receptor-mediated transcription by promoting its monoubiquitination. J Biol Chem 2004;279:17524–34.
36. Metivier R, Penot G, Hubner MR, et al. Estrogen receptor-α directs ordered, cyclical, and combinatorial recruitment of cofactors on a natural target promoter. Cell 2003;115:751–63.
37. Young CY, Montgomery BT, Andrews PE, et al. Hormonal regulation of prostate-specific antigen messenger RNA in human prostatic adenocarcinoma cell line LNCaP. Cancer Res 1991;51:3748–52.

Original Article

Combined Androgen Blockade With Bicalutamide for Advanced Prostate Cancer

Long-Term Follow-Up of a Phase 3, Double-Blind, Randomized Study for Survival

Hideyuki Akaza, MD[1]; Shiro Hinotsu, MD[2]; Michiyuki Usami, MD[3]; Yoichi Arai, MD[4]; Hiroshi Kanetake, MD[5]; Seiji Naito, MD[6]; Yoshihiko Hirao, MD[7], and Study Group for the Combined Androgen Blockade Therapy of Prostate Cancer

BACKGROUND: A previously reported, double-blind, randomized, multicenter phase 3 trial in 205 patients with stage C/D prostate cancer compared combined androgen blockade (CAB) with luteinizing hormone-releasing hormone agonist (LHRH-A) plus bicalutamide 80 mg versus LHRH-A plus bicalutamide-matching placebo (LHRH-A monotherapy). The analysis at a median follow-up of 2.4 years indicated that CAB significantly ($P < .001$) prolonged the time to progression and the time to treatment failure. In the current report, survival data from a long-term follow-up (median, 5.2 years) were analyzed. **METHODS:** All deaths irrespective of cause and all prostate cancer-specific deaths were recorded. The data were analyzed using Cox regression analysis and the log-rank test. **RESULTS:** At a median follow-up of 5.2 years, a significant overall survival advantage was observed in favor of CAB over LHRH-A monotherapy (Cox regression analysis: hazard ratio, 0.78; 95% confidence interval, 0.60-0.99; $P = .0498$; log-rank test: $P = .0425$). The difference in cause-specific survival between the 2 groups was not significant. The achievement of a prostate-specific antigen (PSA) nadir concentration ≤1 ng/mL was a prognostic factor for improved survival. More patients attained

Corresponding author: Hideyuki Akaza, MD, Department of Urology, Institute of Clinical Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba-shi, Ibaraki-ken, 305-8577, Japan; Fax: (011) 81-29-853-3169; akazah@md.tsukuba.ac.jp

[1]Department of Urology, Institute of Clinical Medicine, University of Tsukuba, Ibaraki, Japan; [2]Department of Pharmacoepidemiology, Graduate School of Medicine and Public Health, Kyoto University, Kyoto, Japan; [3]Department of Urology, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka, Japan; [4]Department of Urology, Tohoku University School of Medicine, Miyagi, Japan; [5]Department of Urology, Nagasaki University School of Medicine, Nagasaki, Japan; [6]Department of Urology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; [7]Department of Urology, Nara Medical University, Nara, Japan

We thank the patients and their families for their participation in this study. We also thank the physicians and support staff in each institute and Dr. Sharon Gladwin from Complete Medical Communications, who provided medical writing support.

Institutions participating in the study: Hokkaido University Hospital, Sapporo Medical University Hospital, Hirosaki University School of Medicine and Hospital, Tohoku University Hospital, Yamagata University Hospital, Tsukuba University Hospital, Teikyo University Chiba Medical Center, Asahi Hospital, Nihon University Itabashi Hospital, Toranomon Hospital, Keio University Hospital, Showa University Hospital, the University of Tokyo Hospital, the Jikei University School of Medicine, Tokyo Medical University Hospital, Tokyo Women's Medical University Hospital, Yokohama City University Hospital, Kitasato University Hospital, Fujieda Municipal General Hospital, Niigata University Medical and Dental Hospital, Niigata Cancer Center Hospital, Kanazawa University Hospital, Nagoya Daini Red Cross Hospital, Nagoya City University Hospital, Gifu University Hospital, University Hospital Kyoto Prefectural University of Medicine, Kyoto University Hospital, Nara Medical University Hospital, Osaka University Hospital, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka City University Hospital, Kansai Medical University Takii Hospital, Kobe University Hospital, Nishi-Kobe Medical Center, Okayama University Hospital, Kawasaki Medical School Hospital, Kurashiki Central Hospital, Hiroshima University Hospital, Shimane University Hospital, Tokushima University Hospital, Shikoku Cancer Center, Kochi Medical School Hospital, Kyushu University Hospital, Harasanshin Hospital, Nagasaki University Hospital of Medicine and Dentistry, Sasebo General Hospital, and Nagasaki Medical Center.

See also on pages 3376-8.

Received: September 8, 2008; **Revised:** November 14, 2008; **Accepted:** November 19, 2008

Published online: June 17, 2009

DOI: 10.1002/cncr.24395, www.interscience.wiley.com

PSA nadir concentrations ≤1 ng/mL with CAB compared with patients who received LHRH-A monotherapy (81.4% vs 33.7%; $P < .001$). **CONCLUSIONS:** CAB with bicalutamide 80 mg offered a significant overall survival benefit compared with LHRH-A monotherapy without reducing tolerability in patients with locally advanced or metastatic prostate cancer. **Cancer 2009;115:3437–45. © 2009 American Cancer Society.**

KEY WORDS: prostate cancer, survival, combined androgen blockade, bicalutamide, luteinizing hormone-releasing hormone agonist, clinical trial, immediate combined androgen blockade, deferred combined androgen blockade.

Combined androgen blockade (CAB), consisting of a nonsteroidal antiandrogen plus either a luteinizing hormone-releasing hormone agonist (LHRH-A) or bilateral orchiectomy, is a standard treatment for advanced prostate cancer. Furthermore, early initiation of CAB is a potential option for patients who are at high risk of prostate cancer-specific death after failing radical treatment for clinically localized disease.[1,2]

The use of CAB therapy over castration alone has been widely debated because of conflicting efficacy data from individual clinical trials as well as tolerability and cost issues. In 2000, the Prostate Cancer Trialists' Collaborative Group (PCTCG) published a large meta-analysis of all randomized trials initiated before 1991 that compared CAB with castration alone in patients with advanced prostate cancer (27 studies; n = 8275).[3] The results demonstrated that CAB with a nonsteroidal antiandrogen (flutamide or nilutamide) reduced the risk of death by 8% compared with castration alone ($P = .005$). However, the survival benefit was so small that CAB could not be widely recommended in clinical practice.

Bicalutamide (Casodex; Astra-Zeneca Pharmaceuticals, Wilmington, Del), a nonsteroidal antiandrogen with a better tolerability profile than flutamide and nilutamide,[4,5] was not included in the PCTCG meta-analysis, because no randomized trial data for CAB with bicalutamide versus castration alone were available at the time. However, Klotz and colleagues[6] estimated the efficacy of CAB with bicalutamide 50 mg using validated statistical methodology[7] to combine PCTCG meta-analysis data with data from a phase 3 trial of 2 CAB regimens (LHRH-A plus bicalutamide 50 mg vs LHRH-A plus flutamide). That analysis suggested that CAB with bicalutamide 50 mg could reduce the risk of death by 20% compared with castration alone (hazard ratio [HR] 0.80; 95% confidence interval [CI], 0.66-0.98).

A multicenter, double-blind, controlled trial has compared CAB directly with bicalutamide 80 mg (the 80 mg dose is licensed for CAB and monotherapy in Japan) versus castration alone.[8,9] In that phase 3 trial, 205 Japanese patients with stage C/D prostate cancer were randomized to receive either CAB with an LHRH-A plus bicalutamide 80 mg (n = 102) or LHRH-A monotherapy (LHRH-A plus bicalutamide-matching placebo; n = 103). The proportion of patients who achieved a prostate-specific antigen (PSA) level ≤4 ng/mL at 12 weeks was significantly greater for CAB compared with LHRH-A monotherapy (79.4% vs 38.6%, respectively; $P < .001$).[8] CAB also improved the 12-week overall tumor response rate versus LHRH-A monotherapy (77.5% vs 65.3%, respectively; $P = .063$).[8] At a median follow-up of 2.4 years, CAB significantly prolonged the median time to treatment failure (117.7 weeks vs 60.3 weeks; $P < .001$) and time to progression (not reached vs 96.9 weeks; $P < .001$), compared with LHRH-A monotherapy.[9] It is noteworthy that the incidence of adverse events and withdrawals was similar between the 2 treatment groups.[8,9]

Results from that trial, along with those from the analysis by Klotz and colleagues,[6] are cited in guidelines published by the American Society of Clinical Oncology to support their recommendation that CAB with bicalutamide should be considered for patients with advanced prostate cancer.[10] A subsequent exploratory analysis of the phase 3 study revealed that, in terms of the median time to progression, the benefit of CAB versus LHRH-A monotherapy was greater in patients with stage C disease (median not reached vs 134.1 weeks; $P < .001$)[9] than in patients with stage D disease (98.4 weeks vs 64.1 weeks; $P = .024$) at diagnosis.[11] Furthermore, in patients with stage C disease, CAB significantly prolonged the median time to progression compared with LHRH-A monotherapy, irrespective of histologic grade, patient age, or PSA level at diagnosis.[11] Those data support the use of CAB with bicalutamide in patients with locally advanced, metastasis-free prostate cancer.

When the phase 3 study was completed at a median follow-up of 2.4 years, survival data were immature and, consequently, no significant differences were observed between the 2 treatment groups in terms of overall or cause-specific survival.[9] We instigated the Study Group for the Combined Androgen Blockade Therapy of Prostate Cancer, comprised of the investigators who had participated in the original study, to conduct a long-term follow-up of patients who were enrolled in the original study and remained alive after the trial was completed. The current article presents the results of the survival analysis from the long-term follow-up study.

MATERIALS AND METHODS

Study Design

Detailed methods for the original phase 3 study were published previously[8,9] and are briefly summarized here. Patients with histologically confirmed, previously untreated, advanced (stage C/D[12]) prostate cancer were recruited at 49 centers in Japan between February 2000 and December 2001. All patients received an LHRH-A according to the investigator's choice, either goserelin acetate (Zoladex; Astra-Zeneca Pharmaceuticals) 3.6 mg or leuprorelin acetate (Leuprin; Takeda Chemical Industries, Osaka, Japan) 3.75 mg, given as a subcutaneous injection every 4 weeks. In addition, patients were randomized in a 1:1 ratio to receive either oral bicalutamide 80 mg or matching placebo once daily. Randomized treatment was given in a double-blind manner until September 2002, when the code was broken for ethical reasons. Subsequently, patients in the LHRH-A monotherapy group discontinued placebo, and patients in the CAB group continued their treatment in an open-label manner. Patients continued to receive randomized treatment until the end of November 2003 or until there was evidence of disease progression or any other event leading to withdrawal. Patients who had disease progression during LHRH-A monotherapy were treated at the investigator's discretion, with the option of adding bicalutamide 80 mg (deferred CAB). For patients who experienced disease progression in the CAB group, bicalutamide was discontinued, and the patient was monitored for antiandrogen withdrawal syndrome at the investigator's discretion. The primary endpoints were PSA normalization rate and overall tumor response at 12 weeks and the percentage of withdrawals because of adverse drug reactions. Secondary endpoints included time to treatment failure, time to progression, survival, quality of life (QoL), time to PSA normalization, and the incidence of adverse events and adverse drug reactions.

After the original phase 3 study, the Study Group for the Combined Androgen Blockade Therapy of Prostate Cancer conducted a follow-up study of patients who were enrolled in the original study and were still alive when the original study was completed (from December 2003). The institutional review board at each medical center approved the follow-up study, and all patients provided written informed consent.

Assessments and Statistical Analyses

All deaths, irrespective of cause, and all prostate cancer-specific deaths were recorded for 3 years from the completion of the original study (the follow-up period ended in March 2007). Confirmed deaths from the original study plus deaths that were recorded during the follow-up period were analyzed at the University of Tsukuba. Overall survival and cause-specific survival were assessed using a Cox proportional hazards regression model with covariates for randomized treatment, clinical stage, age, performance status, PSA level at diagnosis, histologic grade, and the type of LHRH-A received. An additional Cox regression analysis of overall survival was performed with covariates for the PSA nadir level achieved during the original study irrespective of whether randomized treatment had been discontinued ($\leq$1 ng/mL or $>$1 ng/mL), randomized treatment, clinical stage, age, performance status, histologic grade, and the type of LHRH-A received. Results from the Cox regression analyses were confirmed using the log-rank test.

For patients who achieved a PSA nadir of $\leq$1 ng/mL during the original study, the time taken to reach the nadir level was assessed. The number of patients achieving PSA nadir levels $>$4 ng/mL, from $\leq$4 to $>$1 ng/mL, from $\leq$1 to $>$0.2 ng/mL, and $\leq$0.2 ng/mL during the administration of randomized treatment only also was investigated.

RESULTS

Patients

Of 205 patients who entered the original study, 203 patients (CAB, n = 102; LHRH-A monotherapy, n =

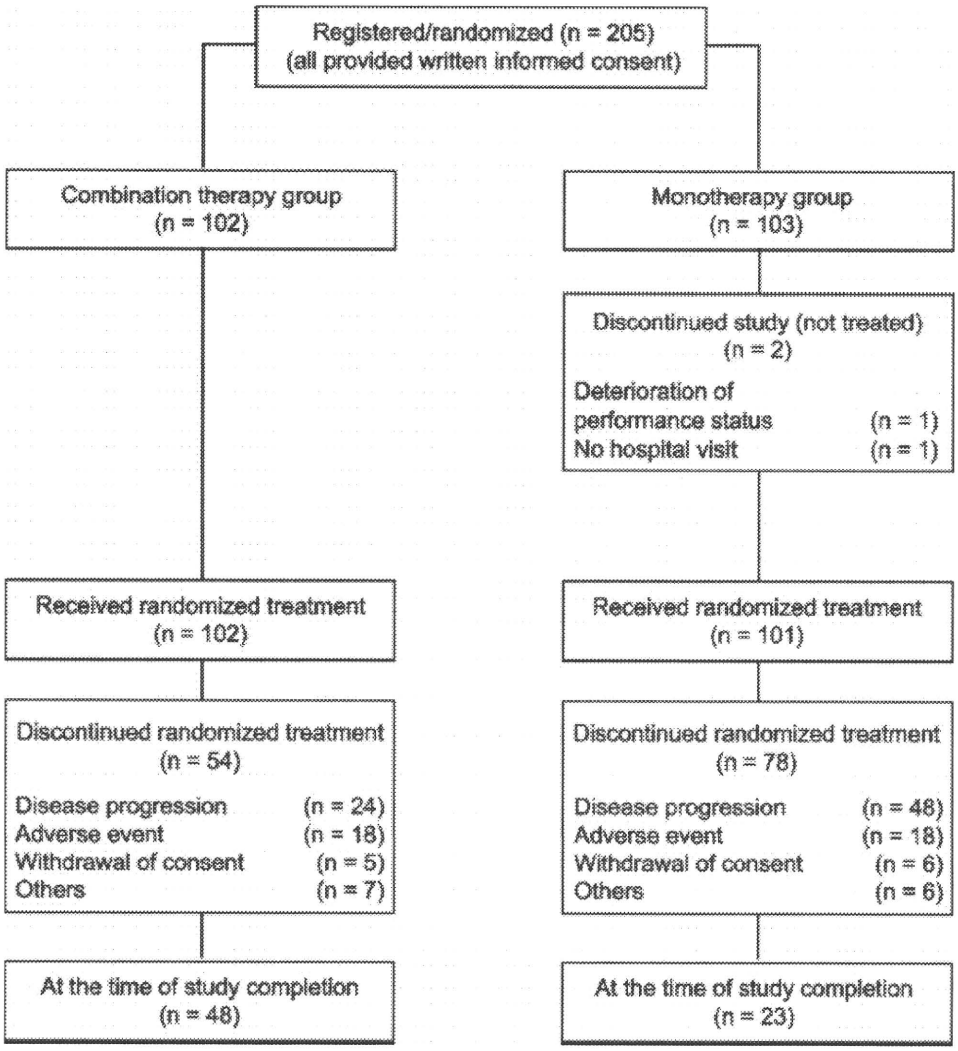


FIGURE 1. Outline of original phase 3 study. Reprinted with permission from Usami M, Akaza H, Arai Y, et al. Bicalutamide 80 mg combined with a luteinizing hormone-releasing hormone agonist (LHRH-A) versus LHRH-A monotherapy in advanced prostate cancer: findings from a phase III randomized, double-blind, multicenter trial in Japanese patients. *Prostate Cancer Prostatic Dis.* 2007;10:194-201.

101) received randomized treatment (Fig. 1).[9] Two patients in the LHRH-A monotherapy group withdrew from the study before commencing treatment (because of deterioration of performance status and failure to attend a hospital visit, respectively). The demographics and baseline characteristics of the patients who received randomized treatment were similar between the 2 treatment arms and are summarized in Table 1.[9] For the majority of men in both treatment arms, the choice of LHRH-A was goserelin acetate (75.5% and 78.2% for the CAB and LHRH-A arms, respectively).

In total, 172 patients (CAB, n = 89; LHRH-A monotherapy, n = 83) remained alive in December 2003 after the original study had completed. At the end of March 2007, in total, 139 patients (CAB, n = 76; LHRH-A monotherapy, n = 63) remained alive.

Overall Survival

At a median follow-up of 5.2 years, there were fewer overall deaths with CAB than with LHRH-A monotherapy (26 deaths vs 38 deaths, respectively). A significant overall survival advantage was observed in favor of CAB over LHRH-A monotherapy (Cox regression analysis: HR, 0.78; 95% CI, 0.60-0.99; $P = .0498$; log-rank test: $P = .0425$) (Fig. 2). The 5-year overall survival rate estimated by the Kaplan-Meier method was 75.3% for CAB versus

Table 1. Demographic and Baseline Characteristics of Patients in the Original Study Population*

Characteristic	No. of Patients (%)	
	CAB With Bicalutamide 80 mg	LHRH-A Monotherapy
All patients	102 (100)	101 (100)
Age, y		
<75	53 (52)	50 (49.5)
≥75	49 (48)	51 (50.5)
PSA level, ng/mL		
<60	40 (39.2)	37 (36.6)
≥60	62 (60.8)	64 (63.4)
Histological grade		
Well differentiated	3 (2.9)	6 (5.9)
Moderately differentiated	52 (51)	55 (54.5)
Poorly differentiated	47 (46.1)	40 (39.6)
Clinical stage		
C,D1	59 (57.8)	57 (56.4)
D2	43 (42.2)	44 (43.6)
Disease stage		
T2	3 (2.9)	1 (1)
T3	83 (81.4)	77 (76.2)
T4	16 (15.7)	23 (22.8)
Nodal stage		
N0	74 (72.5)	63 (62.4)
N1	28 (27.5)	38 (37.6)
Metastatic stage		
M0	59 (57.8)	58 (57.4)
M1	43 (42.2)	43 (42.6)
Location of metastases†		
Bone	40 (39.2)	40 (39.6)
Lymph node	28 (27.5)	38 (37.6)
Other	2 (2)	3 (3)
LHRH-A		
Goserelin acetate	77 (75.5)	79 (78.2)
Leuprorelin acetate	25 (24.5)	22 (21.8)
Performance status		
0, 1	99 (97.1)	99 (98)
2	3 (2.9)	2 (2)

CAB indicates combined androgen blockade; LHRH-A, luteinizing hormone-releasing hormone agonist; PSA, prostate-specific antigen.
* See Usami 2007.[9]
† Some patients had metastases at more than 1 site.

63.4% for LHRH-A monotherapy. The results from the subgroup analysis according to disease stage (stage C/D1 and stage D2) are shown in Figure 3.

Cause-specific Survival

CAB also was associated with fewer cause-specific deaths compared with LHRH-A monotherapy (14 deaths vs 22 deaths, respectively). The difference in cause-specific survival between the 2 groups was not significant (Cox regression analysis: HR, 0.79; 95% CI, 0.55-1.11; $P = .1703$; log-rank test: $P = .0918$) (Fig. 4).

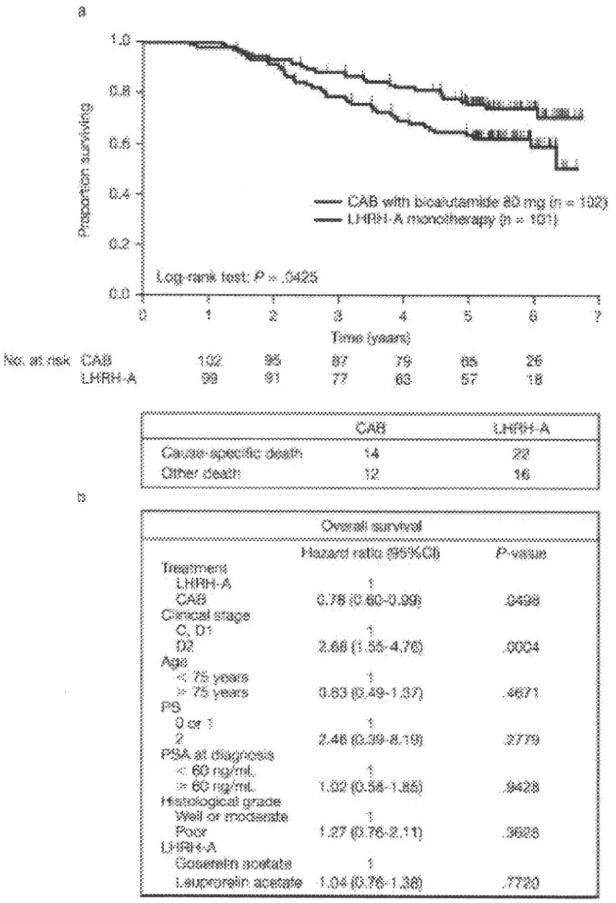


FIGURE 2. Overall survival was analyzed at a median follow-up of 5.2 years: (a) Kaplan-Meier curve by randomized treatment; (b) results of multivariate analysis. CAB indicates combined androgen blockade; LHRH-A, luteinizing hormone-releasing hormone agonist; CI, confidence interval; PS, performance status; PSA, prostate-specific antigen.

Overall Survival and Prostate-Specific Antigen Nadir Level

During the original study, PSA levels decreased to ≤1 ng/mL in 137 of 203 patients (67%). Overall survival was prolonged significantly in patients who attained a PSA nadir ≤1 ng/mL compared with those who did not (death rate: 19.7% [27 of 137 patients] vs 56.1% [37 of 66 patients], respectively; HR, 0.34; 95% CI, 0.20-0.59; $P = .0001$; log-rank test: $P < .0001$) (Fig. 5). In total, 75%

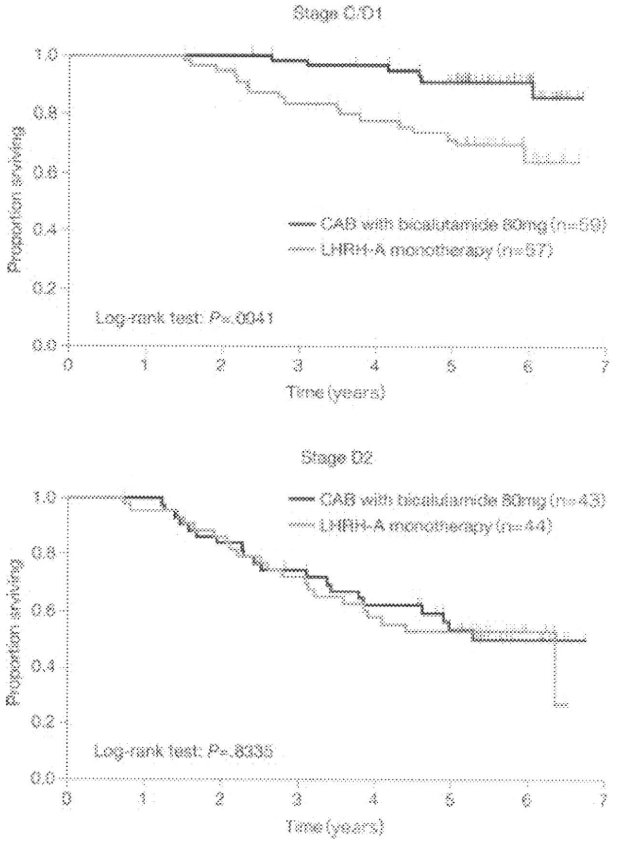


FIGURE 3. Overall survival was analyzed according to disease stage in patients with (*Top*) stage C/D1 disease and (*Bottom*) stage D2 disease at a median follow-up of 5.2 years. CAB indicates combined androgen blockade; LHRH-A, luteinizing hormone-releasing hormone agonist.

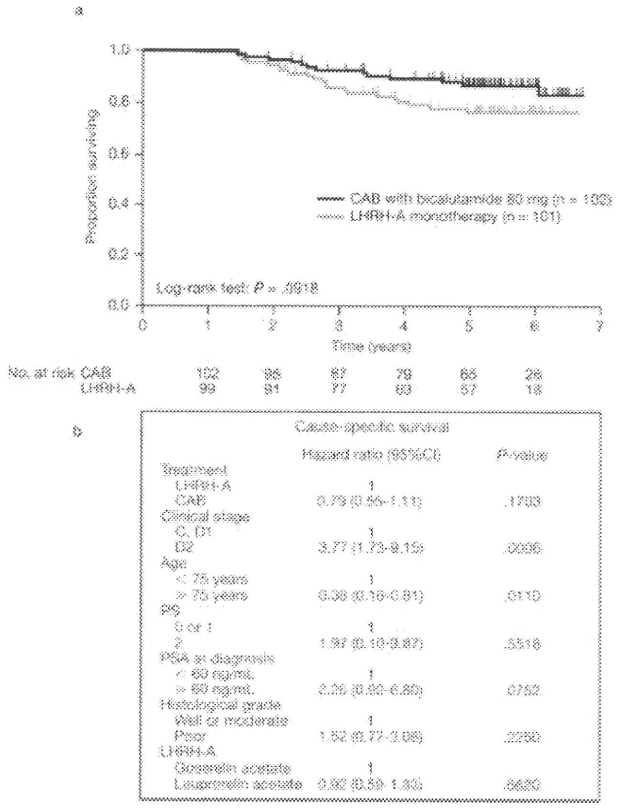


FIGURE 4. Cause-specific survival was analyzed at a median follow-up of 5.2 years: (a) Kaplan-Meier curve by randomized treatment; (b) results of multivariate analysis. CAB indicates combined androgen blockade; LHRH-A, luteinizing hormone-releasing hormone agonist; CI, confidence interval; PS, performance status; PSA, prostate-specific antigen.

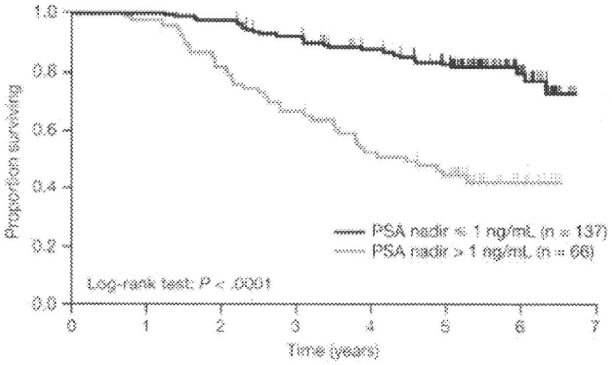
of patients who achieved a PSA nadir ≤ 1 ng/mL had reached that level within the first 192 days of the study (Fig. 6). During randomized treatment, PSA nadir concentrations ≤ 1 ng/mL were achieved by 83 of 102 patients (81.4%) who received CAB and by 34 of 101 patients (33.7%) who received LHRH-A monotherapy (Fisher exact test: $P < .001$) (Table 2).

DISCUSSION

In this report, long-term follow-up data from a phase 3 study of CAB with bicalutamide 80 mg versus LHRH-A monotherapy alone have demonstrated a significant overall survival advantage in favor of CAB. The overall survival advantage for CAB is consistent with previous observations from this study of prolonged time to treatment failure and time to progression.[9] In particular, the magnitude of the reduction in risk of death reported for CAB with bicalutamide 80 mg (22%) concurs with that estimated by Klotz and colleagues[6] for CAB with bicalutamide 50 mg (20%). In most countries, bicalutamide is licensed at a dose of 50 mg daily for use in CAB. However, based on pharmacokinetic and pharmacodynamic data,[13] the only approved dose of bicalutamide in Japanese men is 80 mg per day for monotherapy. A previous pilot study of LHRH-A in combination with bicalutamide 80 mg identified no safety concerns[14]; therefore, the 80 mg dose of bicalutamide is used both for monotherapy and for CAB in Japan. A comparison between our study results and Western CAB data with bicalutamide 50 mg should be considered as the next step.

In total, 30 patients in the CAB group experienced disease progression during the original phase 3 study, including at least 18 patients who were observed for antiandrogen withdrawal syndrome, and 7 patients (39%) responded (median response duration, 58 weeks).[9] Of 57

a



	CAB	LHRH-A
PSA nadir ≤ 1 ng/mL	85*	52*
PSA nadir > 1 ng/mL	17	49

*These numbers include the patients who achieved PSA nadir ≤ 1 ng/mL after randomized treatment

b

Overall survival (using PSA nadir)		
	Hazard ratio (95%CI)	P-value
PSA nadir		
> 1 ng/mL	1	
≤ 1 ng/mL	0.34 (0.20-0.59)	.0001
Treatment		
LHRH-A	1	
CAB	0.82 (0.48-1.40)	.4716
Clinical stage		
C, D1	1	
D2	2.22 (1.31-3.76)	.0032
Age		
< 75 years	1	
≥ 75 years	0.80 (0.48-1.33)	.3875
PS		
0 or 1	1	
2	2.78 (0.66-11.73)	.1640
Histological grade		
Well or moderate	1	
Poor	1.01 (0.99-1.04)	.3673
LHRH-A		
Goserelin acetate	1	
Leuprorelin acetate	0.81 (0.46-1.48)	.4985

FIGURE 5. The relation between prostate-specific antigen (PSA) nadir and overall survival was analyzed at a median follow-up of 5.2 years: (a) Kaplan-Meier curve by PSA nadir; (b) results of multivariate analysis using PSA nadir level as a covariate. CAB indicates combined androgen blockade; LHRH-A, luteinizing hormone-releasing hormone agonist; CI, confidence interval; PS, performance status.

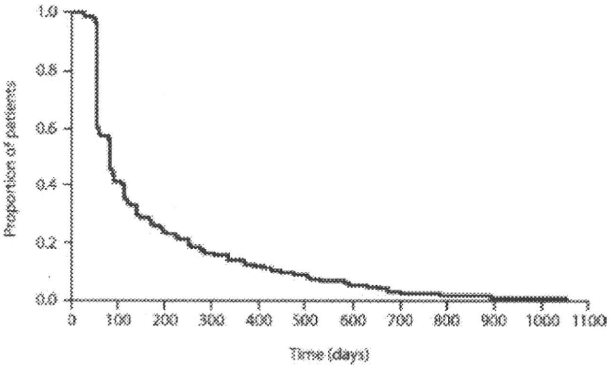


FIGURE 6. This graph illustrates the time to achieve a prostate-specific antigen level ≤1 ng/mL (n = 137).

Table 2. Patients Who Achieved Defined Prostate-Specific Antigen Nadir Levels During Randomized Treatment in the Original Phase 3 Study

PSA Nadir, ng/mL	No. of Patients	
	CAB With Bicalutamide, 80 mg (n = 102)	LHRH-A Monotherapy (n = 101)
>4	6	42
From ≤4 to >1	13	25
From ≤1 to >0.2	9	20
≤0.2	74	14

PSA indicates prostate-specific antigen; CAB, combined androgen blockade; LHRH-A, luteinizing hormone-releasing hormone agonist.

patients in the LHRH-A monotherapy group who had disease progression during the phase 3 study, at least 40 patients subsequently received second-line CAB with bicalutamide 80 mg, and 31 patients (78%) responded to that treatment (median response duration, 40 weeks).[9] Currently, CAB is used widely in Japan and accounts for approximately 70% of primary hormone therapy for prostate cancer.[15] For patients who receive LHRH-A monotherapy as initial treatment and subsequently experience disease progression, second-line therapy is usually the addition of an antiandrogen to their regimen (deferred CAB therapy). Because the majority of patients who progressed in the LHRH-A monotherapy group received second-line CAB therapy, our study can be considered a comparison of immediate versus deferred CAB. Consequently, results from the current follow-up study suggest that immediate CAB may be superior to deferred CAB in terms of prolonging overall survival.

Although it was not predefined in the protocol, a subgroup analysis of overall survival by clinical stage was performed for reference. Consequently, the difference in overall survival between CAB and LHRH-A monotherapy was greater in the patients who had stage C/D1 disease. In the original phase 3 study, the same tendency was observed in the time to progression for CAB in the patients who had stage C disease, suggesting that the long-term prognosis for patients who have stage C disease and

are treated with CAB can be expected to be markedly better than that of the patients who are treated with LHRH-A monotherapy.[9] Sylvester et al. reported that, among patients with stage D2 prostate cancer who either underwent orchiectomy or received CAB (goserelin + flutamide), the survival benefit of CAB was greater for patients who had mild bone metastasis than for those who had more advanced disease.[16] On the basis of these results, the survival benefit of CAB versus LHRH-A monotherapy is expected to be much greater for patients who have early stage disease.

Our follow-up study revealed no significant difference in cause-specific survival between CAB and LHRH-A monotherapy ($P = .0918$). This is unsurprising, because the analysis lacked statistical power to detect a significant difference in cause-specific mortality in light of the low number of prostate cancer-related deaths (14 patients on CAB and 22 on LHRH-A monotherapy). To observe a treatment difference in cause-specific survival, longer follow-up or a larger patient population may be necessary.

Previous studies have suggested that the normalization of PSA by hormone therapy may be associated with prolonged time to progression and survival.[17,18] Because of an exploratory multivariate analysis with PSA cutoff levels of 4 ng/mL, 2 ng/mL, 1 ng/mL, 0.5 ng/mL, and 0.2 ng/mL, the use of 1 ng/mL produced a stable and better fitting model with a small P value and variance of estimated values. Therefore, we used a cutoff level of 1 ng/mL for our analysis of overall survival. Data from our study indicated that patients who attained a PSA nadir $\leq$1 ng/mL survived significantly longer than patients who had PSA levels that remained >1 ng/mL. It also was apparent that patients who received CAB achieved lower PSA nadir levels than patients who received LHRH-A monotherapy. It is noteworthy that PSA levels fell below 0.2 ng/mL (the detection limit) in 89% of patients who had a PSA nadir $\leq$1 ng/mL in the CAB group, compared with only 41% of patients who had a PSA nadir $\leq$1 ng/mL in the LHRH-A monotherapy group. Therefore, the PSA reduction associated with CAB appears to be important clinically in terms of prolonging overall survival. Among the patients who achieved a PSA nadir $\leq$1 ng/mL in the original study, 75% had attained this nadir within approximately 6 months (192 days). This suggests that, if no therapeutic effect is observed within the first 6 months of treatment, then a change of therapy should be considered.

A primary obstacle to the wider use of CAB is the potential for increased side effects and costs compared with castration alone. Indeed, compared with castration alone, CAB with flutamide is associated with an increased incidence of gastrointestinal disorders and hepatotoxicity, whereas CAB using nilutamide is associated with an increased incidence of visual disorders.[19] However, in the phase 3 study of CAB with bicalutamide 80 mg versus LHRH-A monotherapy, there was no difference between the 2 treatment arms regarding the percentage of withdrawals because of adverse drug reactions (primary safety endpoint) or adverse drug reaction profiles.[8,9] QoL was assessed as a secondary endpoint in this study using the Japanese version of the Functional Assessment of Cancer Therapy-Prostate questionnaire.[20] These data demonstrated that, compared with LHRH-A monotherapy, CAB with bicalutamide did not reduce overall QoL and provided an early improvement in QoL related to micturition disorder and pain.[21]

Nishimura and colleagues[22] conducted a cost-effectiveness analysis of CAB with bicalutamide 80 mg based on efficacy data from the phase 3 study and medical costs in Japan. Those authors concluded that CAB was a cost-efficient therapy with an incremental cost effectiveness ratio of approximately ¥1,560,000 (approximately $14,000 in US dollars). This is consistent with results from similar analyses conducted in the United States. For example, Ramsey and colleagues[23] demonstrated that the incremental cost per quality-adjusted life-year (QALY) gained for CAB with bicalutamide 50 mg versus CAB with flutamide was $22,000 at 5 years and $16,000 at 10 years. Likewise, Penson and colleagues[24] estimated that the cost per QALY of CAB with bicalutamide 50 mg was $33,677 and $20,053 at 5 years and 10 years, respectively, compared with castration alone. These studies support CAB with bicalutamide as a cost-effective treatment strategy for patients with advanced prostate cancer.

In conclusion, the long-term follow-up of the first double-blind controlled study to directly compare CAB with bicalutamide 80 mg versus LHRH-A monotherapy has demonstrated a statistically significant overall survival benefit in favor of CAB. The advantage in overall survival, together with the previously reported significant improvements in time to treatment failure and time to

progression, which were achieved without reducing tolerability, indicate that CAB with bicalutamide is a recommendable first-line therapy option for patients with locally advanced or metastatic prostate cancer.

Conflict of Interest Disclosures

Financial sponsorship for this study and publication was provided by the Advanced Clinical Research Organization (a nonprofit organization).

References

1. Moul JW, Wu H, Sun L, et al. Early versus delayed hormonal therapy for prostate specific antigen only recurrence of prostate cancer after radical prostatectomy. *J Urol.* 2004; 171:1141-1147.
2. Freedland SJ, Humphreys EB, Mangold LA, et al. Risk of prostate cancer-specific mortality following biochemical recurrence after radical prostatectomy. *JAMA.* 2005;294:433-439.
3. Prostate Cancer Trialists' Collaborative Group. Maximum androgen blockade in advanced prostate cancer: an overview of the randomised trials. *Lancet.* 2000;355:1491-1498.
4. Schellhammer PF, Sharifi R, Block NL, et al. Clinical benefits of bicalutamide compared with flutamide in combined androgen blockade for patients with advanced prostatic carcinoma: final report of a double-blind, randomized, multicenter trial. *Urology.* 1997;50:330-336.
5. Fourcade R-O, McLeod D. Tolerability of antiandrogens in the treatment of prostate cancer. *UroOncology.* 2004;4:5-13.
6. Klotz L, Schellhammer P, Carroll K. A re-assessment of the role of combined androgen blockade for advanced prostate cancer. *BJU Int.* 2004;93:1177-1182.
7. Rothmann M, Li N, Chen G, et al. Design and analysis of non-inferiority mortality trials in oncology. *Stat Med.* 2003;22:239-264.
8. Akaza H, Yamaguchi A, Matsuda T, et al. Superior anti-tumor efficacy of bicalutamide 80 mg in combination with a luteinizing hormone-releasing hormone (LHRH) agonist versus LHRH agonist monotherapy as first-line treatment for advanced prostate cancer: interim results of a randomized study in Japanese patients. *Jpn J Clin Oncol.* 2004;34: 20-28.
9. 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.
10. Loblaw DA, Virgo KS, Nam R, et al. Initial hormonal management of androgen-sensitive metastatic, recurrent, or progressive prostate cancer: 2007 update of an American Society of Clinical Oncology practice guideline. *J Clin Oncol.* 2007;25:1596-1605.
11. Akaza H, Arai Y, Kanetake H, et al. Efficacy of combined androgen blockade (CAB) therapy in stage C prostate cancer: exploratory analyses based on results of a double-blind, randomized, placebo-controlled phase III study of bicalutamide, 07 update. Available at: http://www.asco.org/ASCO/Abstracts+%26+Virtual+Meeting/Virtual+Meeting?&vmview=vm_session_presentations_view&confID=47&sessionID=358. Accessed on June 5, 2009.
12. Japanese Urological Association and the Japanese Society of Pathology. General rule for clinical and pathological studies on prostatic cancer. 2nd edition. Tokyo: Kanehara. 1992.
13. Kotake T, Usami M, Isaka S, et al. [Clinical early phase II study of bicalutamide (Casodex) in patients with prostatic cancer]. *Hinyokika Kiyo.* 1996;42:157-168.
14. Kotake T, Akaza H, Usami M. Preliminary trial for clinical phase III study of Casodex-combination therapy with LH-RH agonist for prostate cancer. *J N Remed Clin.* 1999;48:1512-1533.
15. Akaza H. Current status and prospects of androgen depletion therapy for prostate cancer. *Best Pract Res Clin Endocrinol Metab.* 2008;22:293-302.
16. Sylvester RJ, Denis L, de Voogt H. The importance of prognostic factors in the interpretation of two EORTC metastatic prostate cancer trials. *Eur Urol.* 1998;33:134-143.
17. Kwak C, Jeong SJ, Park MS, et al. Prognostic significance of the nadir prostate specific antigen level after hormone therapy for prostate cancer. *J Urol.* 2002;168:995-1000.
18. Altwein JE, Schmidt A. Prostate-specific antigen dynamics predict risk of progression in advanced prostate cancer treated with bicalutamide plus castration. *Urol Int.* 2002; 68:220-225.
19. Lukka H, Waldron T, Klotz L, et al. Maximal androgen blockade for the treatment of metastatic prostate cancer—a systematic review. *Curr Oncol.* 2006;13:81-93.
20. Hinotsu A, Niimi M, Akaza H, et al. [Development of Japanese version of QOL questionnaire for bladder and prostate cancer patients using FACT-Bl and P: pilot study]. *Gan To Kagaku Ryoho.* 1999;26:657-666.
21. Arai Y, Akaza H, Deguchi T, et al. Evaluation of quality of life in patients with previously untreated advanced prostate cancer receiving maximum androgen blockade therapy or LHRHa monotherapy: a multicenter, randomized, double-blind, comparative study. *J Cancer Res Clin Oncol.* 2008;134: 1385-1396.
22. Nishimura S, Arai Y, Usami M, et al. [Cost-effectiveness analysis of maximum androgen blockade for Japanese men with advanced prostate cancer]. *Gan To Kagaku Ryoho.* 2007; 34:589-595.
23. Ramsey S, Veenstra D, Clarke L, et al. Is combined androgen blockade with bicalutamide cost-effective compared with combined androgen blockade with flutamide? *Urology.* 2005;66:835-839.
24. Penson DF, Ramsey S, Veenstra D, et al. The cost-effectiveness of combined androgen blockade with bicalutamide and LHRH agonist in men with metastatic prostate cancer. *J Urol.* 2005;174:547-552.

Original Article

Single Infusion of Zoledronic Acid to Prevent Androgen Deprivation Therapy-induced Bone Loss in Men With Hormone-naive Prostate Carcinoma

Takefumi Satoh, MD[1]; Masaki Kimura, MD[1]; Kazumasa Matsumoto, MD[1]; Ken-ichi Tabata, MD[1]; Hiroshi Okusa, MD[1]; Hideharu Bessho, MD[1]; Masatsugu Iwamura, MD[1]; Hiromichi Ishiyama, MD[2]; Kazushige Hayakawa, MD[2]; and Shiro Baba, MD[1]

BACKGROUND: Androgen-deprivation therapy (ADT) decreases bone mineral density (BMD) and increases fracture risk in patients with prostate carcinoma. The authors investigated the effectiveness of a single infusion of zoledronic acid initiated subsequent to ADT on BMD with hormone-naive prostate carcinoma. **METHODS:** Forty men received either a single infusion of zoledronic acid (4 mg intravenously on Day 1) or no infusion during ADT. BMD of the proximal femur and posteroanterior lumbar spine was measured by dual-energy x-ray absorptiometry and urinary N-telopeptide (u-NTx) at 6 and 12 months. **RESULTS:** At baseline, the overall BMDs demonstrated no significant difference in lumbar spine and hip regions. At 6months, mean (±standard error) BMD of the posteroanterior lumbar spine decreased 4.6% ± 1.0% in control patients and increased 5.1% ± 1.2% in patients receiving zoledronic acid, a significant difference ($P = .0002$). At 12 months, the change in BMD between the 2 groups was statistically significantly different at the lumbar region ($P = .0004$), indicating that zoledronate preserved BMD. For u-NTx, bone turnover was statistically significantly decreased in the zoledronate group compared with controls at 6 months ($P < .0001$), but returned to pretreatment levels at 12 months in the zoledronate group. **CONCLUSIONS:** Bone loss begins at 6 months with ADT. A single infusion of zoledronic acid in patients receiving ADT reduces bone mineral loss and maintains BMD at least at 12 months during ADT. Further study is needed to determine the best dosing schedule to prevent ADT-induced bone loss in men with hormone-naive prostate carcinoma. **Cancer 2009;115:3468–74. © 2009 American Cancer Society.**

KEY WORDS: prostate carcinoma, androgen-deprivation therapy, bone mineral density, bisphosphonate.

Current data from the Prostate Strategic Urologic Research Endeavor (CaPSURE) and Surveillance, Epidemiology, and End Results–Medicare database of the United States have demonstrated an increase in

Corresponding author: Takefumi Satoh, MD, Department of Urology, Kitasato University School of Medicine, 1-15-1 Kitasato, Sagamihara, Kanagawa 228-8555, Japan; Fax: (011) 81-42-778-9374; tsatoh@kitasato-u.ac.jp

[1]Department of Urology, Kitasato University School of Medicine, Kanagawa, Japan; [2]Department of Radiology, Kitasato University School of Medicine, Kanagawa, Japan

We thank Yukitoshi Ohta, RT, Erina Sato, CRC, and Mineko Uemae, RT, for helpful data management.

Received: August 11, 2008; **Revised:** January 10, 2009; **Accepted:** January 13, 2009

Published online: May 29, 2009 © 2009 American Cancer Society

DOI: 10.1002/cncr.24404, www.interscience.wiley.com

recent years in the proportion of patients with localized and advanced prostate carcinoma for whom androgen-deprivation therapy (ADT) is being selected.[1,2] Data on the current treatment of prostate cancer in Japan indicate that ADT is chosen to treat localized/advanced prostate cancer in an extremely high proportion of cases.[3]

Testosterone is the primary male hormone and is important in establishing and maintaining the typical male characteristics. Possible adverse effects (AEs) of ADT, in the form of gonadotropin-releasing hormone (GnRH) agonists, are generally related to changing levels of hormones, such as hot flushes, loss of muscle mass, erectile dysfunction, fatigue, anemia, and osteoporosis.

The results of several prospective studies show that a rapid loss of bone mineral density (BMD) occurs within the first 6 to 12 months of ADT.[4,5] The risk of skeletal fracture associated with ADT was recently reported,[6] and it is important to note that a skeletal fracture in a patient with prostate carcinoma is an independent and adverse predictor of survival.[7] Recent studies have shown that bisphosphonates, such as alendronate, pamidronate, risedronate, and zoledronic acid, will maintain the increased BMD in patients on ADT.[8-12] However, the durable or long-term efficacy of zoledronic acid is still unknown.

We investigated the effectiveness of a single infusion of zoledronic acid initiated subsequent to ADT on BMD and biochemical markers of bone turnover in patients with hormone-naive prostate carcinoma. The main focus of this study was to evaluate the near-term effectiveness of a single infusion of zoledronic acid during the 12 months after ADT.

MATERIALS AND METHODS

Patients

Study participants were recruited at Kitasato University Hospital between September 2006 and March 2007. All patients had prostate adenocarcinoma with bone metastasis and did not receive any hormonal therapy (hormone naive) previously. Treatment with GnRH agonist was initiated at study entry in all patients. Men with metabolic bone disease, history of treatment for osteoporosis, a serum calcium level <8.4 mg/dL or >10.6 mg/dL, or a serum creatinine concentration >1.5 mg/dL were also excluded. At the screening visit, BMD of the posteroanterior lumbar spine and proximal femur was determined by dual-energy x-ray absorptiometry (DXA). T score was calculated from a Japanese male reference database.[13] Patients with T score of −2.5 or less were excluded.

Study Design

This study was a randomized, prospective controlled pilot study over 12 months. Eligible patients were simply randomized by random numbers that are readily generated by computer software (Excel version 2003; Microsoft, Redmond, Wash). Forty eligible patients were randomly assigned to receive either zoledronic acid at a dose of 4 mg (Zometa; Novartis Pharmaceuticals Inc, Basel, Switzerland) intravenously on Day 1 only (n = 20) or no treatment (n = 20). The patients received zoledronic acid simultaneously with the initiation of ADT. Patients were evaluated at baseline and at 6 months and 12 months. Serum samples were obtained at each visit and stored at −80°C. BMD was measured by DXA at baseline, 6 months, and 12 months. All patients provided written informed consent.

Safety Assessment

The AEs were monitored every 3 months. Physical examinations and serum creatinine/calcium levels were monitored every 3 months. AEs were scored using the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0 (NCI-CTCAE v.3.0).

Study Endpoints

BMD of the posteroanterior lumbar spine and proximal femur was determined by DXA using a Hologic QDR 4500A/SL densitometer (Hologic Inc, Waltham, Mass) in all patients. The DXA device was standardized and calibrated using the Anthropomorphic Spine Phantom (Hologic Inc). In vivo precision assessment was performed according to the International Society for Clinical Densitometry recommendation.[14] By determining precision error (0.012 g/cm^2) and least significant change (0.034 g/cm^2 at 95% confidence interval [95% CI]), it was

Table 1. Clinical Characteristics of the Patients

Characteristics	Zoledronic Acid Group			Control Group			P
	Mean	No.	SD	Mean	No.	SD	
Patients treated		20			20		
Age, y (range)	70.5 (53-81)			70 (60-82)			NS
Serum testosterone, ng/mL	4.08		0.85	4.12		0.63	NS
Body mass index, kg/m^2	23.7		2.23	22.3		2.8	NS
Urinary NTx, nmol BCE/nmol Cr	44.6		35.6	33.2		14.2	NS
Bone mineral density, g/cm^2							
Posteroanterior lumbar spine	1.026		0.23	0.936		0.183	NS
Total hip	0.856		0.138	0.859		0.174	NS
Femoral neck	0.726		0.13	0.719		0.148	NS
T score							
Posteroanterior lumbar spine	−0.23		1.42	−0.41		1.19	NS
Total hip	−0.66		1.11	−0.39		1.30	NS
Femoral neck	−1.06		1.00	−1.14		1.17	NS

SD indicates standard deviation; NS, not significant; NTx, N-telopeptide; BCE, bone collagen equivalents; Cr, creatinine.

confirmed that sufficiently precise assessment was done in our hospital. Serum concentrations of testosterone (SRL Inc., Tokyo, Japan) were measured by radioimmunoassays. Urine concentrations of N-telopeptide (NTx; SRL Inc.) were measured by enzyme immunoassays.

Statistical Analysis

The primary study endpoint was the percentage change in the BMD of the posteroanterior lumbar spine from baseline to Months 6 and 12. Statistical analyses were performed using SPSS statistical software (version 13.0; SPSS Japan Inc., Tokyo, Japan). Values are reported as means ± standard error [SE] unless otherwise specified. All P values were 2-sided, and $P < .05$ was considered statistically significant.

RESULTS

Forty eligible patients were randomly assigned to receive either zoledronic acid (n = 20) or no drug treatment (n = 20). Table 1 lists the baseline characteristics of patients in both groups. All patients were hormone naive and received treatment with a GnRH agonist after study entry.

The mean percentage changes in BMD of the posteroanterior lumbar spine, the total hip, and the femoral neck differed significantly between groups (Fig. 1). At 6 months, the mean (±SE) BMD of the posteroanterior lumbar spine decreased 4.6% ± 1.0% from baseline in control men and increased 5.1% ± 1.2% from baseline in men given zoledronic acid ($P = .0002$). At 12 months, the mean (±SE) BMD of the posteroanterior lumbar spine decreased 8.2% ± 1.8% from baseline in the control men and increased 3.5% ± 0.8% from baseline in the men receiving zoledronic acid ($P = .0004$). The between-group differences in percent change from baseline to 6 months and to 12 months were 9.7% (95% CI, 7.0%-12.4%) and 11.7% (95% CI, 9.6%-13.4%), respectively.

At 6 months, the mean (±SE) BMD of the total hip decreased 2.2% ± 0.5% from baseline in the controls and increased 1.1% ± 0.7% from baseline in the treatment group ($P = .0025$). At 12 months, the mean (±SE) BMD of the total hip decreased 4.6% ± 1.0% from baseline in controls and increased 1.1% ± 0.6% from baseline in those given zoledronic acid ($P = .0008$). The between-group differences in percent change from baseline to 6 months and to 12 months were 3.3% (95% CI, 2.2%-4.4%) and 5.7% (95% CI, 4.6%-6.9%), respectively.

At 6 months, the mean (±SE) BMD of the femoral neck decreased 0.7% ± 0.1% from baseline in the controls and increased 1.8% ± 0.8% from baseline in the treatment group ($P = .0063$). At 12 months, the mean (±SE) BMD of the femoral neck decreased 1.8% ± 0.4% from baseline in controls and increased 5.1% ± 1.3% from baseline in

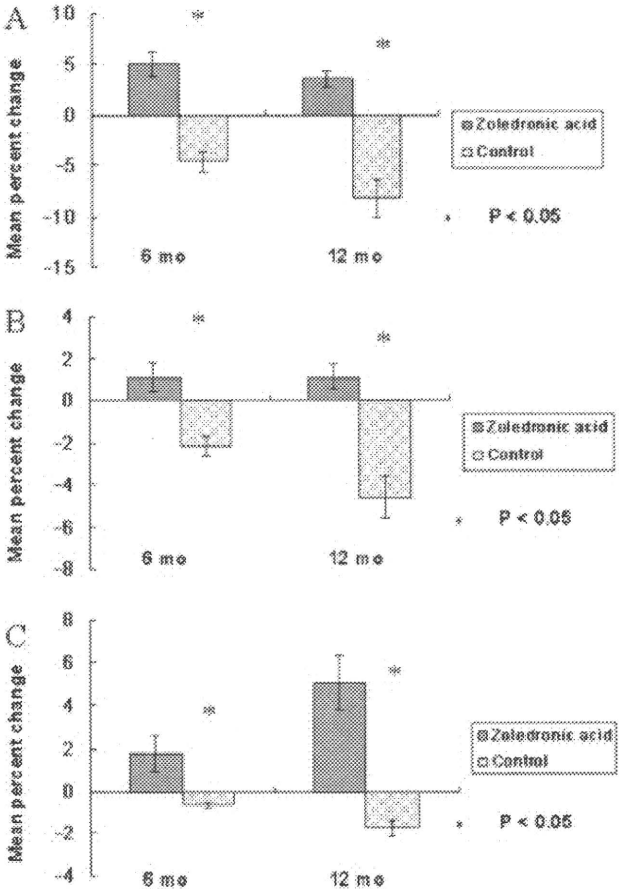


FIGURE 1. Geometric mean percent changes from baseline for bone mineral density for the (A) lumbar spine, (B) total hip, and (C) femoral neck are shown. *P* values are between-group comparisons of the percentage change from baseline to 12 months after treatment.

those given zoledronic acid ($P = .0393$). The between-group differences in percent change from baseline to 6 months and to 12 months were 2.5% (95% CI, 1.3%-3.7%) and 6.9%.(95% CI, 4.6%-9.2%), respectively.

Changes from baseline to 6 months in urine NTx differed significantly between the groups (Fig. 2). Mean (±SE) urine NTx increased by 70.3% ± 15.7% in control men and decreased by 9.5% ± 10.8% in the zoledronic acid group, a statistically significant difference ($P < .0001$). At 12 months, the mean (±SE) urine NTx increased 63.9% ± 14.3% from baseline in the control group and increased 19.7% ± 19.3% from baseline in the zoledronic acid group; these differences did not reach statistical significance ($P = .0703$).

AEs related to treatment in each group were never higher than grade 3 (using NCI-CTCAE v.3.0). Neither

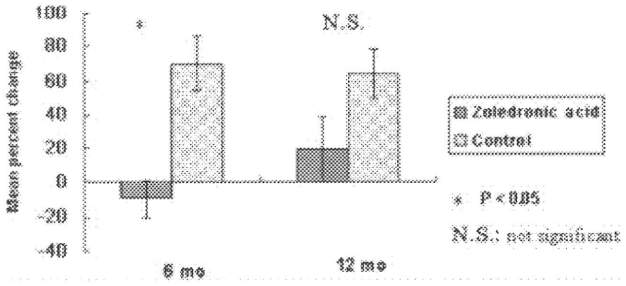


FIGURE 2. Geometric mean percent changes from baseline for urine N-telopeptide are shown. *P* values are between-group comparisons of the percentage change from baseline to 12 months after treatment.

azotemia nor osteonecrosis of the jaw was reported in either group.

DISCUSSION

The incidence and mortality of prostate carcinoma is rapidly increasing in Japan. In 2000, the Japanese Urological Association (JUA) launched a system for registering patients who were newly diagnosed with prostate carcinoma at institutions authorized by the JUA. The compilation results of 2000 were published and surprisingly indicated that primary ADT was used in 40% of patients with T1c disease and in >50% of patients with T2 disease.[3]

Conversely, the US National Cancer Institute Physician Data Query and American Urological Association guidelines do not recommend hormonal therapy for treatment of localized prostate carcinoma. However, CaP-SURE surveillance data have demonstrated that in recent years the use of ADT has increased for patients with all stages of prostate carcinoma.[1] Furthermore, several randomized controlled trials show an overall survival benefit of neoadjuvant ADT as well as adjuvant ADT, and this combination treatment has had a large impact.[15-17] Thus, ADT for prostate carcinoma is being adopted and the number of patients undergoing ADT may increase worldwide in the future. However, among men surviving at least 5 years after the diagnosis of prostate carcinoma, 19.4% of those who received ADT had skeletal fractures, and there was a statistically significant relationship between the number of doses of GnRH received during the 12 months after diagnosis and subsequent risk of fracture.[18] In addition, the occurrence of skeletal-related events (SREs), including fractures, contributes

significantly to the cost of care for patients with advanced prostate carcinoma.[19] The average total cost of treatment was €13,051/patient over the 24-month follow-up period, which includes an average cost of €6973/patient to treat SREs. Treatment of SREs more than doubled total treatment costs, and these data suggest that bisphosphonates can reduce SREs and healthcare costs.

Recent studies have shown that bisphosphonates, such as alendronate, pamidronate, risedronate, and zoledronic acid, will maintain the increased BMD in patients on ADT.[8-12] Greenspan et al reported the effect of the oral bisphosphonate alendronate given orally (70 mg) once weekly on BMD, and markers of bone turnover in patients with nonmetastatic prostate carcinoma recently initiating ADT or receiving ADT for ≥6 months evaluated in a prospective, randomized, double-blind, placebo-controlled, partial crossover trial.[20] In patients treated with alendronate, BMD increased over 12 months by 3.7% ($P < .001$) at the spine and 1.6% ($P = .008$) at the femoral neck. Conversely, patients in the placebo group had losses of 1.4% ($P = .045$) at the spine and 0.7% ($P = .081$) at the femoral neck. At 12 months, the difference between the 2 groups was 5.1% ($P < .001$) at the spine and was 2.3% ($P < .001$) at the femoral neck.

Intravenous bisphosphonates also increase BMD in GnRH agonist–treated men.[8,10-12] To our knowledge, Smith et al were the first to assess the effect of zoledronic acid on BMD during ADT for nonmetastatic prostate carcinoma.[8] Patients with prostate carcinoma (no metastases) who were beginning ADT were randomly assigned to receive zoledronic acid at a dose of 4 mg or placebo intravenously every 3 months for 1 year. The mean BMD in the lumbar spine increased by 5.6% in patients receiving zoledronic acid and decreased by 2.2% in those given placebo (mean difference, 7.8%; $P < .001$). The mean BMD of the femoral neck, trochanter, and total hip also increased in the zoledronic acid group and decreased in the placebo group after 1 year of therapy.

Israeli et al assessed the benefit of zoledronic acid in patients with pre-existing bone loss. Patients were randomized to receive zoledronic acid at a dose of 4 mg or placebo intravenously every 3 months when initiated during the first year of ADT in patients with locally advanced prostate carcinoma.[11] Although all patients receiving zoledronic acid in their study experienced increases in lumbar spine and total hip BMD, patients with low baseline T scores (−1 or less and −2 or greater) experienced a greater magnitude of increase in lumbar spine BMD than patients with normal baseline T scores (more than −1) (5.8% vs 4.4%, respectively). A similar difference in the magnitude of NTx suppression was observed. Zoledronic acid–treated patients with low baseline T scores experienced greater NTx suppression than patients with normal baseline T scores (−82.7% vs −58.4%, respectively). These results suggest that patients with pre-existing bone loss may experience a greater benefit of zoledronic acid treatment.

In patients with hormone-refractory metastatic prostate carcinoma, frequent treatment with zoledronic acid (4 mg every 3 weeks) reportedly decreases the risk of SREs.[21]

Patients with hormone-refractory prostate carcinoma and a history of bone metastases were randomly assigned to a double-blind treatment regimen of intravenous zoledronic acid at a dose of 4 mg, zoledronic acid at a dose of 8 mg (subsequently reduced to 4 mg; 8/4), or placebo every 3 weeks for 15 months. The median time to first SRE (defined as pathologic bone fractures, spinal cord compression, surgery to bone, radiation therapy to bone, or a change of antineoplastic therapy to treat bone pain) was 321 days for patients who received placebo, was not reached for patients who received zoledronic acid at a dose of 4 mg ($P = .011$ vs placebo), and was 363 days for those who received zoledronic acid at a dose of 8/4 mg ($P = .491$ vs placebo). Given the results of this study, zoledronic acid (4 mg every 3-4 weeks) was approved to treat patients with hormone-refractory prostate carcinoma metastatic to bone, and this treatment remains the only known effective schedule to prevent SRE complications in patients with metastatic prostate carcinoma.

The results of the current study demonstrate that a single infusion of zoledronic acid within the first 12 months of ADT prevents bone loss and increases BMD in men with hormone-naive prostate carcinoma. Compared with the control group, zoledronic acid significantly increased lumbar spine and femoral neck BMD by 11.7% and 5.7%, respectively ($P = .0004$ and $P = .0008$, respectively). A single infusion of zoledronic acid can provide a durable effect for the hormone-naive patient for at least 12 months after ADT. When initiating ADT for hormone-naive patients with prostate carcinoma, the simultaneous use of a single infusion of zoledronic acid