2- to 29-fold increase in reporter transcription compared with the *1G* construct. In the present study, despite the increased expression of MMP-1 in specimens with the *2G* allele, progression of the disease did not seem to be affected by the existence of the *2G* allele alone. Since the 11q22 region contains a cluster of *MMP* genes including *MMP-1*, *3*, *7*, *8*, *10*, *12*, *13*, *20*, and *27* (33), the amplification of 11q22 region may induce co-overexpression of other MMPs concurrent with MMP-1 and cooperatively facilitates tumor invasion.

In this study, the tumor progression was significantly associated with an AI, especially retention of the *2G* allele of the *MMP-1* promoter region, whereas our case-control study showed that the presence of the *2G* allele alone did not seem to have an effect on the tumor progression. Noll *et al* also demonstrated that 83% of metastatic melanoma with an AI of the region had retention of the *2G* allele (34). Our study, however, did not reveal whether each AI case had a loss or gain of either allele. A previous study using comparative genomic hybridization (CGH) analyses showed that 11q22 was one of the most frequently amplified regions in localized prostate cancer (27). In the study, 5 (22.7%) of 22 cases with pT2 disease and 6 (75.0%) of 8 with pT3 disease had a gain in 11q22 region, an observation consistent with our findings. The finding that advanced tumors have a disproportionate representation of the *2G* allele implies that the retention and probably amplification of the *2G* allele has a selective advantage for tumor cells to acquire metastatic or invasive potential. Further studies combined with fluorescence *in situ* hybridization or CGH analysis are warranted to determine the gene dosage effect of the *2G* allele on prostate cancer progression. In renal cell cancer, although AI of the 11q22 region was not detected, the *2G/2G* genotype showed a significant association with cancer susceptibility (17). The contribution of the *2G* allele dosage to carcinogenesis or tumor progression varies among different cancer types (17).

There is only one small-scale study regarding prostate cancer, which showed that the *MMP-1* promoter polymorphism was not associated with susceptibility to the disease (29). Our relatively large-scale study also did not find any association with susceptibility to prostate cancer. Some potential explanations for the lack of association include the fact that *MMP-1* polymorphism is not involved in carcinogenesis of prostate cancer, but contributes to tumor invasion or progression. Another reason could be that the sample size in this case-control study did not have sufficient statistical power to detect minimal differences in genotype frequency between the control and patient groups. A larger-scale study is therefore needed to validate our results.

It is unclear whether the polymorphism possesses direct effects on the development of malignant tumors or is only a genetic marker predicting susceptibility to cancers. The *MMP-1* promoter polymorphism is known to be in linkage disequilibrium with the *5A/6A* polymorphism in the promoter region of the *MMP-3*, (35,36) and it is known that spontaneous breast cancer develops in *MMP-3* transgenic mice (37). Although a previous study indicated that the *2G* allele, which creates ETS binding site, induced higher ERK-mediated MMP-1 expression (11), there has been no apparent evidence that higher expression of MMP-1 itself promotes carcinogenesis. Moreover, the amplified 11q22 region harbors several important genes involved in carcinogenesis and/or cancer progression such as other *MMPs*, *BIRC2*, and *BIRC3*. Thus, when we evaluate the effect of the *MMP-1* polymorphism or AI on the cancer susceptibility or progression, consideration should be given to the relative contribution of linkage disequilibrium between polymorphisms and other genes located in the co-amplified region.

In conclusion, the presence of the *2G* allele was associated with higher expression of MMP-1 in prostate cancer tissue and AI of the *MMP-1* promoter region, specifically in retention of the *2G* allele, was suggested to be involved in the progression of prostate cancer. However, the *MMP-1* polymorphism itself did not influence susceptibility nor progression of the prostate cancer.

## Acknowledgements

This study was supported by Japan Society for Promotion of Science (19390411, 19591832, 19591833, 19659406). We are grateful to Ms. Y. Mitobe and Ms. T. Shibata (Department of Urology, Akita University School of Medicine, Japan) for their excellent technical assistance.

## References

1. Dvorak HF: Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med 315: 1650-1659, 1986.
2. Pulukuri SM and Rao JS: Matrix metalloproteinase-1 promotes prostate tumor growth and metastasis. Int J Oncol 32: 757-765, 2008.
3. Murray GI, Duncan ME, O'Neil P, Melvin WT and Fothergil JE: Matrix metalloproteinase-1 is associated with poor prognosis in colorectal cancer. Nat Med 2: 461-462, 1996.
4. Kanamori Y, Matsushima M, Minaguchi T, Kobayashi K, Sagae S, Kudo R, Terakawa N and Nakamura Y: Correlation between expression of the matrix metalloproteinase-1 gene in ovarian cancers and an insertion/deletion polymorphism in its promoter region. Cancer Res 59: 4225-4227, 1999.
5. Murray GI, Duncan ME, O'Neil P, McKay JA, Melvin WT and Fothergill JE: Matrix metalloproteinase-1 is associated with poor prognosis in oesophageal cancer. J Pathol 185: 256-261, 1998.
6. Ala-Aho R and Kahari VM: Collagenases in cancer. Biochimie 87: 273-286, 2005.
7. Murray GI, Duncan ME, Arbuckle E, Melvin WT and Fothergill JE: Matrix metalloproteinases and their inhibitors in gastric cancer. Gut 43: 791-797, 1998.
8. Nikkola J, Vihinen P, Vlaykova T, Hahka-Kemppinen M, Kahari VM and Pyrhonen S: High expression levels of collagenase-1 and stromelysin-1 correlate with shorter disease-free survival in human metastatic melanoma. Int J Cancer 97: 432-438, 2002.
9. Pardo A and Selman M: MMP-1: the elder of the family. Int J Biochem Cell Biol 37: 283-288, 2005.
10. Rutter JL, Mitchell TI, Buttice G, Meyers J, Gusella JF, Ozelius LJ and Brinckerhoff CE: A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. Cancer Res 58: 5321-5325, 1998.
11. Tower GB, Coon CI and Brinckerhoff CE: The 2G single nucleotide polymorphism (SNP) in the MMP-1 promoter contributes to high levels of MMP-1 transcription in MCF-7/ADR breast cancer cells. Breast Cancer Res Treat 82: 75-82, 2003.
12. Nishioka Y, Kobayashi K, Sagae S, Ishioka S, Nishikawa A, Matsushima M, Kanamori Y, Minaguchi T, Nakamura Y, Tokino T and Kudo R: A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter in endometrial carcinomas. Jpn J Cancer Res 91: 612-615, 2000.

13. Ghilardi G, Biondi ML, Mangoni J, Leviti S, DeMonti M, Guagnellini E and Scorza R: Matrix metalloproteinase-1 promoter polymorphism 1G/2G is correlated with colorectal cancer invasiveness. Clin Cancer Res 7: 2344-2346, 2001.
14. Zhu Y, Spitz MR, Lei L, Mills GB and Wu X: A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter enhances lung cancer susceptibility. Cancer Res 61: 7825-7829, 2001.
15. Su L, Zhou W, Park S, Wain JC, Lynch TJ, Liu G and Christiani DC: Matrix metalloproteinase-1 promoter polymorphism and lung cancer risk. Cancer Epidemiol Biomarkers Prev 14: 567-570, 2005.
16. Hinoda Y, Okayama N, Takano N, Fujimura K, Suehiro Y, Hamanaka Y, Hazama S, Kitamura Y, Kamatani N and Oka M: Association of functional polymorphisms of matrix metalloproteinase (MMP)-1 and MMP-3 genes with colorectal cancer. Int J Cancer 102: 526-529, 2002.
17. Hirata H, Naito K, Yoshihiro S, Matsuyama H, Suehiro Y and Hinoda Y: A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter is associated with conventional renal cell carcinoma. Int J Cancer 106: 372-374, 2003.
18. Elander N, Soderkvist P and Fransen K: Matrix metalloproteinase (MMP) -1, -2, -3 and -9 promoter polymorphisms in colorectal cancer. Anticancer Res 26: 791-795, 2006.
19. Nishizawa R, Nagata M, Noman AA, Kitamura N, Fujita H, Hoshina H, Kubota T, Itagaki M, Shingaki S, Ohnishi M, Kurita H, Katsura K, Saito C, Yoshie H and Takagi R: The 2G allele of promoter region of matrix metalloproteinase-1 as an essential pre-condition for the early onset of oral squamous cell carcinoma. BMC Cancer 7: 187, 2007.
20. Tasci AI, Tugcu V, Ozbek E, Ozbay B, Simsek A and Koksal V: A single-nucleotide polymorphism in the matrix metalloproteinase-1 promoter enhances bladder cancer susceptibility. BJU Int 101: 503-507, 2008.
21. Nishioka Y, Sagae S, Nishikawa A, Ishioka S and Kudo R: A relationship between Matrix metalloproteinase-1 (MMP-1) promoter polymorphism and cervical cancer progression. Cancer Lett 200: 49-55, 2003.
22. Matsumura S, Oue N, Kitadai Y, Chayama K, Yoshida K, Yamaguchi Y, Toge T, Imai K, Nakachi K and Yasui W: A single nucleotide polymorphism in the MMP-1 promoter is correlated with histological differentiation of gastric cancer. J Cancer Res Clin Oncol 130: 259-265, 2004.
23. Zinzindohoue F, Lecomte T, Ferraz JM, Houllier AM, Cugnenc PH, Berger A, Blons H and Laurent-Puig P: Pro-gnostic significance of MMP-1 and MMP-3 functional promoter polymorphisms in colorectal cancer. Clin Cancer Res 11: 594-599, 2005.
24. Carneiro A, Isinger A, Karlsson A, Johansson J, Jonsson G, Bendahl PO, Falkenback D, Halvarsson B and Nilbert M: Prognostic impact of array-based genomic profiles in esophageal squamous cell cancer. BMC Cancer 8: 98, 2008.
25. Lassmann S, Weis R, Makowiec F, Roth J, Danciu M, Hopt U and Werner M: Array CGH identifies distinct DNA copy number profiles of oncogenes and tumor suppressor genes in chromosomal- and microsatellite-unstable sporadic colorectal carcinomas. J Mol Med 85: 293-304, 2007.
26. Narayan G, Bourdon V, Chaganti S, Arias-Pulido H, Nandula SV, Rao PH, Gissmann L, Durst M, Schneider A, Pothuri B, Mansukhani M, Basso K, Chaganti RS and Murty VV: Gene dosage alterations revealed by cDNA microarray analysis in cervical cancer: identification of candidate amplified and overexpressed genes. Genes Chromosomes Cancer 46: 373-384, 2007.
27. Kasahara K, Taguchi T, Yamasaki I, Kamada M and Shuin T: Genetic changes in localized prostate cancer of Japanese patients shown by comparative genomic hybridization. Cancer Genet Cytogenet 159: 84-88, 2005.
28. Cardillo MR, Di Silverio F and Gentile V: Quantitative immunohistochemical and in situ hybridization analysis of metalloproteinases in prostate cancer. Anticancer Res 26: 973-982, 2006.
29. Albayrak S, Canguven O, Goktas C, Aydemir H and Koksal V: Role of MMP-1 1G/2G promoter gene polymorphism on the development of prostate cancer in the Turkish population. Urol Int 79: 312-315, 2007.
30. Tsuchiya N, Mishina M, Narita S, Kumazawa T, Inoue T, Horikawa Y, Kakinuma H, Yuasa T, Matsuura S, Satoh S, Ogawa O and Habuchi T: Association of XRCC1 gene polymorphisms with the susceptibility and chromosomal aberration of testicular germ cell tumors. Int J Oncol 28: 1217-1223, 2006.
31. D'Amico AV, Whittington R, Malkowicz SB, Schultz D, Schnall M, Tomaszewski JE and Wein A: A multivariate analysis of clinical and pathological factors that predict for prostate specific antigen failure after radical prostatectomy for prostate cancer. J Urol 154: 131-138, 1995.
32. Shimizu Y, Kondo S, Shirai A, Furukawa M and Yoshizaki T: A single nucleotide polymorphism in the matrix metalloproteinase-1 and interleukin-8 gene promoter predicts poor prognosis in tongue cancer. Auris Nasus Larynx 35: 381-389, 2008.
33. Puente XS, Sanchez LM, Overall CM and Lopez-Otin C: Human and mouse proteases: a comparative genomic approach. Nat Rev Genet 4: 544-558, 2003.
34. Noll WW, Belloni DR, Rutter JL, Storm CA, Schned AR, Titus-Ernstoff L, Ernstoff MS and Brinckerhoff CE: Loss of heterozygosity on chromosome 11q22-23 in melanoma is associated with retention of the insertion polymorphism in the matrix metalloproteinase-1 promoter. Am J Pathol 158: 691-697, 2001.
35. Pendas AM, Matilla T, Estivill X and Lopez-Otin C: The human collagenase-3 (CLG3) gene is located on chromosome 11q22.3 clustered to other members of the matrix metalloproteinase gene family. Genomics 26: 615-618, 1995.
36. Ye S, Dhillon S, Turner SJ, Bateman AC, Theaker JM, Pickering RM, Day I and Howell WM: Invasiveness of cutaneous malignant melanoma is influenced by matrix metalloproteinase 1 gene polymorphism. Cancer Res 61: 1296-1298, 2001.
37. Sternlicht MD, Bissell MJ and Werb Z: The matrix metalloproteinase stromelysin-1 acts as a natural mammary tumor promoter. Oncogene 19: 1102-1113, 2000.

# Clinical Significance of Polymorphism and Expression of Chromogranin A and Endothelin-1 in Prostate Cancer

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**Abbreviations and Acronyms**

BPH = benign prostatic hyperplasia
BPHcont = nonprostate cancer specimen BPH region
BPHpca = prostate cancer specimen BPH region
CHGA = chromogranin A
ET = endothelin
IHC = immunohistochemistry
LD = linkage disequilibrium
PCapca = prostate cancer region
PCR = polymerase chain reaction
PSA = prostate specific antigen
RRP = radical retropubic prostatectomy

Submitted for publication October 17, 2009.
Study received institutional ethics committee approval.
Supported by Japan Society for Promotion of Science, Japan (19390411, 19591832, 19591833, 19659406 and 21592029).
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**Purpose:** We investigated the clinical significance of chromogranin A and endothelin-1 polymorphism and expression in prostate cancer.
**Materials and Methods:** We analyzed 2 *CHGA* polymorphisms by polymerase chain reaction-restriction fragment length polymorphism in DNA samples of 435 patients with prostate cancer and 316 age matched male controls. Chromogranin A and endothelin-1 expression was evaluated by immunohistochemistry in prostate specimens of 114 men with prostate cancer who underwent radical retropubic prostatectomy and in 27 with bladder cancer who underwent radical cystectomy and served as controls.
**Results:** For the *CHGA* Glu264Asp polymorphism men with the *GG* genotype were at 2.05 times higher risk for prostate cancer than men with the *CC* genotype ($p = 0.014$). In men with prostate cancer higher chromogranin A immunohistochemistry grade was associated with higher stage and higher Gleason score ($p = 0.011$ and 0.044, respectively). Multivariate analysis showed that chromogranin A immunohistochemistry grade was an independent variable for predicting biochemical failure after radical prostatectomy ($p = 0.023$). Higher endothelin-1 expression was observed in prostate cancers ($p = 0.011$), especially those with a higher Gleason score ($p = 0.042$). There was no significant relationship between chromogranin A polymorphisms, and chromogranin A and endothelin-1 expression.
**Conclusions:** Polymorphism and expression of chromogranin A and endothelin-1 have clinical significance in prostate cancer. Chromogranin A expression was an independent predictor of biochemical failure after prostatectomy in patients with localized prostate cancer.

**Key Words:** prostate; prostatic neoplasms; polymorphism, genetic; chromogranin A; endothelin-1

NEUROENDOCRINE cells have an important role in normal prostates and BPH as well as in primary and metastatic prostate cancer.[1,2] Of the biogenic amines and neuropeptides secreted by neuroendocrine cells CHGA is a candidate marker for diagnosing and predicting the prognosis of prostate cancer. Patients with prostate cancer have significantly higher serum CHGA than those with BPH and controls.[2] A group reported that CHGA protein expression determined by IHC is a useful prognostic marker of biochemical failure after radical prostatectomy.[3] To date only 1 group



0022-5347/10/1843-1182/0
THE JOURNAL OF UROLOGY®

Vol. 184, 1182-1188, September 2010
Printed in U.S.A.
DOI:10.1016/j.juro.2010.04.063

has performed IHC analysis and found higher CHGA expression in benign epithelial cells adjacent to prostate cancer lesions than in the BPH region.[4] On the other hand, CHGA polymorphisms can influence CHGA expression, which eventually affects baseline blood pressure,[5] but the relationship between CHGA polymorphisms and prostate cancer remains unclear.

ETs, which are endogenous small peptides secreted by endothelium, exert paracrine and autocrine effects through cell surface receptors and influence cellular processes, such as angiogenesis, cellular proliferation, and tissue repair and development.[6–8] Plasma ET-1 levels in patients with hormone refractory, metastatic prostate cancer are higher than in patients with organ confined prostate cancer or controls.[9] Another IHC study showed ET-1 over expression in cases of advanced prostate cancer and high grade prostatic intraepithelial neoplasia.[10] Recently CHGA and ET-1 interaction was reported in a group of twins as well as in vitro experiments.[11] The study showed that polymorphisms in the CHGA promoter region are associated with serum ET-1 and CHGA stimulated ET-1 secretion in endothelial cells in a dose dependent manner. To our knowledge the association between CHGA and ET-1 in prostate cancer has not been assessed.

We analyzed 7 polymorphisms in the promoter region and the Glu264Asp polymorphism in exon 6 of *CHGA* in a Japanese population to evaluate the relationship to prostate cancer risk and clinical characteristics. We evaluated CHGA and ET-1 protein expression to determine whether they are related to localized prostate cancer pathological features and treatment outcomes. Also, we assessed the relationships among the *CHGA* genotypes, CHGA protein expression and ET-1 protein expression.

## MATERIALS AND METHODS

### Subjects

A total of 751 men, including 435 with prostate cancer and 316 controls, were enrolled in this study. All patients with prostate cancer were diagnosed at Akita University Medical Center, Kyoto University affiliated hospital and related community hospitals. They were pathologically diagnosed using specimens obtained from transrectal needle biopsy or transurethral prostate resection due to lower urinary tract symptoms. Prostate cancer clinical or pathological stage at diagnosis was determined by reviewing the medical records based on the TNM system. Prostate cancer was classified as stage A—T1a-bN0M0, stage B—T1c-2N0M0, stage C—T3-4N0M0 and stage D—T1-4N1M0-1 or T1-4N0-1M1 by the modified Whitmore-Jewett system. Controls were native Japanese men older than 60 years who had undergone health inspection at a community hospital.

IHC was done in prostate specimens from 114 men with stage T2-4 prostate cancer who underwent RRP and in BPH specimens from 27 who underwent radical cystectomy for bladder cancer. Since endocrine therapy may affect the number of neuroendocrine cells, patients with prostate cancer treated with endocrine therapy before RRP were excluded from analysis.[12] Clinical information was reviewed in the medical records. DNA and prostate specimens were collected after obtaining informed consent with approval from the institutional ethics committee.

### CHGA Polymorphism Genotyping

We selected 7 polymorphisms in the *CHGA* promoter region for LD analysis. DNA direct sequencing was done in 200 samples to analyze the genotypes of those polymorphisms. The Appendix lists PCR primer sequences. Genotype data were imported into Haploview, version 3.32 (Daly Laboratory, Board Institute, Cambridge, Massachusetts) to test LD among polymorphisms in the *CHGA* promoter region. D′ greater than 0.8 was considered a strong LD.

Finally, we analyzed 2 *CHGA* polymorphisms, including rs9658635 in the promoter region and Glu264Asp in exon 6, using certain primers (table 1). After confirming successful PCR amplification each product was digested at 37C overnight with 5 U *Bcc* I or *BfuC* I restriction enzymes (New England Biolabs, Beverly, Massachusetts). For the rs9658635 polymorphism restriction fragments were 114 and 21 bp for the *T* allele, and 135 bp for the *C* allele. For the Glu264Asp polymorphism restriction fragments were 129 and 106 bp for the *G* allele, and 235 bp for the *C* allele. To avoid genotyping errors caused by incomplete digestion or other technical failures we repeated the experiment at least twice for all samples and compared the genotype with the DNA sequencing results in 100 randomly selected samples.

**Table 1.** *PCR primers*

| | Reference Single Nucleotide Polymorphism | |
|---|---|---|
| | rs9658635 | rs9658655 |
| Polymorphism | T-415C | Glu264Asp |
| Primers | Forward-5′ CCTAGATATTGGAGAGAGCCATGAGTGA 3′<br>Reverse-5′ CCATGTGTACTGAGGTCCCTGGCAG 3′ | Forward-5′ AGGGTGGCAGGCAAAGAG 3′<br>Reverse-5′ AAGGTGGAATGAGGTTATGG 3′ |
| Length (bp) | 135 | 235 |
| Enzyme | *BccI* | *BfuCI* |
| Fragments (bp) | 21 + 114 | 106 + 129 |

### IHC Staining and Evaluation

We performed IHC staining for CHGA and ET-1 using a certain protocol. Briefly, deparaffinized, rehydrated sections were steamed for 20 minutes to enhance antigen retrieval. Immunohistochemical labeling with mouse anti-human CHGA antibody (DakoCytomation, Glostrup, Denmark) (×800) or ET-1 antibody (Alexis Biochemicals, Lausen, Switzerland) (×250) was done overnight at 4C. Slides were labeled with the anti-mouse EnVision™+ system labeled with horseradish peroxidase for 30 minutes. The liquid DAB+ Substrate-Chromogen System (DakoCytomation) was applied at room temperature for 30 minutes. Slides were counterstained with hematoxylin solution for nuclear staining. Specimens were examined by 2 independent researchers blinded to sample background data.

CHGA positive stained cells were counted in 10 high power visual fields at 200× magnification to determine which had the most positive cells (fig. 1, *A*). Since the number of CHGA positive cells in the BPH region was greatly different than that in the prostate cancer region, CHGA positive cells in 3 regions were counted, including BPHcont, BPHpca and PCapca. Counting was done 3 times per sample and the mean was used for statistics. The mean value of each sample was categorized as grade 1—less than 10, grade 2—10 to 29 and grade 3—30 or greater for the prostate cancer region. Since neuroendocrine cells are consistently found in the periurethral ducts and verumontanum,[13] those regions were excluded from counting.

Cytoplasmic ET-1 staining intensity was scored on a semiquantitative scale as 1—weak, 2—moderate and 3—strong (fig. 1, *B*). The percent of cytoplasmic ET-1 positive cells was divided into 4 groups, including 1—less than 25%, 2—25% to 50%, 3—50% to 75% and 4—greater than 75%. Total immunoreactivity grade was calculated by multiplying the 2 scores[14] and defined as grade 1—6 or less, grade 2—8 and grade 3—greater than 8.

### Statistical Analysis

All data were entered into an Access® database and analyzed using Excel® 2007 and SPSS®, version 16.0J. We examined differences in mean age in the 3 groups using the independent t test. Hardy-Weinberg equilibrium analysis was done to compare observed and expected genotype frequency using the Pearson chi-square test. We used binary logistic regression to assess the association between prostate cancer risk and genotypes by calculating the OR and 95% CI. We hypothesized that the *C* allele of the rs9658635 polymorphism would be an inherent genetic risk factor for prostate cancer and prostate cancer progression. Statistical modeling was done independently on the relative risk of the *CC* or *CT* genotype against the *TT* genotype for rs9658635 using the logistic regression model adjusted by age. For Glu264Asp the *G* allele was hypothesized as an inherent genetic risk factor for prostate cancer and prostate cancer progression.

We used 1-way ANOVA to compare the number of CHGA IHC positive cells among the 3 groups and Kendall's $\tau$-b rank correlation coefficients to examine the relationship between IHC grade and Gleason score or clinical stage. The biochemical failure-free interval was defined as the time from the date of RRP to the date when PSA increased to more than 0.4 ng/ml. We estimated relationships between polymorphisms or IHC grade and biochemical failure-free survival in stage T2-4 prostate cancer cases by the Kaplan-Meier method and evaluated them by the log rank test. The Cox multivariate proportional hazards model was used for multivariate analysis. We examined relationships between polymorphisms and IHC grades in patients with prostate cancer using Fisher's exact test. All statistical tests and p values were 2-tailed with results considered significant at $p < 0.05$.

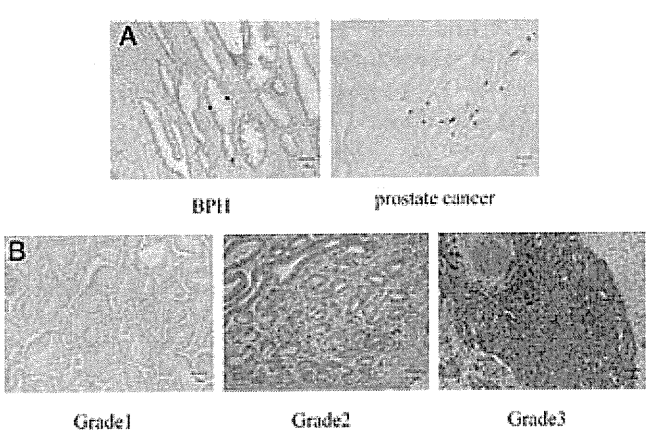


**Figure 1.** IHC in BPH and prostate cancer regions. *A*, CHGA cytoplasmic staining pattern. *B*, representative ET-1 IHC stains of different grades.

## RESULTS

### Characteristics

Mean age ± SD in patients with prostate cancer and male controls was 70.28 ± 7.43 and 69.46 ± 7.22 years, respectively ($p = 0.289$). Stage was A to C, D1 and D2 in 10, 191, 83, 25 and 126 patients with prostate cancer, respectively. In the prostate cancer group Gleason score was less than 7, 7, greater than 7 and unavailable in 14, 202, 164 and 55 patients, respectively.

### CHGA Associations

***Polymorphism genotypes vs prostate cancer risk and clinicopathological factors.*** Genotype distributions in all groups were consistent with Hardy-Weinberg equilibrium. Since more than 90% of D′ values in the 7 polymorphisms in the *CHGA* promoter region equaled 1 (fig. 2), the rs9658635 polymorphism, which was reported to be associated with CHGA expression,[5] was chosen as a representative polymorphism for further analysis. Statistical analysis of genotype frequency showed no relationship between the rs9658635 polymorphism and the prostate cancer risk ($p > 0.05$, table 2). For the Glu264Asp polymorphism we found a significantly increased prostate cancer risk in men with the *GG*

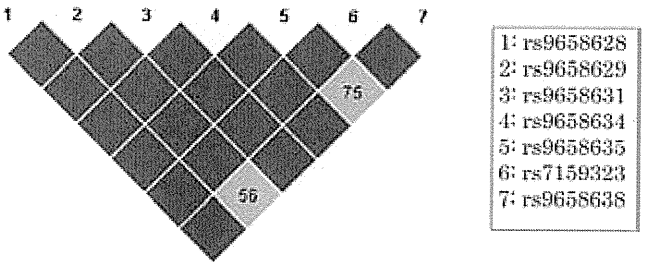


**Figure 2.** There were strong LDs among 7 CHGA promoter region polymorphisms. Red diamonds indicate D′ = 1. Gray diamonds indicate D′ less than 1.

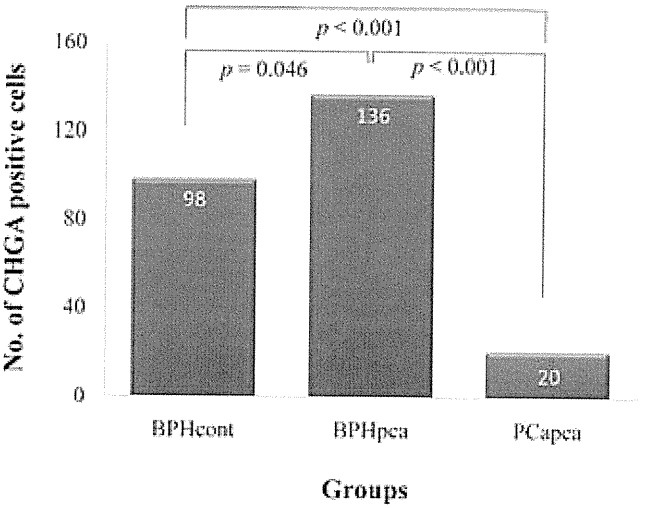


**Figure 3.** There were significant differences among mean number of CHGA IHC positive cells in BPHcont, BPHpca and PCapca.

genotype and the *GC* genotype than in those with the *CC* genotype (OR 2.05, 95% CI 1.16–3.63; p = 0.014 and OR 1.97, 95% CI 1.10–3.52; p = 0.023, respectively, table 2). There was no significant association of the rs9658635 or the Glu264Asp *CHGA* polymorphism with prostate cancer clinical stage or Gleason score (p >0.05).

***IHC grade vs prostate cancer clinicopathological factors and prognosis.*** BPHcont, BPHpca and PCapca showed a mean ± SD of 97 ± 81, 136 ± 109 and 20 ± 48 CHGA IHC positive cells, respectively (p <0.001, fig. 3). Compared with BPHcont BPHpca had more and PCapca had fewer CHGA positive cells (p = 0.046 and <0.001, respectively). In patients with prostate cancer a higher CHGA IHC grade was more often found in those with pT3-4 than pT2 disease (p = 0.011). There was a significant association between CHGA IHC grade and Gleason score (p = 0.044). On univariate analysis a higher probability of biochemical failure after RRP was significantly associated with higher CHGA IHC grade (p = 0.001, fig. 4), higher Gleason score (p = 0.039), higher stage (p = 0.025) and higher PSA at diagnosis (p <0.001). On multivariate analysis CHGA IHC grade was an independent factor predicting possible biochemical failure after RRP (p = 0.023, table 3).

### ET-1 IHC Grade

Prostate cancer showed a higher ET-1 IHC grade than BPH (chi-square 9.030, p = 0.011). Of patients with prostate cancer we noted a higher ET-1 IHC grade in those with a higher Gleason score (chi-square 4.149, p = 0.042, fig. 5). There was no statistically significant relationship between ET-1 IHC grade and clinical stage or biochemical failure after RRP (p = 0.661 and 0.230, respectively).

### CHGA Polymorphism Genotypes vs CHGA and ET-1

Cross-tabulation results showed no significant association of the *CHGA* rs9658635 or the Glu264Asp polymorphism with CHGA or ET-1 IHC grade (table 4). We found no significant relationship between CHGA and ET-1 expression (p >0.05).

**Table 2.** *CHGA polymorphisms vs prostate cancer risk and clinicopathological factors*

| | Prostate Ca vs Control | | Clinical Stage D vs A + B + C | | Gleason Score 8 or Greater vs Less Than 8 | |
|---|---|---|---|---|---|---|
| Genotype Polymorphism | OR (95% CI) | p Value | OR (95% CI) | p Value | OR (95% CI) | p Value |
| CHGA promoter rs9658635: | | | | | | |
| TT | 1 | | 1 | | 1 | |
| CT | 1.12 (0.81–1.55) | 0.502 | 1.41 (0.87–2.31) | 0.167 | 1.26 (0.79–1.98) | 0.331 |
| CC | 0.83 (0.55–1.25) | 0.363 | 1.24 (0.65–2.39) | 0.512 | 1.03 (0.56–1.91) | 0.914 |
| CT + CC | 1.02 (0.76–1.38) | 0.892 | 1.37 (0.86–2.18) | 0.187 | 1.19 (0.77–1.84) | 0.424 |
| TT + CT:CC | 1.29 (0.89–1.86) | 0.185 | 0.98 (0.55–1.76) | 0.954 | 1.10 (0.64–1.91) | 0.729 |
| Exon 6 Glu264Asp: | | | | | | |
| CC | 1 | | 1 | | 1 | |
| GC | 1.97 (1.10–3.52) | 0.023 | 1.80 (0.64–5.10) | 0.268 | 2.98 (0.96–9.22) | 0.059 |
| GG | 2.05 (1.16–3.63) | 0.014 | 2.11 (0.76–5.87) | 0.154 | 2.62 (0.86–8.04) | 0.091 |
| GC + CC | 2.01 (1.15–3.51) | 0.014 | 1.98 (0.72–5.43) | 0.817 | 2.77 (0.91–8.37) | 0.072 |
| CC + GC:GG | 0.86 (0.64–1.15) | 0.314 | 0.80 (0.54–1.20) | 0.279 | 1.02 (0.69–1.51) | 0.904 |

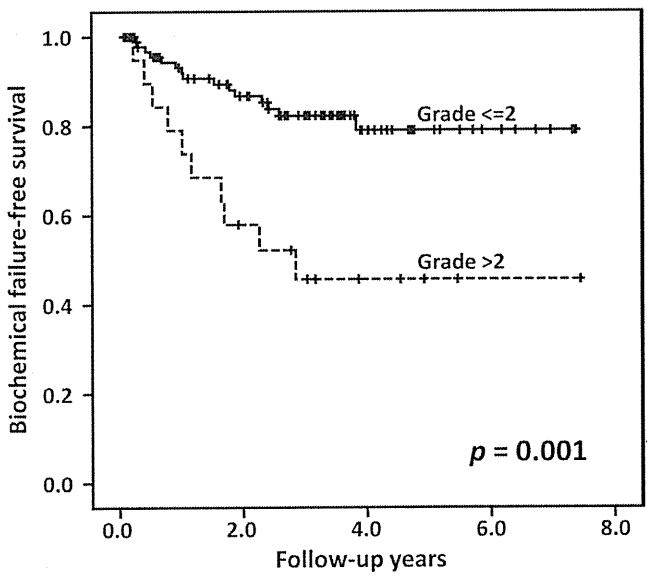


**Figure 4.** Higher CHGA IHC grade was associated with higher probability of biochemical failure after RRP.

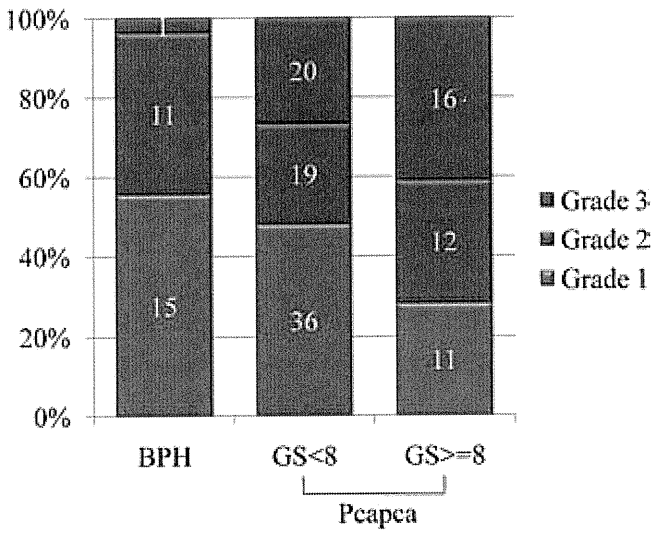


**Figure 5.** Compared with BPH prostate cancer showed higher ET-1 IHC grade. Higher ET-1 IHC grade was associated with higher Gleason score (*GS*) prostate cancer.

## DISCUSSION

We first investigated the influence of CHGA polymorphisms on prostate cancer clinicopathological factors. Results revealed a significant association between the *G* allele of the *CHGA* Glu264Asp polymorphism and the risk of prostate cancer in a native Japanese population, suggesting that the *CHGA* Glu264Asp polymorphism may be a useful marker for estimating the prostate cancer risk. To our knowledge this is the first study to investigate whether *CHGA* gene variants influence prostate cancer. The *G* to *C* allele variant of the Glu264Asp polymorphism caused the 264 amino acid CHGA to change from glutamic to aspartic acid. Pancreastatin, an impairing glucose metabolism peptide of 52 amino acids, is located in this CHGA encoding region.[15] Pancreastatin inhibits the release of glucose stimulated insulin from pancreatic islet $\beta$ cells.[16] Since insulin has an important role in prostate cancer pathogenesis,[17] it is reasonable that the *CHGA* Glu264Asp polymorphism affects prostate cancer carcinogenesis through functional alteration of pancreastatin by regulating insulin secretion. Also, the importance of the pancreastatin polymorphism was supported by the observation that the Gly297Ser polymorphism influences glucose uptake.[18] Pancreastatin is a useful prognostic indicator in patients with neuroendocrine tumors[19] but there was no association between the CHGA Glu264Asp polymorphism and prostate cancer prognosis.

The CHGA rs9658634 polymorphism is reportedly associated with serum CHGA and the ET-1 level.[5,11] However, the CHGA polymorphism showed no relationship with CHGA or ET-1 IHC expression in our study, which could have been due to several reasons. 1) The different methods of measuring CHGA expression may have led to different results. Since CHGA is secreted by other tissues as well as the prostate, serum CHGA represents total CHGA expression in the whole body. We used prostate surgical specimen IHC grade instead of the serum level to more specifically reflect prostate CHGA expression in the prostate. In support of our findings another research group found no correlation between serum CHGA and immunohistochemical results.[20] 2) The discrepancy may have been be due to our population. Only men older than 60 years with prostate cancer were enrolled in our study

**Table 3.** *Biochemical failure and clinical factor Cox proportional hazards model*

| Clinical Factors | Univariate | | Multivariate | |
|---|---|---|---|---|
| | HR (95% CI) | p Value | HR (95% CI) | p Value |
| CHGA IHC grade greater than 2 or not | 3.585 (1.609–7.987) | 0.002 | 2.713 (1.149–6.407) | 0.023 |
| Gleason score 8 or greater or not | 2.237 (1.020–4.908) | 0.044 | 1.512 (0.650–3.517) | 0.337 |
| T stage greater than 2 or not | 2.422 (1.088–5.394) | 0.030 | 0.969 (0.375–2.504) | 0.948 |
| PSA greater than 10 ng/ml or not | 5.682 (1.950–16.557) | 0.001 | 4.611 (1.483–14.336) | 0.008 |

**Table 4.** *CHGA polymorphisms vs CHGA and ET-1 expression*

| | CHGA IHC Grade | | | | ET-1 IHC Grade | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | p Value | 1 | 2 | 3 | p Value |
| rs9658635: | | | | 0.964 | | | | 0.497 |
| TT | 29 | 6 | 9 | | 19 | 14 | 11 | |
| CT | 32 | 10 | 9 | | 18 | 14 | 19 | |
| CC | 13 | 3 | 3 | | 10 | 3 | 6 | |
| Glu264Asp: | | | | 0.423 | | | | 0.272 |
| CC | 4 | 0 | 0 | | 1 | 2 | 1 | |
| GC | 27 | 11 | 10 | | 17 | 17 | 14 | |
| GG | 43 | 8 | 11 | | 29 | 12 | 21 | |

whereas other studies included subjects without cancer regardless of age or gender. This may result in the lack of a significant relationship between CHGA polymorphisms and expression in patients with prostate cancer.

However, our study shows that in patients with localized prostate cancer and no history of endocrine therapy a higher CHGA IHC grade was associated with worse tumor stage and higher Gleason score. A group also reported that IHC staining for CHGA is significantly associated with Gleason score,[20] although a contradictory result was reported.[21] Furthermore, the Cox multivariate regression model showed that CHGA IHC grade was an independent variable for predicting biochemical failure after RRP. In a study of lymph node positive cases of prostate cancer the investigators found that CHGA expression is associated with biochemical failure after RRP.[22] However, the history of endocrine therapy before RRP, which was associated with CHGA expression,[12] was not controlled in that study. Other studies in D2 prostate cancer cases showed that higher CHGA IHC grade is associated with a worse prognosis.[21,23] Taken together, CHGA IHC grade, which represents neuroendocrine differentiation, could predict the prognosis in patients with prostate cancer.

Compared with BPHcont, the number of CHGA IHC positive cells was higher in BPHpca and lower in PCapca (fig. 3). This agrees with the result that CHGA positive cells had more prominent expression in benign epithelial cells adjacent to prostate cancer lesions than in the prostate cancer region.[4] Also, there is a tendency toward a decreased number of neuroendocrine cells in untreated patients with prostate cancer compared with that in patients with BPH and male controls with a normal prostate.[24] Hence, higher serum CHGA in patients with prostate cancer may result from BPHpca, which has many more neuroendocrine cells than in patients with BPH. This indicates that to predict prostate cancer susceptibility more efficiently we should focus on the cancerous region and the adjacent noncancerous region.

Neuroendocrine cells in the BPH region are negative for $\alpha$-methylacyl coenzyme A racemase while neuroendocrine cells in prostate cancer are positive for $\alpha$-methylacyl coenzyme A racemase.[25] In vitro cells of the androgen dependent line LNCaP were induced to show neuroendocrine differentiation by androgen deprivation[26] or agents that increase intracellular cyclic adenosine monophosphate.[27] Results indicate that PCapca neuroendocrine cells, which have hormone insensitive characteristics, may differentiate from prostate cancer cells. Patients with the worst prostate cancer stages had a higher CHGA IHC grade, indicating that more prostate cancer cells had transformed into neuroendocrine cells with hormone insensitive characteristics and resulting in a worse prognosis. Whether our patients with hormone refractory prostate cancer had many more hormone insensitive neuroendocrine cells than our patients with localized prostate cancer should be explored in the future.

Serum ET-1 has no value for estimating prostate cancer prognosis.[28] We noted no association of ET-1 expression with clinical stage or biochemical failure after RRP. However, prostate cancer showed significantly higher ET-1 expression than the BPH region and higher ET-1 IHC grade was associated with a higher Gleason score.

## CONCLUSIONS

A *CHGA* genetic variant may modify prostate cancer carcinogenesis and CHGA expression may be a useful biomarker to predict the higher malignant potential of localized prostate cancer and biochemical failure after RRP. Thus, results suggest that CHGA is involved in prostate cancer carcinogenesis and progression.

## ACKNOWLEDGMENTS

Mrs. Mitobe provided technical assistance.

## APPENDIX

**DNA Sequencing Primers for CHGA Promoter Region**

| Fragment No. (polymorphisms) | Reference Single Nucleotide Polymorphism | Primers | |
|---|---|---|---|
| | | PCR | DNA Sequencing |
| 1: | | Forward-5′ CAGGTTCTCATTTAGGGACA 3′<br>Reverse-5′ AAAGGTCAGTTTCCTGGTTG 3′ | Forward-5′ TTTAGGGACAGGCGTGAGCACAGGT 3′<br>Reverse-5′ TCAGTTTCCTGGTTGGCTTCCCTT 3′ |
| G-1106A | rs9658628 | | |
| A-1018T | rs9658629 | | |
| T-998G | rs9658631 | | |
| 2: | | Forward-5′ CATCAGTTACCTGTCAAGTGCGT 3′<br>Reverse-5′ CCCCGTGCTATTTTTCCTAAGT 3′ | Forward-5′ TGTCAAGTGCGTTTCCTCTGT 3′<br>Reverse-5′ TTCCTAAGTGCCCTCTGCCT 3′ |
| G-462A | rs9658634 | | |
| T-415C | rs9658635 | | |
| 3: | | Forward-5′ GCCCAGGGACACAAGGCAAAT 3′<br>Reverse-5′ TCGGCGTGCGTCCGTCTGTC 3′ | Forward-5′ CACCTCTTGGAAACCAGATACC 3′<br>Reverse-5′ TGCGTCCGTCTGTCGGTCGATG 3′ |
| C-89A | rs7159323 | | |
| C-57T | rs9658638 | | |

## REFERENCES


1. di Sant'Agnese PA and de Mesy Jensen KL: Neuroendocrine differentiation in prostatic carcinoma. Hum Pathol 1987; **18:** 849.
2. Ranno S, Motta M, Rampello E et al: The chromogranin-A (CgA) in prostate cancer. Arch Gerontol Geriatr 2006; **43:** 117.
3. May M, Siegsmund M, Hammermann F et al: Prognostic significance of proliferation activity and neuroendocrine differentiation to predict treatment failure after radical prostatectomy. Scand J Urol Nephrol 2007; **41:** 375.
4. Sion-Vardy N, Tzikinovsky A, Bolotyn A et al: Augmented expression of chromogranin A and serotonin in peri-malignant benign prostate epithelium as compared to adenocarcinoma. Pathol Res Pract 2004; **200:** 493.
5. Chen Y, Rao F, Rodriguez-Flores JL et al: Common genetic variants in the chromogranin A promoter alter autonomic activity and blood pressure. Kidney Int 2008; **74:** 115.
6. Schiffrin EL, Deng LY, Sventek P et al: Enhanced expression of endothelin-1 gene in resistance arteries in severe human essential hypertension. J Hypertens 1997; **15:** 57.
7. Li JS, Lariviere R and Schiffrin EL: Effect of a nonselective endothelin antagonist on vascular remodeling in deoxycorticosterone acetate-salt hypertensive rats. Evidence for a role of endothelin in vascular hypertrophy. Hypertension 1994; **24:** 183.
8. Kopetz ES, Nelson JB and Carducci MA: Endothelin-1 as a target for therapeutic intervention in prostate cancer. Invest New Drugs 2002; **20:** 173.
9. Nelson JB, Hedican SP, George DJ et al: Identification of endothelin-1 in the pathophysiology of metastatic adenocarcinoma of the prostate. Nat Med 1995; **1:** 944.
10. Montironi R, Mazzucchelli R, Barbisan F et al: Immunohistochemical expression of endothelin-1 and endothelin-A and endothelin-B receptors in high-grade prostatic intraepithelial neoplasia and prostate cancer. Eur Urol 2007; **52:** 1682.
11. Lillie EO, Mahata M, Khandrika S et al: Heredity of endothelin secretion: human twin studies reveal the influence of polymorphism at the chromogranin A locus, a novel determinant of endothelial function. Circulation 2007; **115:** 2282.
12. Ahlgren G, Pedersen K, Lundberg S et al: Regressive changes and neuroendocrine differentiation in prostate cancer after neoadjuvant hormonal treatment. Prostate 2000; **42:** 274.
13. di Sant'Agnese PA, de Mesy Jensen KL, Churukian CJ et al: Human prostatic endocrine-paracrine (APUD) cells. Distributional analysis with a comparison of serotonin and neuron-specific enolase immunoreactivity and silver stains. Arch Pathol Lab Med 1985; **109:** 607.
14. Liao Y, Abel U, Grobholz R et al: Up-regulation of insulin-like growth factor axis components in human primary prostate cancer correlates with tumor grade. Hum Pathol 2005; **36:** 1186.
15. Taupenot L, Harper KL and O'Connor DT: The chromogranin-secretogranin family. N Engl J Med 2003; **348:** 1134.
16. Sanchez-Margalet V, Gonzalez-Yanes C, Santos-Alvarez J et al: Pancreastatin. Biological effects and mechanisms of action. Adv Exp Med Biol 2000; **482:** 247.
17. Nandeesha H: Insulin: a novel agent in the pathogenesis of prostate cancer. Int Urol Nephrol 2008; **41:** 267.
18. O'Connor DT, Cadman PE, Smiley C et al: Pancreastatin: multiple actions on human intermediary metabolism in vivo, variation in disease, and naturally occurring functional genetic polymorphism. J Clin Endocrinol Metab 2005; **90:** 5414.
19. Stronge RL, Turner GB, Johnston BT et al: A rapid rise in circulating pancreastatin in response to somatostatin analogue therapy is associated with poor survival in patients with neuroendocrine tumours. Ann Clin Biochem 2008; **45:** 560.
20. Grimaldi F, Valotto C, Barbina G et al: The possible role of chromogranin A as a prognostic factor in organ-confined prostate cancer. Int J Biol Markers 2006; **21:** 229.
21. Kokubo H, Yamada Y, Nishio Y et al: Immunohistochemical study of chromogranin A in stage D2 prostate cancer. Urology 2005; **66:** 135.
22. Quek ML, Daneshmand S, Rodrigo S et al: Prognostic significance of neuroendocrine expression in lymph node-positive prostate cancer. Urology 2006; **67:** 1247.
23. Kamiya N, Suzuki H, Kawamura K et al: Neuroendocrine differentiation in stage D2 prostate cancers. Int J Urol 2008; **15:** 423.
24. Guate JL, Escaf S, Menendez CL et al: Neuroendocrine cells in benign prostatic hyperplasia and prostatic carcinoma: effect of hormonal treatment. Urol Int 1997; **59:** 149.
25. Huang J, Yao JL, di Sant'Agnese PA et al: Immunohistochemical characterization of neuroendocrine cells in prostate cancer. Prostate 2006; **66:** 1399.
26. Burchardt T, Burchardt M, Chen MW et al: Transdifferentiation of prostate cancer cells to a neuroendocrine cell phenotype in vitro and in vivo. J Urol 1999; **162:** 1800.
27. Cox ME, Deeble PD, Lakhani S et al: Acquisition of neuroendocrine characteristics by prostate tumor cells is reversible: implications for prostate cancer progression. Cancer Res 1999; **59:** 3821.
28. Zhou WQ, Sun YH, Xu CL et al: Plasma endothelin-1 in patients with prostate cancer and its clinical significance. Zhonghua Nan Ke Xue 2006; **12:** 315.

# Relationship Between Bone Mineral Density and Androgen-deprivation Therapy in Japanese Prostate Cancer Patients

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**OBJECTIVES** To examine Japanese patients who had received androgen-deprivation therapy (ADT) for longer periods, as it is known that ADT of patients with prostate cancer reduces their bone mineral density (BMD). However, our previous cross-sectional study revealed that short-term ADT (average, 23.5 months) does not significantly increase the prevalence of osteoporosis in Japanese patients.

**METHODS** The subjects consisted of 201 native Japanese patients with prostate cancer. They comprised 113 ADT-treated and 88 hormone-naive patients. Lumbar spine, total hip, and femoral neck BMDs were measured by dual-energy x-ray absorptiometry and expressed in standard deviation units relative to the scores of young adult men (*T*-score) or age-matched men (Z-score). Serum levels of bone metabolism markers were also measured.

**RESULTS** The ADT-treated patients had significantly lower BMD values, *T*-scores, and even Z-scores than the hormone-naive patients ($P < .001$). For patients who were hormone-naive, ADT-treated for less than 2 years, and ADT-treated for more than 2 years, the osteoporosis prevalence was 4.5% (4/88), 12.1% (4/33), and 10.8% (4/37), respectively. The ADT-treated patients had significantly higher serum amino-terminal telopeptide levels than the hormone-naive patients ($P = .014$), but significantly lower serum carboxy-terminal telopeptide of type-I collagen levels than the ADT-treated patients with bone metastasis ($P < .001$).

**CONCLUSIONS** Our cross-sectional study confirmed that both ADT-treated and hormone-naive Japanese patients with prostate cancer have low rates of osteoporosis. These findings are different from those of studies in western countries. Genetic and hormonal or other environmental factors may result in population differences in the characteristics of prostate cancer and BMD. UROLOGY 75: 1131–1137, 2010. © 2010 Elsevier Inc.

Cancer treatment-induced bone loss is one of the complications associated with androgen-deprivation therapy (ADT) of patients with prostate cancer. It is of particular concern because it can lead to osteoporosis and bone fractures, which not only negatively impact patient quality of life but also overall survival.[1-3] However, these studies were performed in western countries. This is of relevance because several studies, including a breast cancer study of patients treated with aromatase inhibitor, revealed that Caucasian and Japanese women show racial differences in bone mineral density (BMD) and the incidence of bone fracture.[4-6] Moreover, in our previous study of Japanese men with prostate cancer, we found that they had a low prevalence of osteoporosis that was not increased by ADT.[7] These observations led us to hypothesize that Caucasian and Japanese men with prostate cancer may differ in terms of the influence of ADT on BMD due to racial and/or environmental differences. Because our previous study only examined a relatively small number of Japanese patients with prostate cancer, and the 58 ADT-treated patients had been treated for only a relatively short duration, in the current study we recruited larger numbers of Japanese patients with prostate cancer (n = 201) who had received ADT for a longer duration (average, 35.3 months). We then analyzed the effect of ADT on their BMD. In addition, to determine the influence of ADT on serum bone metabolic variables, we measured

*This work was partly supported by the Takeda Science Foundation, the Kobayashi Institute for Innovative Cancer Chemotherapy, the Shimadzu Science Foundation, the Sagawa Foundation for Promotion of Cancer Research, the Japan-China Sasakawa Medical Fellowship, Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and the GCOE program of the Ministry of Education, Culture, Sports, Science and Technology, Japan.*

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*Submitted: August 01, 2009, accepted (with revisions): October 23, 2009*



0090-4295/10/$34.00
doi:10.1016/j.urology.2009.10.075

amino-terminal telopeptide (NTx) and carboxy-terminal telopeptide of type-I collagen (ICTP).

## MATERIAL AND METHODS

### Subjects

This cross-sectional study consisted of 201 native Japanese patients with prostate cancer. These patients comprised 113 ADT-treated and 88 hormone-naive patients, who were treated at the Akita University Medical Center from December 2006 through to March 2009. Of the 113 ADT-treated patients, 43 and 70 did and did not have bone metastasis, respectively. Patients with bone metastases in the lumbar spine or the hip joint were not included in this study as these were the regions we used to measure BMD. All prostate cancer patients were diagnosed according to histologic analysis of specimens obtained by transrectal needle biopsy or transurethral resection of the prostate performed to alleviate voiding symptoms. The ADT patients without bone metastasis were treated with combined androgen blockade (CAB) therapy using bicalutamide (80 mg/d) together with luteinizing hormone-releasing hormone (LH-RH) analog and/or surgical castration (n = 34), LH-RH analog monotherapy (n = 28), or bicalutamide monotherapy (n = 6). Among the patients, 2 were treated with CAB therapy using LH-RH analog and estramustine phosphate for a maximum of 3 months. In addition, 27 of the 43 ADT-treated patients with bone metastasis (62.8%) had also received estramustine phosphate during their treatment process. Among these ADT-treated patients, 5 patients (11.6%) with bone metastasis and 27 (38.6%) without metastasis underwent local radiation therapy in their earlier disease course. None of the hormone-naive patients underwent radiation therapy. The prostate cancers were pathologically graded according to Gleason's histologic grading and the Tumor-Node-Metastatic system.[8,9]

### BMD Measurements

BMD was measured in our hospital in 2006-2009 by dual-energy x-ray absorptiometry using a Delphi QDR (Hologic, Bedford, MA), as previously described.[7] The area of BMD in grams per square centimeter was measured at the posteroanterior spine (L2-L4) and the nondominant hip (total hip and femoral neck). Peak BMD, age-specific BMD, peak standard deviation (SD), and age-specific SD for dual-energy x-ray absorptiometry values were derived from the Hologic database for East Asian ethnicity (version 2.0; Hologic, Bedford, MA). BMD was expressed in SD units relative to the BMDs of young adult men (*T*-score) and age-matched men (*Z*-score). According to World Health Organization criteria, a normal BMD is defined as a *T*-score greater than −1 SD, osteopenia as a *T*-score between −1 and −2.5 SD, and osteoporosis as a *T*-score of −2.5 SD or less.[10] In this study, we used the *T*-score of the worst site to classify the patient, as previously described.[7]

### Measurement of Biochemical Values

The serum levels of NTx (normal range, 9.5-17.7 nmol/L), ICTP (normal range, 1.6-3.8 ng/mL), intact parathyroid hormone (normal range, 15-65 pg/mL), prostate-specific antigen (PSA; normal range, <4 ng/mL), and testosterone (normal range, 2.0-7.6 ng/mL), and common laboratory blood and serum data were measured before breakfast at the time BMD was measured, as described previously.[7]

### Statistical Analysis

Differences in the clinical and serum variables of the hormone-naive patients, the ADT-treated patients without bone metastasis, and the ADT-treated patients with bone metastasis were evaluated using the Student *t* test. Alternatively, the Mann–Whitney *U* test was used if the group variances were unequal or the dependent variables were non-normally distributed and not transformable. The groups were also compared with regard to the prevalence of normal BMDs, osteopenia, osteoporosis and the respective treatment groups, and analyzed statistically by using the Kruskal–Wallis test. Nonparametric Spearman rank-order correlation coefficients were calculated to investigate the relationship between BMD, *T*-score, and *Z*-score, and treatment period. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS version 13.0; SPSS, Inc., Chicago, IL) and two-sided $P < .05$ was considered to indicate statistical significance.

## RESULTS

### Patient Characteristics

The 201 patients were divided into hormone-naive patients (n = 88), ADT-treated patients without bone metastasis (n = 70), and ADT-treated patients with bone metastasis (n = 43), and their characteristics, including their age, body mass index, biopsy Gleason score, and serum or blood levels of PSA, hemoglobin, albumin, lactate dehydrogenase, glucose, and testosterone, and the presence and duration of ADT, are summarized in Table 1. The ADT-treated patients were grouped into those who did and did not have bone metastasis because bone metastasis can artificially elevate BMD measurements, even in patients who do not seem to have bone metastases in the areas being measured. The ADT-treated patients without bone metastasis were significantly older than the other 2 groups ($P < .001$ and .038, respectively), while the ADT-treated patients with bone metastasis had higher serum PSA values and lower hemoglobin, albumin, and blood glucose levels than those without bone metastasis ($P = .014$, $< .001$, $< .001$, and .008, respectively). The hormone-naive patients had significantly higher body mass index and serum levels of testosterone than the ADT-treated patients without bone metastasis ($P = .034$ and $< .001$, respectively). However, all 3 groups were similar in biopsy Gleason scores (Table 1).

### Comparison of the BMD, *T*-Scores, and *Z*-Scores of the Hormone-Naive and ADT-treated Patients With or Without Bone Metastasis

The ADT-treated patients without bone metastasis had significantly lower BMD values and *T*-scores at all 3 sites than the hormone-naive patients (Table 2). The ADT-treated patients without bone metastasis also had significantly lower *Z*-scores at the lumbar spine and total hip than the hormone-naive patients (Table 2). These results suggest ADT decreased BMD in these patients. In contrast, the ADT-treated patients with bone metastasis had significantly higher BMD values, *T*-scores, and *Z*-scores

**Table 1.** Comparison of the characteristics of the 3 groups of patients with prostate cancer

| | Hormone-Naive Prostate Cancer Patients | ADT-treated Prostate Cancer Patients Without Bone Metastasis | ADT-treated Prostate Cancer Patients With Bone Metastasis | P* | P† |
|---|---|---|---|---|---|
| N | 88 | 70 | 43 | | |
| Age (y) | 65.1 ± 9.7 | 74.1 ± 6.2 | 71.1 ± 7.7 | <.001 | .038 |
| BMI (kg/m$^2$) | 24.2 ± 3.1 | 23.2 ± 3.0 | 23.9 ± 3.9 | .034 | .336 |
| Biopsy Gleason score | 7.4 ± 1.2 | 7.7 ± 1.4 | 8.0 ± 1.3 | .244 | .291 |
| PSA (ng/mL) at diagnosis | 12.6 ± 10.3 | 25.2 ± 28.5 | 851 ± 2124 | .271 | .014 |
| Hemoglobin (g/dL) | 12.8 ± 1.6 | 12.3 ± 1.6 | 10.8 ± 1.9 | .111 | <.001 |
| Albumin (g/dL) | 4.1 ± 0.4 | 4.1 ± 0.4 | 3.7 ± 0.4 | .334 | <.001 |
| LDH (IU/L) | 174 ± 32 | 201 ± 62 | 444 ± 1101 | <.001 | .161 |
| Blood sugar (g/dL) | 117 ± 39 | 125 ± 35 | 106 ± 35 | .148 | .008 |
| Testosterone (ng/mL) | 4.1 ± 2.1 | 0.8 ± 0.8 | 0.8 ± 0.7 | <.001 | .46 |
| Duration of ADT (mon) | — | 30.7 ± 25.8 | 40.3 ± 19.4 | — | .014 |

BMD = bone mineral density.
Values indicate means ± SD.
* Probability values of the differences between the hormone-naive patients and the ADT-treated patients without bone metastasis.
† Probability values of the differences between the ADT-treated patients with and without bone metastasis.

**Table 2.** Comparison of the BMDs, *T*-scores, and *Z*-scores of the 3 groups of patients with prostate cancer

| | Hormone-Naive Prostate Cancer Patients | ADT-treated Prostate Cancer Patients Without Bone Metastasis | ADT-treated Prostate Cancer Patients With Bone Metastasis | P* | P† |
|---|---|---|---|---|---|
| N | 88 | 70 | 43 | | |
| Age (y) | 65.1 ± 9.7 | 74.1 ± 6.2 | 71.1 ± 7.7 | <.001 | .038 |
| Lumbar spine | | | | | |
| BMD | 1.060 ± 0.21 | 0.973 ± 0.212 | 0.973 ± 0.136 | .008 | .164 |
| *T*-score | 0.102 ± 1.502 | −0529 ± 1.519 | −0.585 ± 0.943 | .006 | .691 |
| *Z*-score | 0.663 ± 1.199 | 0.288 ± 1.101 | 0.156 ± 0.820 | .034 | .966 |
| Femoral neck | | | | | |
| BMD | 0.774 ± 0.133 | 0.716 ± 0.112 | 0.779 ± 0.109 | .002 | .002 |
| *T*-score | −0.701 ± 1.049 | −1.161 ± 0.833 | −0.656 ± 0.855 | .004 | .003 |
| *Z*-score | 0.511 ± 1.090 | 0.280 ± 0.915 | 0.674 ± 0.967 | .054 | .034 |
| Total hip | | | | | |
| BMD | 0.916 ± 0.155 | 0.824 ± 0.130 | 0.911 ± 0.125 | <.001 | .001 |
| *T*-score | −0.333 ± 1.163 | −1.007 ± 0.977 | −0.365 ± 0.934 | <.001 | <.001 |
| *Z*-score | 0.721 ± 1.110 | 0.332 ± 0.945 | 0.795 ± 0.983 | <.001 | .007 |

BMD = bone mineral density.
Values indicate means ± SD.
* Probability values of the differences between the hormone-naive patients and the ADT-treated patients without bone metastasis.
† Probability values of the differences between the ADT-treated patients with and without bone metastasis.

at the femoral neck and total hip than the ADT-treated patients without bone metastasis (Table 2). Notably, the average Z-scores of the ADT-treated patients (with or without bone metastasis) and the hormone-naive patients were positive in this study. We also observed this in our previous study.[7]

### Prevalence of Osteoporosis and Osteopenia in the Hormone-Naive and ADT-treated Patients

We compared the prevalence of osteoporosis and osteopenia in the hormone-naive patients and the ADT-treated patients without bone metastasis (Fig. 1A). On the basis of the worst site showing a low *T*-score, the prevalence of osteoporosis and osteopenia for the hormone-naive prostate cancer patients (n = 88) were 4.5% (4 patients) and 31.8% (26 patients), respectively. In contrast, for the prostate cancer patients who had been treated with ADT for less than 2 years (n = 33), the respective prevalence were 12.1% (4 patients) and 48.4% (16 patients). For the prostate cancer patients who had been treated with ADT for more than 2 years (n = 37), the respective prevalence were 10.8% (4 patients) and 54.1% (20 patients). The prevalence did not differ significantly. Thus, although the ADT-treated patients without bone metastasis had significantly lower BMD values, *T*-scores, and even Z-scores than the hormone-naive patients, their ADT treatment (on average 30.7 months) did not elevate the prevalence of osteoporosis. The BMDs of the CAB- and LH-RH monotherapy–treated patients did not differ significantly.

### Association Between Duration of ADT and BMDs, *T*-Scores, and *Z*-Scores of Patients With Nonmetastatic Prostate Cancer

We investigated the association between the duration of ADT and the BMDs, *T*-scores, and Z-scores of the ADT-treated patients with nonmetastatic prostate cancer (Fig. 1B). The duration of ADT did not correlate significantly

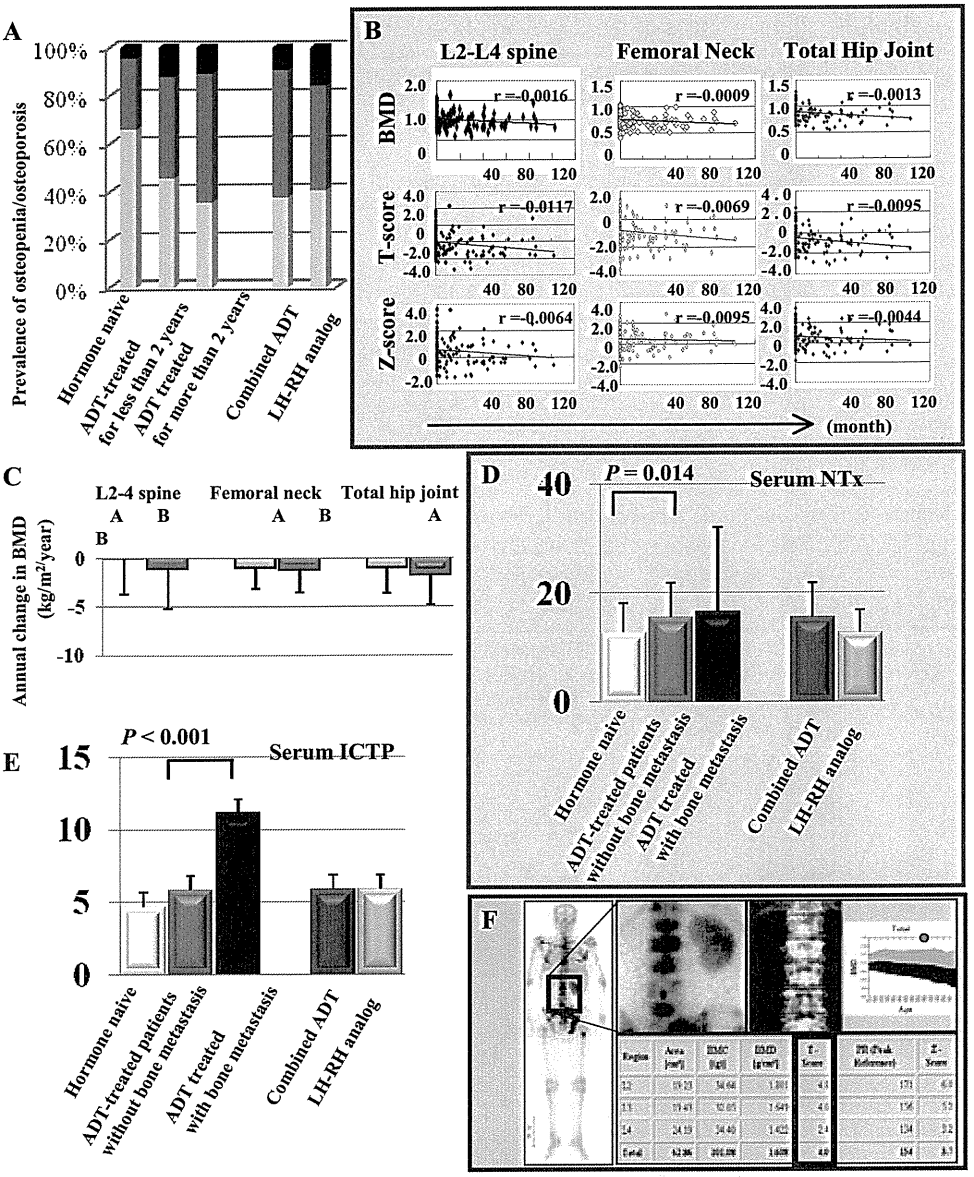


**Figure 1.** Relationship between androgen-deprivation (ADT) therapy and bone mineral density (BMD) of patients with prostate cancer. **(A)** The frequencies of osteoporosis (black bars), osteopenia (dark gray bars), and normal BMD (light gray bars) of the ADT-treated patients without bone metastasis and the hormone-naive patients. **(B)** Association between the duration of ADT of patients without bone metastasis and their BMDs **(B)**, *T*-scores (**T**), and *Z*-scores (**Z**) of the lumbar spine, femoral neck, and total hip joints. **(C)** Comparison of the annual BMD loss in the preceding year at the lumbar spine, femoral neck, and total hip joints of the hormone-naive patients (light gray bars) and the ADT-treated patients without bone metastasis (dark gray bars). Comparison of the serum levels of the bone resorption markers, NTx **(D)** and ICTP **(E)**, of the hormone-naive patients, the ADT-treated patients without metastasis, and the ADT-treated patients with bone metastasis. **(F)** Typical BMD measurement of the prostate cancer patient with bone metastasis.

with BMD for all 3 sites measured (L2-L4 spine, femoral neck, and total hip join; $r = -0.0016$, $-0.0009$, and $-0.0013$, respectively). We also analyzed the bone loss during 1 year in the ADT-treated patients (n = 25) and hormone-naive patients (n = 16) whose BMD had been measured twice with a 1-year interval in the preceding year. In both groups, their average BMDs had decreased over the year, but the 2 groups did not change significantly with regard to annual change in BMD (Fig. 1C).

### Influence of ADT on Serum Variables and Bone Metabolism

Next, to determine the effect of ADT on serum variables associated with bone metabolism, we compared the hormone-naive patients, the ADT-treated patients without metastasis, and the ADT-treated patients with metastasis with regard to these variables. As shown in Fig. 1D, the ADT-treated patients without metastasis had significantly higher serum levels of NTx, which reflects bone resorption, than the hormone-naive patients ($P = .014$).

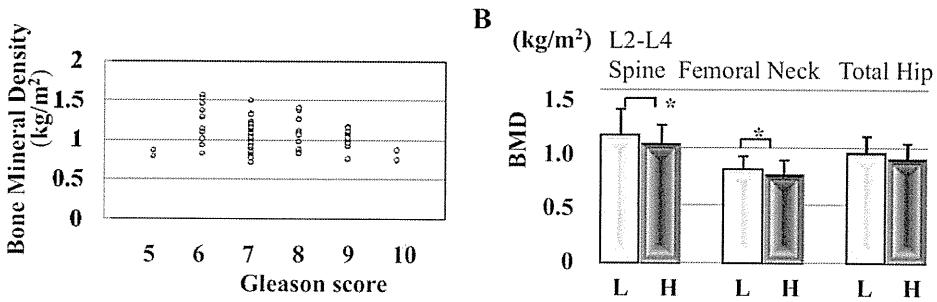


**Figure 2.** Relationship between the bone mineral densities and Gleason scores of the hormone-naive patients with prostate cancer. **(A)** Relationship between the bone mineral densities and Gleason scores. **(B)** Comparison of the bone mineral densities of patients with low (**L**) and high (**H**) Gleason scores. Gleason scores ≤7 (low, n = 49) vs >7 (high, n = 31). * $P < .05$.

However, these 2 groups did not differ significantly in any of the other serum variables. In addition, the ADT-treated patients with bone metastasis had significantly higher serum levels of ICTP, which also reflects bone resorption, than the ADT-treated patients without bone metastasis (Fig. 1E, $P < .001$). The CAB-treated patients and the patients treated with LH-RH analog alone did not differ in either NTx or ICTP levels.

### Relationship Between Serum Testosterone Levels, BMDs, and Biopsy Gleason Scores

That all groups exhibited average Z-scores in this study suggested that these prostate cancer patients had higher BMDs than age-matched East Asian men. Therefore, we investigated the BMDs and other characteristics of these patients further. Of the 88 hormone-naive patients, the serum testosterone levels, BMDs, and biopsy Gleason scores were available for 80 patients. We first examined the relationship between the serum testosterone level and biopsy Gleason score but did not find a significant association (data not shown). However, patients with high Gleason scores tended to have lower BMDs than those with low Gleason scores (Fig. 2A). Patients with high-risk locally advanced prostate cancer are defined by PSA >20 ng/mL, Gleason score >7, and clinical stage T3/T4. In this group of patients, reported rates of disease-free survival after local therapy range from 30% to 50%.[11,12] Therefore, we compared the BMDs of the patients who had Gleason scores of ≤7 with those whose Gleason scores exceeded 7. The patients who had high Gleason scores had significantly lower BMDs than those with lower Gleason scores (Fig. 2B).

## COMMENT

Our previous study showed that both ADT-treated and hormone-naive Japanese patients with prostate cancer have low rates of osteoporosis.[7] Here, we found that, although the ADT-treated patients did have significantly lower BMD values, T-scores, and Z-scores than the hormone-naive patients (Table 2), there was no concomitant significant increase in the prevalence of osteoporosis in the ADT-treated patients (Fig. 1A) who had been treated with ADT for an average of 30.7 months. On the contrary, other studies of ADT-treated patients with prostate cancer in western countries found they had a high incidence of osteoporosis,[13-15] For example, Morote et al[14] reported that 35.4% and 45.2% of hormone-naive patients had osteoporosis and osteopenia, respectively, while 42.9% and 39.3% of patients who had been treated with ADT for 2 years suffered from osteoporosis and osteopenia, respectively. In this study, we again found that this was not true for Japanese patients with prostate cancer.

Osteoblastic metastatic lesions, which frequently occur in patients with bone metastasis and prostate cancer, may artificially increase the BMD score (Fig. 1F). This observation led us to exclude several typical cases with bone metastasis from the current and previous studies.[7] However, although we did not detect any significant differences between ADT-treated prostate cancer patients with and without bone metastasis with regard to BMD or serum levels of bone metabolic markers in our previous study,[7] in the present study, we found that the ADT-treated patients with bone metastasis had significantly higher BMD values than the ADT-treated patients without bone metastasis (Table 2). These results suggest that bone scans may fail to detect small metastatic bone lesions in the measured sites that can artificially elevate the BMD measurements. Alternatively, because 62.8% of the patients with bone metastasis were given estramustine phosphate during their treatment process, it is possible that this nitrogen mustard-conjugated estrogen may have helped to prevent ADT-induced bone loss in these patients.

The fact that the hormone-naive and ADT-treated patients with and without bone metastasis had positive average Z-scores indicates that the average BMDs of the patients in this study were higher than those of age-matched East Asian controls. Because the total hormonal environment during life affects BMD, this observation suggests that Japanese patients with prostate cancer may have had increased testosterone levels that led to higher than normal BMDs. These abnormal hormonal levels

may also have predisposed our patients to the development of prostate cancer. We and others have shown a significant association between low serum testosterone levels and high Gleason score,[16,17] advanced pathologic stage,[18,19] and poor outcome.[20] Moreover, recent results from the Prostate Cancer Prevention Trial suggest that while inhibiting the conversion of testosterone to the more potent dihydrotestosterone by finasteride treatment reduces the number of prostate cancer cases, it also increases the risk of high-grade cancer.[21] Recently, a large multicenter longitudinal study evaluating risk factors of fractures (the Osteoporotic Fractures in Men: MrOS Study) disclosed that a significant inverse relationship was observed for total body BMD with high-grade prostate cancer.[22] Notably, in our study, the Gleason scores correlated inversely with BMD (Fig. 2A,B), which is consistent with the observations described earlier. BMD has been regarded as a possible surrogate marker for lifetime exposure to endogenous sex hormones, insulin-like growth factor I, and calcium intake.[22,23] The relationships between hormonal levels, BMD, and prostate cancer carcinogenesis and malignant potential may be further complicated by racial differences, which have been highlighted by our observation that western and Asian (Japanese) men with prostate cancer differ markedly in the prevalence of osteoporosis. Further research comparing the BMDs and occurrence and characteristics of prostate cancer in these different populations is needed.

In addition, it should be noted that the serum levels of these bone markers in patients with prostate cancer are affected by 2 different influences, namely, the occurrence and progression of bone metastasis, and treatment with ADT. Patients with prostate cancer are frequently subjected to both influences simultaneously. Two recent studies that measured these bone resorptive markers of zoledronic acid–treated prostate cancer patients found that the NTx serum levels rapidly decreased after the zoledronic acid–induced prevention of bone resorption started, whereas the ICTP levels did not alter.[24,25] Thus, there might be a discrepancy between these markers. To provide valuable information regarding both the treatment-induced and disease-dependent bone loss of patients with prostate cancer, further investigation of these bone-turnover markers is necessary.

In conclusion, our cross-sectional study confirmed our previous observation[7] that both hormone-naive and ADT-treated Japanese patients with prostate cancer have similarly low rates of osteoporosis. This is true even for patients treated with ADT for more than 2 years. These findings are quite different from those of studies examining patients in western countries. Genetic and hormonal or other environmental factors may be responsible for differences in not only BMD but also the characteristics of prostate cancer between these populations. To confirm these differences in BMD and the occurrence and characteristics of prostate cancer in these different populations, larger scale and prospective studies will be necessary.

**Acknowledgments.** We thank Yoko Mitobe, Yuka Izumida, and Tomomi Kawamura for their technical assistance.

## References

1. Oefelein MG, Ricchiuti VS, Conrad PW, et al. Clinical predictors of androgen-independent prostate cancer and survival in the prostate-specific antigen era. *Urology.* 2002;60:120-124.
2. Smith MR. Osteoporosis and obesity in men receiving hormone therapy for prostate cancer. *J Urol.* 2004;172:S52-S56.
3. Shahinian VB, Kuo YF, Freeman JL, et al. Risk of fracture after androgen deprivation for prostate cancer. *N Engl J Med.* 2005;352:154-164.
4. Shimizu H, Ross RK, Bernstein L, et al. Serum oestrogen levels in postmenopausal women: comparison of American whites and Japanese in Japan. *Br J Cancer.* 1990;62:451-453.
5. Ito M, Lang TF, Jergas M, et al. Spinal trabecular bone loss and fracture in American and Japanese women. *Calcif Tissue Int.* 1997;61:123-128.
6. Yoneda K, Tanji Y, Okishiro M, et al. Influence of adjuvant anastrozole on bone mineral density in Japanese postmenopausal breast cancer patients: is there a racial difference? *Ann Oncol.* 2006;17:1175-1176.
7. Wang W, Yuasa T, Tsuchiya N, et al. Bone mineral density in Japanese prostate cancer patients under androgen-deprivation therapy. *Endocr Relat Cancer.* 2008;15:943-952.
8. Gleason DF. Histological grading and clinical staging of prostate carcinoma. In: Tannenbaum M, ed. *Urologic Pathology.* Philadelphia, PA: Lea & Febiger; 1977:171-197.
9. International Union Against Cancer, urological tumors, prostate. In: Sobin LH, Wittekind C, eds. *TNM Classification of Malignant Tumors.* 6th ed. New York, NY: Wiley-Liss; 2002:184-187.
10. Miller PD. Guidelines for the diagnosis of osteoporosis: T-scores vs fractures. *Rev Endocr Metab Disord.* 2006;1:775-789.
11. D'Amico AV, Chen MH, Renshaw AA, et al. Androgen suppression and radiation vs radiation alone for prostate cancer: a randomized trial. *JAMA.* 2008;299:289-295.
12. Joniau S, Van Poppel H. Localized prostate cancer: can we better define who is at risk of unfavourable outcome? *BJU Int.* 2008;101:S5-S10.
13. Bruder JM, Ma JZ, Basler JW, et al. Prevalence of osteopenia and osteoporosis by central and peripheral bone mineral density in men with prostate cancer during androgen-deprivation therapy. *Urology.* 2006;67:152-155.
14. Morote J, Morin JP, Orsola A, et al. Prevalence of osteoporosis during long-term androgen deprivation therapy in patients with prostate cancer. *Urology.* 2007;69:500-504.
15. Morote J, Orsola A, Abascal JM, et al. Bone mineral density changes in patients with prostate cancer during the first 2 years of androgen suppression. *J Urol.* 2006;175:1679-1683.
16. Hoffman MA, DeWolf WC, Morgentaler A. Is low serum free testosterone a marker for high grade prostate cancer? *J Urol.* 2000;163:824-827.
17. San Francisco IF, Regan MM, Dewolf WC, et al. Low age adjusted free testosterone levels correlate with poorly differentiated prostate cancer. *J Urol.* 2006;175:1341-1345.
18. Imamoto T, Suzuki H, Fukasawa S, et al. Pretreatment serum testosterone level as a predictive factor of pathological stage in localized prostate cancer patients treated with radical prostatectomy. *Eur Urol.* 2005;47:308-312.
19. Teloken C, Da Ros CT, Caraver F, et al. Low serum testosterone levels are associated with positive surgical margins in radical retropubic prostatectomy: hypogonadism represents bad prognosis in prostate cancer. *J Urol.* 2005;174:2178-2180.

20. Yamamoto S, Yonese J, Kawakami S, et al. Preoperative serum testosterone level as an independent predictor of treatment failure following radical prostatectomy. *Eur Urol.* 2007;52:696-701.
21. Lucia MS, Epstein JI, Goodman PJ, et al. Finasteride and high-grade prostate cancer in the Prostate Cancer Prevention Trial. *J Natl Cancer Inst.* 2007;99:1375-1383.
22. Farhat GN, Taioli E, Cauley JA, et al; Osteoporotic Fractures in Men (MrOS) Study Group. The association of bone mineral density with prostate cancer risk in the Osteoporotic Fractures in Men (MrOS) Study. *Cancer Epidemiol Biomarkers Prev.* 2009; 18:148-154.
23. Zmuda JM, Cauley JA, Ljung BM, et al. Bone mass and breast cancer risk in older women: differences by stage at diagnosis. *J Natl Cancer Inst.* 2001;93:930-936.
24. Lein M, Wirth M, Miller K, et al. Serial markers of bone turnover in men with metastatic prostate cancer treated with zoledronic acid for detection of bone metastases progression. *Eur Urol.* 2007;52: 1381-1387.
25. Lein M, Miller K, Wirth M, et al. Bone turnover markers as predictive tools for skeletal: complications in men with metastatic prostate cancer treated with zoledronic acid. *Prostate.* 2009;69:624-632.

## EDITORIAL COMMENT

The adverse metabolic effects of androgen deprivation therapy (ADT) in men with metastatic prostate cancer are clear. This study by Wang et al discusses the relationship between ADT and bone mineral density (BMD) in Japanese men with prostate cancer, a group previously reported to have a lower incidence of baseline and ADT-induced osteoporosis compared with Caucasians.[1] Yuasa et al[2] provided an interesting article that may lead to additional studies to understand the impact of prostate cancer and its treatment on bone metabolism. This article should also serve as a cautionary note about the appropriate use of ADT for prostate cancer and the use of biomarkers in clinical trials.

To assess the clinical applicability of data in this retrospective study, it is necessary to clearly understand the patient characteristics. As we try to minimize use of ADT due to adverse effects, only 40% of patients receiving ADT in this study had metastatic disease to the bone, the most frequent site of metastatic disease. It would be informative to know why the other 60% of patients were receiving ADT. Was this for metastatic disease to lymph nodes? With adjuvant radiation for locally advanced disease? For an increasing PSA without evidence for metastatic disease? Did patients without metastatic disease to bone have negative bone imaging or was presence of metastatic disease not documented? Each of these populations has a much different likelihood for disease- or treatment-related changes in bone metabolism and, therefore, BMD due to differences in the duration of disease or treatment.

The measurement of markers of bone metabolism in this study adds more confusion than insight and points to the uncertainty associated with using unvalidated markers in clinical trials. There are many markers for bone turnover, including total and bone-specific alkaline phosphatase (tALP and bALP), cross-linked N- and C-terminal telopeptides of type-I collagen (NTx and CTx), amino-terminal procollagen propeptides of type-I collagen (PINP), and C-terminal telopeptides of type-I-collagen (ICTP). It is unclear why NTx and ICTP were chosen for this study. Both NTx and ICTP are appropriately described in the results as measures of bone resorption with a significant increase in ICTP, but not NTx in patients with bone metastasis receiving ADT, purportedly due to a proposed differential effect of ADT and progression of metastatic disease on bone markers. It is not clear why one marker of resorption would be influenced differently than the other. More importantly, the effects of ADT on both NTx and ICTP in patients with bone metastasis would be significantly different from hormone-naive patients with less variation in the serum NTx data.

Differences in the effects of ADT on BMD in Japanese and Caucasian men with prostate cancer may provide a unique opportunity to understand the mechanisms by which hormonal therapies affect bone metabolism. In vitro and clinical trials, with carefully selected markers of bone metabolism, comparing the effects of ADT or bisphosphonates on bone metabolism in these patients may uncover differences in cellular receptors or cell signaling pathways, which could be explored as future therapeutic targets.

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### Reference

1. Wang W, Yuasa T, Tsuchiya N, et al. Bone mineral density in Japanese prostate cancer patients under androgen-deprivation therapy. *Endocr Relat Cancer.* 2008;15:943.

doi:10.1016/j.urology.2009.11.061

*Jpn J Clin Oncol 2009*
*doi:10.1093/jjco/hyp146*

**Original Article**

# A Phase II Study of Sunitinib in Japanese Patients with Metastatic Renal Cell Carcinoma: Insights into the Treatment, Efficacy and Safety

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Received June 23, 2009; accepted October 1, 2009

**Objective:** This study aims to assess the efficacy and safety of sunitinib in Japanese patients with metastatic renal cell carcinoma (RCC).
**Methods:** Fifty-one Japanese patients with prior nephrectomy, 25 treatment-naive patients (first-line group) and 26 cytokine-refractory patients (pretreated group) were enrolled in this phase II trial. Patients received sunitinib 50 mg orally, once daily, in repeated 6-week cycles (4 weeks on treatment, 2 weeks off). The primary endpoint was RECIST-defined objective response rate (ORR) with tumour assessments every 6 weeks via computed tomography or magnetic resonance imaging. Toxicity was assessed regularly. In the primary efficacy analysis of the intent-to-treat (ITT) population, ORR and 95% confidence interval were calculated based on independent review. Secondary time-to-event endpoints, such as progression-free survival (PFS), were estimated using the Kaplan–Meier method.
**Results:** In the ITT population, ORR was 48.0% in the first-line group (after a median 4 cycles), 46.2% in the pretreated group (5 cycles) and 47.1% overall, with median times to tumour response of 7.1, 10.7 and 10.0 weeks, respectively. Median PFS was 46.0, 33.6 and 46.0 weeks, respectively. The most common treatment-related grade 3/4 adverse events and laboratory abnormalities were fatigue (20%), hand-foot syndrome (14%) and hypertension (12%), decreased platelet count (55%), decreased neutrophil count (51%), increased lipase (39%) and decreased lymphocyte count (33%).
**Conclusions:** In Japanese patients with RCC, sunitinib is consistently effective and tolerable with similar risk/benefit as that in Western patients, though there was a trend toward greater antitumour efficacy and higher incidence of haematological adverse events in Japanese patients.

*Key words: Japanese – phase II – renal cell carcinoma – sunitinib*

## INTRODUCTION

Renal cell carcinoma (RCC) accounts for approximately 2–3% of all cancers (1,2) and 80–85% of cases of malignant kidney disease (1). The global incidence of RCC is increasing (3), with the highest rates in North America and Scandinavia (4). In Japan, there were an estimated 7400 persons diagnosed with RCC in 2002, up from 6360 persons in 1997, with crude incidence rates of 8.2 men and 3.6 women per 100 000 people (5).

Up to 30% of patients present with metastatic disease (6,7), and approximately 40% of patients treated for localized RCC eventually relapse (6,8). Until recently, cytokine therapy with interferon-alpha (IFN-α) and/or interleukin-2 (IL-2) had provided the mainstay of systemic RCC treatment, but with limited success and high corresponding rates of adverse events (9,10). More recently, increased understanding of RCC biology has led to the development of targeted agents that block proliferative, dysregulated tumour pathways and have demonstrated superior efficacy over cytokines and changed the RCC treatment paradigm (11,12).

Sunitinib malate (SUTENT®, Pfizer Inc., New York, NY, USA) is an oral, multitargeted tyrosine kinase inhibitor of vascular endothelial growth factor receptors 1–3 and platelet-derived growth factor receptors $\alpha$ and $\beta$ (13). Single-agent sunitinib showed unprecedented antitumour activity in two consecutive single-arm phase II trials of patients with cytokine-refractory metastatic RCC (14–16), demonstrating an objective response rate (ORR) of 33% (per independent, third-party, core imaging laboratory review), a median time to tumour progression (TTP) of 10.7 months, and a median overall survival (OS) of 23.9 months (as recently reported for the second phase II trial) (16). In addition, sunitinib subsequently demonstrated superior first-line efficacy over IFN-α, with significantly greater progression-free survival (PFS) and ORR (11 vs. 5 months and 47% vs. 12%, respectively; both $P < 0.001$) in an international, randomized phase III trial of 750 patients with metastatic RCC (17). Median OS was greater in the sunitinib group (26.4 months) vs. the IFN-$\alpha$ group (21.8 months).

These data have established sunitinib monotherapy as a standard of care for RCC treatment. Sunitinib is now approved multinationally for the treatment of first- and second-line advanced RCC. Here, we report the efficacy and safety results of the first Japanese phase II study of single-agent sunitinib in both treatment-naïve and cytokine-pretreated Japanese patients with metastatic RCC.

## PATIENTS AND METHODS

### Patient Population

The study population comprised patients aged ≥20 years with histologically proven RCC, with a clear-cell component and metastases; evidence of unidimensionally measurable disease per Response Evaluation Criteria in Solid Tumours (RECIST) (18) and prior nephrectomy. Other inclusion criteria included: Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1; resolution of all acute toxic effects of prior therapy to ≤1 severity (classified according to the National Cancer Institute Common Terminology Criteria for Adverse Events [NCI-CTCAE] version 3.0); and adequate organ function. Patients with the following were excluded: any prior systemic treatment since RCC diagnosis (first-line population); any prior systemic therapy other than one cytokine-based regimen that could include multiple cytokines (pretreated population); prior treatment within 4 weeks of the start of study treatment; any secondary malignancy within the previous 5 years; uncontrolled hypertension; history of/known brain metastases; and cardiovascular disease. All patients gave written informed consent. This study was approved by the institutional review board at each participating centre and was conducted in accordance with the International Conference on Harmonization Guideline for Good Clinical Practice.

### Study Design

This was a multicentre, open-label, non-randomized, single-arm, phase II study of sunitinib. Sunitinib was self-administered at a starting dose of 50 mg orally, once daily, in the morning, without regard to meals, in repeated 6-week cycles according to Schedule 4/2 (4 weeks on treatment followed by 2 weeks off).

Patients were monitored for toxicity, and doses were adjusted according to individual patient tolerance according to the protocol. Doses were reduced to 37.5 mg/day in cases of treatment-related grade ≥3 adverse events, and by an additional 12.5 mg/day if toxicities persisted to a minimum dose of 25 mg/day. Treatment was continued until one of the following occurred: disease progression; requirement for additional anticancer treatment; development of left ventricular systolic dysfunction; or treatment withdrawal.

### Study Assessments

Patients were screened within 21 days prior to treatment initiation. Baseline evaluations included physical examination; ECOG PS; haematology; biochemistry; tumour assessment (scanned by computed tomography [CT] or magnetic resonance imaging [MRI]); 12-lead electrocardiography; and echocardiography or MUGA scan.

The primary efficacy endpoint was ORR, defined as the proportion of patients with a confirmed complete response (CR) or partial response (PR) according to RECIST (18). Tumour assessments were obtained every 6 weeks by investigators via CT or MRI scan with or without X-ray with response confirmed by an Extramural Review Committee (independent review). Secondary endpoints included PFS, TTP, duration of response, time to tumour response, and OS.

Safety and tolerability were assessed at regular intervals with adverse event monitoring, using NCI CTCAE version 3.0

to document adverse events and classify severity; haematology and biochemistry; body weight; vital signs; ECOG PS; 12-lead electrocardiography; and echocardiography or MUGA scan. Health-related quality of life was assessed using two measures from the EuroQol Group's EQ-5D self-report questionnaire, a population preference-based health state utility score (EQ-5D Index) and a visual analogue scale (EQ-VAS) for assessing a patient's overall health state (19–21).

Trough plasma concentrations of sunitinib, its active metabolite (SU12662) and total drug (sunitinib + SU12662) were assessed. Plasma samples for pharmacokinetic (PK) analysis were collected prior to treatment on days 1, 14 and 28 of cycle 1, days 1 and 28 of cycle 2, and day 28 of cycle 3.

### Statistical Methods

In the primary efficacy analysis of the intent-to-treat (ITT) population, the ORR and 95% confidence interval (CI) were calculated for the first-line and pretreated populations based on independent review (the same analysis was performed for investigators' assessments). Sample sizes were calculated based on response rates for each population in which it was concluded that efficacy was confirmed if the lower limit of the 95% CI was greater than or equal to the threshold rate in each population. The thresholds were set at levels considered to be clinically ineffective for each population (5% for the pretreated population and 10% for the first-line population). Based on these assumptions, sample sizes of 26 and 25 patients were required for the pretreated and first-line populations, respectively, to provide a power of 80% with an alpha level of 2.5%. In the analysis of secondary endpoints, PFS, TTP and OS assessed by investigators were summarized using the Kaplan–Meier method (22). In the PK analysis, descriptive statistics were calculated for trough plasma concentrations of sunitinib, SU12662 and total drug.

## RESULTS

### Patients

As of February 2007 (time of data cutoff), 51 patients were enrolled at 12 sites in Japan and had completed at least four cycles of sunitinib treatment. The mean age was 56.6 years (range: 33–76) in the first-line population ($n = 25$) and 61.1 years (range: 34–77) in the pretreated population ($n = 26$). All patients had an ECOG PS of 0–1 at baseline. The most prevalent site of metastasis was the lung (82%). Baseline characteristics are summarized in Table 1.

In the second-line population, 22 patients (85%) had received previous treatment with IFN-α. The best responses to previous treatment were CR in one patient (4%) and PR in 11 patients (42%); 13 patients (50%) exhibited progressive disease. Cytokine-based treatment was discontinued because of tumour progression in 22 patients (85%), intolerance in two patients (8%) and other reasons in two patients (8%).

**Table 1.** Patient characteristics at baseline

| Characteristic | First-line population ($n = 25$) | Pretreated population ($n = 26$) |
|---|---|---|
| Male, *n* (%) | 11 (44.0) | 21 (80.8) |
| Mean age, years (range) | 56.6 (33–76) | 61.1 (34–77) |
| ≥65 years, *n* (%) | 7 (28) | 11 (42) |
| Mean weight, kg (range) | 57.7 (40.0–77.5) | 63.7 (42.1–92.4) |
| Median time since initial diagnosis, months (range) | 2.69 (0.3–157.5) | 16.69 (1.8–221.2) |
| ECOG PS, *n* (%) | | |
| 0 | 20 (80) | 22 (85) |
| 1 | 5 (20) | 4 (15) |
| Common sites of metastases, *n* (%) | | |
| Lung | 19 (76) | 23 (88) |
| Lymph nodes | 8 (32) | 11 (42) |
| Visceral organs | 7 (28) | 8 (31) |
| Bone | 5 (20) | 4 (15) |
| Prior cytokine-based therapy, *n* (%) | | |
| IFN-alpha | – | 22 (85) |
| IL-2 | – | 1 (4) |
| IFN-alpha + IL-2 | – | 2 (8) |
| IFN-alpha + IL-2 + tegafur-uracil | – | 1 (4) |

ECOG PS, Eastern Cooperative Oncology Group performance status; IFN, interferon; IL, interleukin.

### Treatment and Disposition

At the time of analysis, patients in the first-line population had received a median of four cycles of treatment (range: 1–8) and patients in the pretreated population had received five cycles (range: 1–7). Eleven patients (44%) in the first-line population and nine patients (35%) in the pretreated population discontinued treatment. One patient in the pretreated population died due to tumour progression, which was considered unrelated to treatment. Sunitinib treatment and disposition by patient population are summarized in Table 2.

### Efficacy

Based on independent review, ORR in the ITT populations was 48.0% (95% CI, 27.8–68.7) in the first-line population, 46.2% (95% CI: 26.6–66.6) in the pretreated population and 47.1% (95% CI: 32.9–61.5) in the overall population (Table 3). Identical ORR values were reported by the investigators. The lower confidence limit for ORR exceeded the threshold value for each population (10% for the first-line population and 5% for the pretreated population). Twelve patients in each population achieved a confirmed PR according to investigator assessment; one patient achieved a confirmed CR (and 11 patients achieved a confirmed PR) in the first-line population based on independent review (Table 3).

Median time to tumour response based on independent review was 7.1 weeks (95% CI: 4.0–10.1) in the first-line population, 10.7 weeks (95% CI: 4.0–16.0) in the pretreated population and 10.0 weeks (95% CI: 4.0–11.0) in the overall population, with similar data reported by the investigators (data not shown).

**Table 2.** Sunitinib treatment and disposition

| | First-line population ($n = 25$) | Pretreated population ($n = 26$) |
|---|---|---|
| Median no. of treatment cycles (range) | 4.0 (1–8) | 5.0 (1–7) |
| Median duration of treatment, days (range) | 148.0 (14–322) | 196.0 (9–280) |
| Patients on treatment ≥6 months, *n* (%) | 10 (40) | 17 (65) |
| Median daily sunitinib dose, mg (range) | 43.29 (30.4–50.0) | 38.17 (27.7–50.0) |
| Treatment change due to adverse events, *n* (%)[a] | 17 (68) | 23 (88) |
| Discontinuations, *n* (%) | 11 (44) | 9 (35) |
| Owing to disease progression | 6 (24) | 4 (15)[b] |
| Owing to adverse event | 5 (20) | 4 (15) |

[a]Treatment change includes dose reduction, temporary discontinuation, or extension of the defined off-treatment period.
[b]One of the four patients discontinued sunitinib treatment because of death due to tumour progression. Note: Adverse events referenced in this table were treatment-related.

Progressive disease during treatment with sunitinib (including 28 days after the completion of treatment) was documented by investigators in eight patients in both the first-line and pretreated populations (32% and 31%, respectively) including three patients who had PR on study, one patient and two patients, respectively. Of these three patients with PR, duration of response was 42.3 weeks for the first-line patient and 21.0 and 30.0 weeks for the pretreated patients.

Median PFS was 46.0 (95% CI: 46.0–NR), 33.6 (95% CI: 25.0–NR) and 46.0 (95% CI: 28.4–NR) weeks in the first-line, pretreated and overall populations, respectively (Figure 1; overall population not shown); PFS and TTP were identical. At the time of analysis, median OS had not been reached in either population.

### Safety

All 51 patients experienced treatment-related adverse events, the majority of which were grade 1 or 2 in severity (Table 4). The most commonly reported treatment-related adverse events in the first-line population were anorexia (68%), skin discoloration (64%), diarrhoea (60%) and pyrexia (60%). In the pretreated population, the most commonly reported adverse events were skin discoloration (81%), fatigue (69%), anorexia (54%), dysgeusia (54%) and rash (54%).

The most common grade 3 adverse events in the first-line population were diarrhoea and hand-foot syndrome, each of which occurred in four patients (16%) and were manageable and reversible; the most common grade 3 adverse event in the pretreated population was fatigue, occurring in six patients (23%). No grade 4 or 5 treatment-related adverse events were reported in the first-line population, and in the pretreated population, one patient experienced grade 4 fatigue; however, this patient recovered and continued study treatment.

The most frequently reported laboratory abnormalities in the first-line population included decreased platelet count (96% of patients), white blood cells (84%), lymphocytes (84%) and neutrophils (72%), as well as increased lactate dehydrogenase (68%), aspartate aminotransferase (56%), lipase (60%), creatinine (56%) and alanine aminotransferase (52%; Table 4). Similar laboratory abnormalities were reported in the pretreated population, all occurring in >50% of patients except for increased creatinine, which occurred in 38%.

In the first-line population, the most common grade 3 laboratory abnormality was decreased platelet count, occurring in 12 patients (48%); the most common grade 4 abnormality was increased lipase, occurring in three patients (12%). In the pretreated population, grade 3 decreased neutrophils and platelets occurred in 15 (58%) and 12 patients (46%), respectively. No grade 5 laboratory abnormalities occurred in either patient population.