TABLE I. *Comparison between tPSA and cPSA at various cutoff values*

	Cutoff Value (ng/mL)					
	Hybritech tPSA	cPSA				
	>4.0	>3.4	>3.0	>2.9	>2.8	>2.7
Cancer (n)	250	237	250	251	252	255
Cancer missed (n)	18	31	18	17	16	13
False-positive (n)	380	326	352	367	378	382
No. vs. Hybritech tPSA						
Cancer missed		+13	±0	−1	−2	−5
False-positive results		−54	−28	−13	−2	+2

KEY: PSA = prostate-specific antigen; tPSA = total PSA; cPSA = complexed PSA.

TABLE II. *Comparison of cPSA and tPSA specificities at 80%, 85%, 90%, and 95% sensitivities in all cases*

	80% Sensitivity		85% Sensitivity		90% Sensitivity		95% Sensitivity	
	Cutoff (ng/mL)	Specificity (%)	Cutoff (ng/mL)	Specificity (%)	Cutoff (ng/mL)	Specificity (%)	Cutoff (ng/mL)	Specificity (%)
cPSA	4.27	49.6	3.75	41.3	3.36	33.7	2.76	22.8
tPSA	5.55	43.5	4.95	36.8	4.35	27.6	3.85	18.5
P value	0.0239		0.0042		0.0439		0.0068	

Abbreviations as in Table I.

4.0 to 6.0, 6.0 to 8.0, 8.0 to 10.0, and greater than 10.0, respectively. In men with cancer, the cPSA value was greater than 8.3 ng/mL in 125 (46.6%) and was the largest subgroup. In contrast, the greatest proportion of men without cancer (29.5%, n = 145; Fig. 1) had a cPSA value of 3.2 to 5.1 ng/mL.

We compared the number of patients with a missed cancer or false-positive result between the tPSA and cPSA values (Table I). The number of cancers missed and number of false-positive results using a tPSA cutoff value of 4.0 ng/mL was 18 and 380, respectively. A cPSA threshold of 3.0 ng/mL provided equivalent sensitivity (93.2%) and a better positive predictive value (39.6% versus 41.5%) to a 4.0-ng/mL cutoff for tPSA in the Japanese population. For the comparison in all cases, the area under the curve for cPSA (0.741) was significantly better than that for tPSA (0.721, $P < 0.001$).

The performance characteristics with respect to sensitivity and specificity for all cases are summarized in Table II. At a sensitivity of 80% to 95%, significant differences were found in the corresponding specificities between tPSA and cPSA. At a sensitivity of 90%, the specificity of cPSA improved 6.1% compared with that of tPSA. In addition, we compared the performance characteristics with respect to sensitivity and specificity in men with a tPSA value between 4.01 and 10.00 ng/mL (Table III). Similar to the results in all cases, significant differences were found in the corresponding specificities between tPSA and cPSA at a sensitivity of 80% to 95%.

COMMENT

To assess the utility of new serum markers, it is necessary to analyze their performance in different ethnic populations. The population of men in this study was 100% Asian. In our study, the median age for those with cancer was 71 years and was 68 years for those without cancer. In the study by Partin *et al.*,[10] the study population was 87.9% white. Consequently, those results mainly reflected the performance in one ethnic group. Comparing the two patient populations, the median age for men with and without cancer was 6 and 7 years older, respectively, in our study. In addition, substantial differences in median age have been reported in studies of different national or ethnic populations.[8,11] Just as the incidence of prostate cancer has been increasing in younger men in the United States,[12] the same trend has been observed with the use of PSA screening in Japan.[13] Despite the trend of the young age migration, the median age in Japanese men with prostate cancer was still older than that in the United States.

The distribution of cPSA and tPSA in the population studied also influences the age distribution, as well as the diagnostic performance of the serum marker. To our knowledge, this is the first report of

TABLE III. *Comparison of cPSA and tPSA specificities at 80%, 85%, 90%, and 95% sensitivities in men with total PSA value between 4.01 and 10.00 ng/mL*

	80% Sensitivity		85% Sensitivity		90% Sensitivity		95% Sensitivity	
	Cutoff (ng/mL)	Specificity (%)	Cutoff (ng/mL)	Specificity (%)	Cutoff (ng/mL)	Specificity (%)	Cutoff (ng/mL)	Specificity (%)
cPSA	3.82	31.8	3.50	22.0	3.36	17.9	3.01	11.8
tPSA	5.05	24.7	4.75	17.6	4.55	12.2	4.25	5.7
P value	0.0030		0.0471		0.0025		<0.0001	

Abbreviations as in Table I.

the distribution of cPSA in Japanese men with and without prostate cancer using a urologic referral population. In American and European countries, PSA levels have been shifting downward and what was once considered the traditional gray zone of tPSA (4 to 10 ng/mL) may now be 2 to 6 ng/mL.[10] In contrast to the study by Partin *et al.*,[10] most cPSA levels in men with cancer were more than 8.3 ng/mL (tPSA more than 10.0 ng/mL) in our study. First, the difference in cPSA distribution between the two studies might be explained by the prostate biopsy indication. In our study, biopsy was indicated for a tPSA cutoff of 4.0 ng/mL if the DRE findings were normal. In addition, the proportion of clinical stage, pathologic outcome, and men with urologic symptoms can be associated with the distribution of tPSA and cPSA in a population. Taneja *et al.*[14] reported the distribution of clinical stage and biopsy Gleason score in 410 men with prostate cancer, with the aim of using cPSA as a diagnostic and staging tool. The proportion of clinical Stage T3 disease and biopsy Gleason score less than 7 in their study was 4.0% and 57.6%, respectively, compared with corresponding values of 29% and 24% in our study. We speculate that the striking difference in clinical stage and biopsy outcomes resulted in the distribution of the cPSA range. Furthermore, the proportion of the total International Prostate Symptom Score of 8 or more was 58% in our study. Filella *et al.*[11] demonstrated the usefulness of cPSA and tPSA in the diagnosis of prostate cancer in patients with urologic symptoms. In their study, prostate symptoms were present in 94 (47%) of 200 men referred to the urologic practice, and the average tPSA level for those with cancer was 11.60 ± 10.12 ng/mL (range 1.86 to 49). In our study, it was 18.3 ± 19.5 ng/mL. Despite the substantial differences in the average tPSA value in those with cancer between the two studies, that the PSA value in those with cancer was based on a population with urinary symptoms results in a relatively greater range compared with a screening population. Also, selection bias and study population variances could potentially influence the optimal assay threshold values.

Previously, we established a preliminary optimal cutoff cPSA value (2.8 ng/mL) compared with a tPSA threshold of 4.0 ng/mL.[9] In this study, a 3.0 ng/mL cPSA threshold should be considered the potential cutoff value for comparative analysis of cancers missed and false-positive cases using the conventional 4.0-ng/mL tPSA threshold. In general, the calculated optimal cutoff value will change depending on the population being studied. On the basis of our previous study[9] and this study, however, we concluded that cPSA can be an alternative to tPSA as the first-line prostate cancer detection test in the selected (hospital referral) Japanese population. This position has been supported by the comparison of cPSA and tPSA specificities at a sensitivity of 80% to 95% (Tables II and III). Our next goal is to assess the optimal cutoff value of cPSA in the screening population.

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Prognostic Significance of Thymidylate Synthase Expression in Patients with Prostate Cancer Undergoing Radical Prostatectomy

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OBJECTIVES Thymidylate synthase (TS), a key enzyme in DNA synthesis, is overexpressed in a variety of cancer cells. 5-Fluorouracil (5-FU), an anticancer agent used clinically against various cancers, including prostate cancer, inhibits DNA synthesis by binding TS. In this study, we investigated the expression of TS in prostate cancer and its prognostic significance. Its association with the expression of dihydropyrimidine dehydrogenase (DPD), a principal enzyme in the degradation of 5-FU and pyrimidine nucleotides, was also examined.

METHODS Fifty-two prostatic tissue specimens were obtained from patients who had undergone radical prostatectomy for prostate cancer without neoadjuvant hormonal therapy. We analyzed the cancerous tissue and normal prostatic tissue specimens for TS expression using immunohistochemistry.

RESULTS TS was expressed at greater levels in the prostate cancer specimens than in the normal prostatic tissue specimens. The patients with prostate cancer with negative TS expression had a longer postoperative recurrence-free rate than did those with positive expression during the 5 years of follow-up. TS expression was significantly decreased in patients who received neoadjuvant hormonal therapy. No relationship was found between the expression of TS and DPD. Patients with prostate cancer with either negative TS or DPD expression had a significantly longer postoperative disease-free rate than those with positive expression of both during the 5 years of follow-up.

CONCLUSIONS The results of the present study have shown for the first time that TS expression could be a prognostic marker for patients with prostate cancer undergoing radical prostatectomy. In addition, the combination of TS and DPD expression might also be helpful for the prediction of the prognosis of patients with prostate cancer. UROLOGY xx: xxx, xxxx. © 2007 Elsevier Inc.

The anticancer agent, 5-fluorouracil (5-FU), is used clinically against various cancers, including prostate cancer.[1,2] Single-agent infusion 5-FU has demonstrated some efficacy against hormone-refractory prostate cancer, and response rates up to 27% have been reported.[3] 5-FU itself is inactive and requires intracellular conversion to 5-fluoro-2′-deoxyuridine 5′-monophosphate. 5-Fluoro-2′-deoxyuridine 5′-monophosphate exerts its cytotoxic activity through the formation of a ternary complex with thymidylate synthase (TS) and 5,10-methylene-tetrahydrofolate, resulting in inhibition of TS and blockage of the DNA synthetic process.[4,5] TS is overexpressed in tumor cells, which show high proliferative activity.[6] Several studies examining the importance of TS expression have indicated that TS expression predicts for overall outcome and the response to 5-FU cytotoxic therapy in several major tumor types.[7–9] Furthermore, the immunohistochemical staining results for TS and dihydropyrimidine dehydrogenase (DPD) predict the response to 5-FU.[10,11]

Our previous studies on renal cell carcinoma and bladder cancer showed that TS activity was greater in the cancerous tissue specimens than in the normal tissue samples and that

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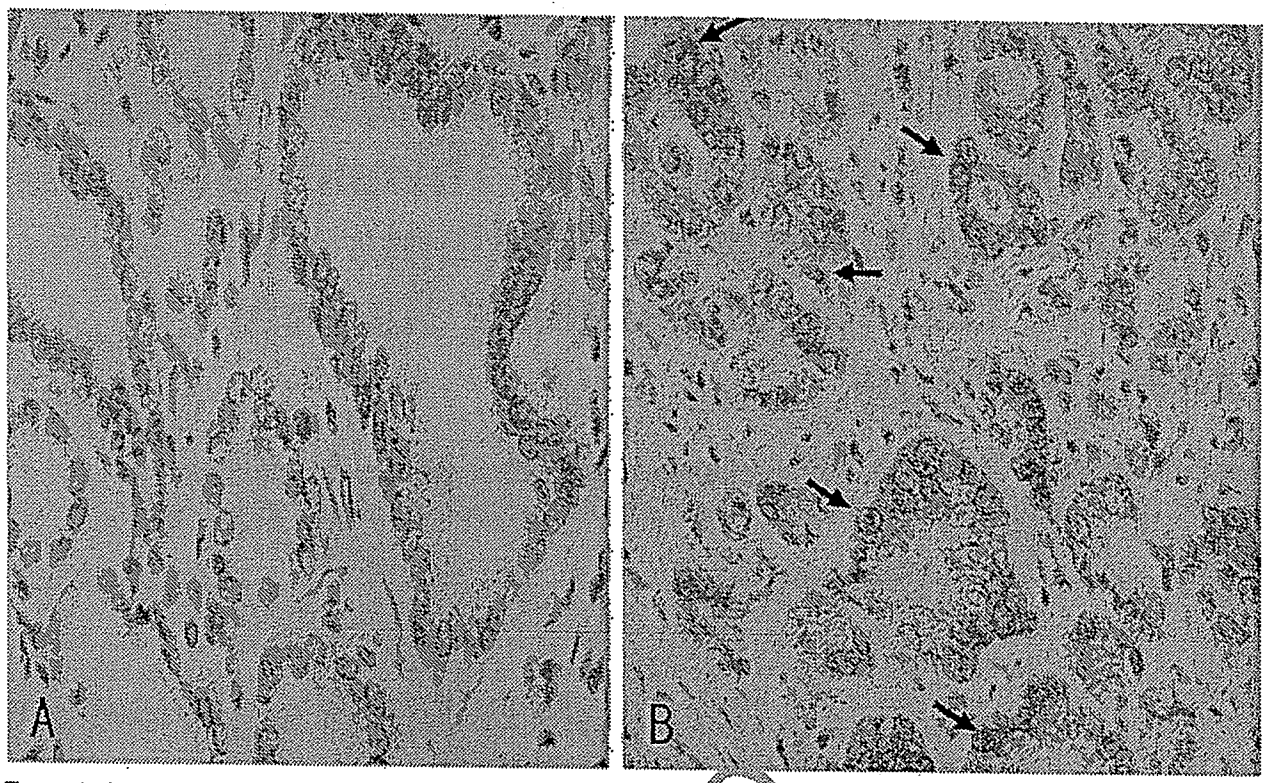


Figure 1. Immunohistochemical staining for TS in normal prostatic tissues and prostate cancer. Representative images of tissue microarray samples with absent staining and strong TS expression were examined. TS expression confined to cytoplasm of cells as demonstrated by immunohistochemistry. Original magnification ×200. **(A)** TS staining negative in normal prostatic tissue. **(B)** Positive TS staining (arrows) observed in cytoplasm of prostate cancer tissue.

AQ: 2

the TS activity level correlated with stage progression and increase in bladder cancer grade.[12] In addition, TS activity is a significant prognostic marker in patients with bladder cancer or renal cell carcinoma.[12,13] Reported data on TS activity in prostate cancer are limited, and little is known about the significance of TS in the biology of prostate cancer. The aim of this study was to define whether TS expression is a prognostic marker for patients with prostate cancer.

MATERIAL AND METHODS

Patients

We obtained 52 prostate cancer specimens with adjacent normal prostatic tissues from 1997 to 2005. The patients had not undergone preoperative androgen-deprivation therapy or radiotherapy. The mean patient age was 65 years (range 53 to 75). The 2002 TNM system was used for pathologic staging.[14] The pathologic stage was T2 in 38 patients and T3 in 14. The Gleason grading system was used to determine the Gleason score.[15] The Gleason score of the 52 specimens was grade 4/5 in 1 patient, 4/4 in 1, 4/3 in 9, 3/5 in 2, 3/4 in 18, 3/3 in 8, 3/2 in 8, 2/3 in 3, 2/1 in 1, and 1/2 in 1 patient. In addition, prostate cancer tissue from 48 patients who had undergone neoadjuvant hormonal therapy were examined.

The local human investigations committee approved this study, and all patients provided informed consent.

Immunohistochemistry for TS and DPD

TS and DPD expression was examined by immunohistochemistry, as previously described.[5,16] The sections were incubated with monoclonal antibody TS106 (1:500, dilution, Taiho Pharmaceutical, Saitama, Japan) or incubated with polyclonal rabbit antibody against human DPD[16] (1:2000 dilution, Taiho Pharmaceutical) overnight at 4°C. The secondary antibody was visualized with diaminobenzedine.

Evaluation of TS and DPD Expression

The intensity of the immunoreactivity for TS and DPD was evaluated in normal prostatic tissue and prostate cancer tissue from the same slide in each case. At least 10 high-power fields at 400× magnification were chosen randomly, and more than 1000 carcinoma cells were counted for each section. A pathologist who was unaware of the clinicopathologic data and clinical outcomes of the patients examined cytoplasmic TS and DPD staining results. The intensity of TS and DPD was graded from 0 to 3, and the extent was graded as focal (less than 25% of tumor staining positive) or diffuse (more than 25% of tumor staining positive).[5] A score of 0 or 1 was regarded as negative expression, and a score of 2 or 3 as positive expression. Figure 1 shows representative examples; Fig. 1A shows a TS-negative normal prostate specimen and Fig. 1B TS-positive prostate cancer. F1

Statistical Analysis

For statistical analysis, the Student *t* test and chi-square test were used. Biochemical recurrence was defined as a postopera-

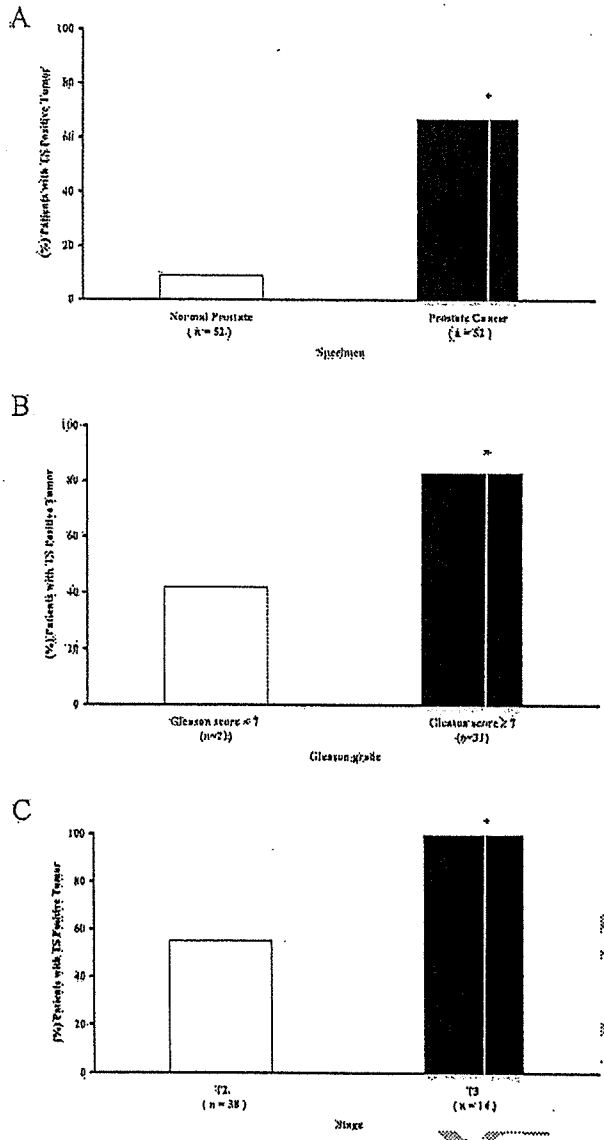


Figure 2. Expression of TS in normal prostatic tissue and prostate cancer. Percentage of TS expression detected by immunohistochemical assay as described in Material and Methods section. **(A)** $^*P < 0.0001$ versus normal prostatic tissue; **(B)** $^*P = 0.0017$ versus Gleason score less than 7 disease; and **(C)** $^*P = 0.0113$ versus Stage T2.

AQ: 3

tive serum prostate-specific antigen (PSA) level of 0.1 ng/mL or more.[17] The postoperative recurrence-free rate was determined using the Kaplan-Meier method. The influence of each variable on the recurrence-free rate was analyzed by multivariate analysis of a Cox proportional hazard model. $P < 0.05$ was considered significant.

RESULTS

TS Expression in Prostate Cancer and Normal Prostatic Tissue

TS was expressed in the cytoplasm of both normal prostatic tissue and prostate cancer cells. TS expression was detected in 35 (67%) of 52 prostate cancer samples (Fig. 2A). In contrast, TS expression was detected in 5 (9%) of the 52 normal prostatic tissue specimens. In addition, the intensity of cells that reacted with TS antibodies was significantly greater in the prostate cancer specimens than in the normal prostate samples ($P < 0.0001$, data not shown).

TS Expression in Relation to Pathologic Features and Tumor Stage

The staining percentage of TS expression was greater in patients with Gleason score 7 or greater disease (26 of 31, 83%) than that for patients with Gleason score less than 7 disease (9 of 21, 42%; Fig. 2B). It was also greater in Stage T3 tumor (14 of 14, 100%) than in Stage T2 tumors (21 of 38, 55%; Fig. 2C).

Relationship Between TS Expression and Postoperative Recurrence-Free Rate and Between TS and DPD Expression and Postoperative Recurrence-Free Rate in Patients with Prostate Cancer

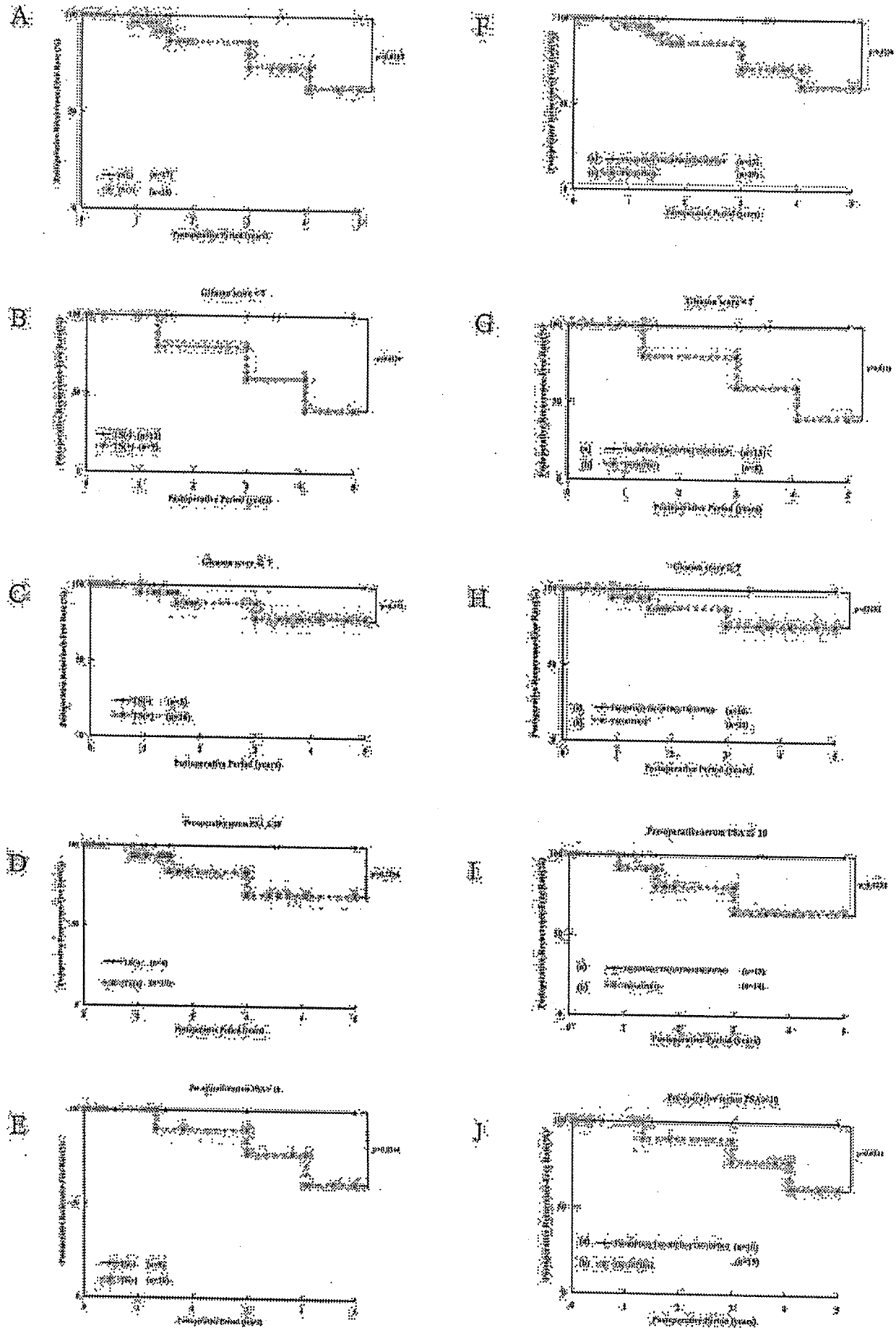
Patients with prostate cancer undergoing radical prostatectomy alone were evaluated to determine the postoperative clinical course. From these results, patients with prostate cancer were divided into two groups—those with positive TS expression and those with negative TS expression. At 5 years of follow-up, patients with negative TS expression had a greater recurrence-free rate compared with those with positive TS expression ($P = 0.0183$; Fig. 3A). Using Cox regression analysis for the 52 patients, TS expression seemed to be independent prognostic indicator ($P = 0.021$ on multivariate analysis). The patients were also divided according to the Gleason grade and preoperative serum PSA level at baseline, and the recurrence-free rate of the groups with different TS expression status were analyzed. In Gleason score less than 7 cancer, the TS-negative group had a significantly greater 5-year recurrence-free rate than did the TS-positive group (Fig. 3B). In those with Gleason score 7 or greater cancer, no significant difference was found (Fig. 3C). In those with a PSA of 10 ng/mL or less at baseline, the 5-year recurrence-free rate of TS-negative patients tended to be greater than that of TS-positive patients. However, statistical significance was not reached (Fig. 3D). In those with a PSA level greater than 10 ng/mL, the TS-negative group had a significantly greater 5-year recurrence-free rate compared with the TS-positive group (Fig. 3E). These findings indicate that the TS expression level in prostate cancer could be a prognostic indicator, with negative TS expression a good prognostic sign.

F3

Effect of Neoadjuvant Hormonal Therapy on TS Expression in Prostate Cancer

The expression of TS in patients with prostate cancer was greater after radical prostatectomy alone than that after radical prostatectomy plus neoadjuvant hormonal therapy (32 of 52, 61% versus 18 of 48, 37%; Fig. 4A). For patients with Stage T2 prostate cancer, positive TS expression in those who underwent neoadjuvant hormonal

F4



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therapy (9 of 38, 23%) was less than that in patients who underwent radical prostatectomy alone (21 of 38, 55%; Fig. 4B). However, no significant difference was found in those with Stage T3 prostate cancer (14 of 14, 100% versus 10 of 10, 100%; Fig. 4C). With Gleason score less than 7 prostate cancer, positive TS expression in patients with radical prostatectomy alone (9 of 21, 42%) was significantly greater than that in patients who had undergone neoadjuvant hormonal therapy (1 of 13, 7%; Fig. 4D). A significant difference was also observed in patients with Gleason score 7 or greater prostate cancer between those who underwent radical prostatectomy alone (26 of 31, 83%) and those who also received neoadjuvant hormonal therapy (18 of 35, 51%; Fig. 4E).

These findings suggest that neoadjuvant hormonal therapy might downregulate TS expression in prostate cancer, especially Stage T2 prostate cancer and Gleason score less than 7 disease.

Prognostic Significance of Combined TS and DPD Evaluation

DPD is the initial and rate-limiting enzyme in the three-step pathway of pyrimidine nucleotide catabolism.[18] In contrast, TS is an important enzyme in pyrimidine nucleotides synthesis. Kornmann *et al.*[19] reported that the combination of TS and DPD expression correlated highly with survival. We examined the association between TS and DPD expression in patients with prostate cancer. No significant association was observed between the levels of TS and DPD expression using the chi-square test (data not shown).

We then examined the prognostic significance of a combination of TS and DPD expression using Kaplan-Meier analysis. On the basis of TS and DPD expression, the 52 patients were stratified categorized as those having positive expression of both TS and DPD and all others. We found a significant difference between these two groups ($P = 0.0108$; Fig. 3F). Patients with positive expression of both TS and DPD had a greater rate of postoperative recurrence. The patients also were divided using the Gleason grade and preoperative serum PSA level at baseline, and the recurrence-free rate of the groups with different TS and DPD expression were analyzed. In those with Gleason score less than 7 cancer, the TS-negative group had a significantly greater 5-year recurrence-free rate than did the TS-positive group (Fig. 3G). In patients with Gleason score 7 or greater cancer, no significant difference was found (Fig. 3H). In patients with a PSA level of 10 ng/mL or less, the 5-year recurrence-free rate of the TS-negative group tended to be greater than that of the TS-positive group. However, statistical significance was not reached (Fig. 3I). In those with a PSA level greater than 10 ng/mL, the TS-negative group had a significantly greater 5-year recurrence-free rate than that of the TS-positive group (Fig. 3J).

COMMENT

The role of TS expression in prostate cancer has not been previously studied. Multivariate analysis revealed that the TS expression profile was an independent prognostic indicator of prostate cancer. These results suggest that TS expression in patients with prostate cancer might provide additional prognostic information beyond the orthodox clinical and pathologic prognostic markers. Ichikawa *et al.*[20] reported that patients with colorectal tumor with both low DPD and low TS survived longer than did patients with tumor having the other patterns of TS and DPD expression. Consistent with our findings, the combined evaluation of TS and DPD expression might predict the prognosis more accurately than using one marker in patients with prostate cancer. These data also reflect the report by Jakob *et al.*[11] that immunohistochemistry such as reverse transcriptase polymerase chain reaction is a suitable method to determine the correlation between TS and DPD expression and histopathologic tumor regression. However, the precise mechanisms for this relationship remain unclear at present. Additional studies are necessary to examine the mechanisms.

The benefit of hormonal therapy against advanced prostate cancer has been widely accepted. In addition, the antitumor effect is very high. Because TS is the key

Figure 3. Relationship between TS expression and postoperative recurrence-free rate in patients with prostate cancer and relationship between TS and DPD expression and postoperative recurrence-free rate in patients with prostate cancer. Postoperative recurrence-free rate of 52 patients with prostate cancer undergoing radical prostatectomy alone was determined using Kaplan-Meier method. **(A)** Patients categorized by TS expression. Recurrence-free rate was significantly greater for patients with negative TS expression than for those with positive expression ($P = 0.0183$). **(B)** Patients categorized by TS expression of Gleason score less than 7 disease. Recurrence-free rate was significantly greater for patients with negative TS expression than for those with positive expression ($P = 0.0256$). **(C)** Patients categorized by TS expression of Gleason score 7 or worse. No significant difference observed between two groups of patients ($P = 0.2722$). **(D)** Patients categorized by TS expression of preoperative serum PSA level of 10 ng/mL or less. No significant difference observed between two groups of patients ($P = 0.2296$). **(E)** Patients categorized by TS expression of preoperative serum PSA level greater than 10 ng/mL. Recurrence-free rate significantly greater for patients with negative TS expression than for those with positive expression ($P = 0.0344$). Patients (n = 52) categorized as having positive expression of both TS and DPD (b) and all others (a). **(F)** Significant difference in recurrence rate between two groups ($P = 0.0108$). **(G)** Patients with Gleason score less than 7 disease. **(H)** Patients with Gleason score of 7 or more. **(I)** Patients with tumor with preoperative serum PSA level of 10 ng/mL or less. **(J)** Patients with tumor and preoperative serum PSA level greater than 10 ng/mL.

AQ: 4

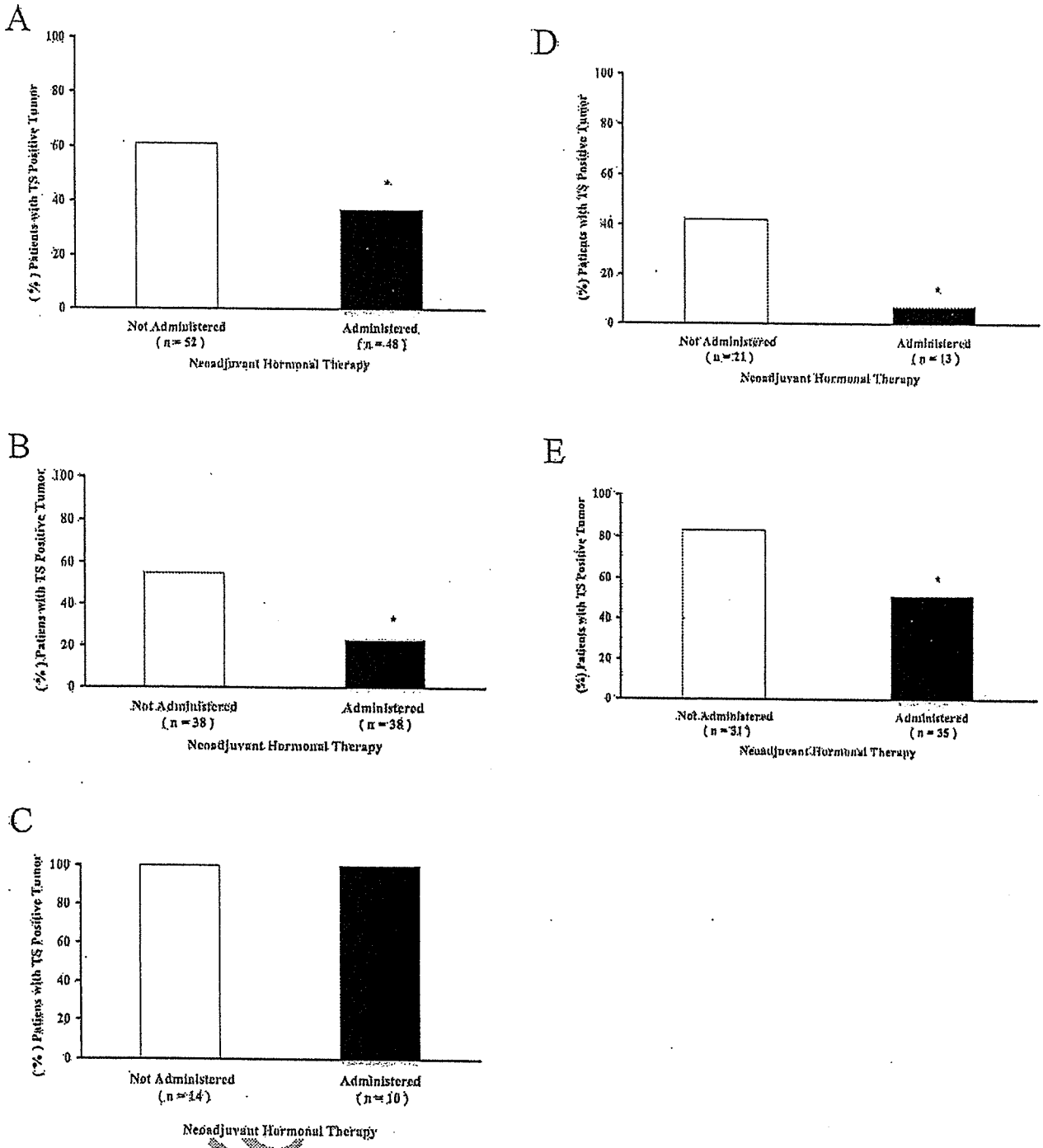

Figure 4. TS expression in prostate cancer with/without neoadjuvant hormonal therapy according to stage and Gleason grade of prostate cancer. TS expression in prostate cancer specimens evaluated by immunohistochemical assay as described in Material and Methods section. **(A)** $^{*}P = 0.0049$ versus not administered. **(B)** Patients with Stage T2 disease ($^{*}P = 0.0078$ versus not administered). **(C)** Patients with Stage T3 tumor. **(D)** Patients with Gleason score less than 7 tumor ($^{*}P = 0.0259$ versus not administered). **(E)** Patients with Gleason score 7 or greater tumor ($^{*}P = 0.0036$ versus not administered).

enzyme in the process of DNA synthesis, we believe it is reasonable to assume that this was the reason the TS expression was greater in the in the prostate cancer specimens of the patients treated without neoadjuvant hormonal therapy than in those treated with surgery and neoadjuvant hormonal therapy. Furthermore, hormonal therapy might directly downregulate TS expression. A study by Ogasawara *et al.*[21] showed that in nude mouse MCF-7 breast cancer xenografts, tumor TS levels were reduced by treatment with a pure antiestrogen. Hung *et al.*[22] also reported that the Ki-67 proliferation index was significantly greater in TS-positive tumors than in TS-negative tumors, suggesting that TS-negative tumors might have a low rate of cell proliferation.

TS is a key enzyme for pyrimidine synthesis. DPD is an important pyrimidine salvage enzyme. No correlation was found between DPD and TS expression in colorectal cancer.[20,23] We reported that no correlation was found between the TS and DPD activity levels in bladder carcinoma[12,24] or renal cell carcinoma.[13] Our data in prostate cancer are consistent with these results.

Immunohistochemical staining for TS and DPD predict the response to 5-FU.[10,11] Previous studies of several cancers have demonstrated that the TS expression level predicted the response to 5-FU-based chemotherapy.[4,5] Greater TS expression was accompanied by a greater response rate to 5-FU-containing chemotherapy. Most of the administrated 5-FU is degraded through the catabolic pathway with DPD.[18] DPD activity is highly associated with 5-FU pharmacokinetics.[25] The efficacy of 5-FU is related to the plasma level of this agent, which is inversely associated with the DPD activity level.[25] Our previous report demonstrated that primary cultured renal cell carcinoma cells with both high TS activity and low DPD activity were more sensitive to 5-FU than those with either low TS activity or high DPD activity.[26] These findings suggest that the TS and DPD expression levels in prostate cancer could be important predictive indicators for 5-FU efficacy. However, other factors might be more important, including the rate of degradation, carrier protein level, and so forth, although the principle TS and DPD expression levels might predict the response to 5-FU.

CONCLUSIONS

The results of our study have demonstrated that TS expression was significantly greater in the cancerous prostate and that positive TS expression was associated with a worse prognosis. These findings suggest that the assessment of TS expression might be useful in the management of prostate cancer. Because TS expression could be used as a prognostic parameter in patients with prostate cancer, an accurate prediction of prognosis might help to select patients for more intensive surgical, hormonal, or chemotherapeutic approaches, including 5-FU. Additional prospective studies are warranted to define the role of TS in selecting patients for adjuvant therapy for prostate cancer.

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Long-term results of first-line sequential high-dose carboplatin, etoposide and ifosfamide chemotherapy with peripheral blood stem cell support for patients with advanced testicular germ cell tumor

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Objective: Standard chemotherapy shows relatively low long-term survival in patients with poor-risk testicular germ cell tumor (GCT). First-line high-dose chemotherapy (HD-CT) may improve the result. High-dose carboplatin, etoposide, ifosfamide chemotherapy followed by autologous peripheral blood stem cell transplantation (PBSCT) was investigated as first-line chemotherapy in patients with advanced testicular GCT.

Methods: Fifty-five previously untreated testicular GCT patients with Indiana 'advanced disease' criteria received three cycles of bleomycin, etoposide and cisplatin (BEP) followed by one cycle of HD-CT plus PBSCT, if elevated serum tumor markers were observed after three cycles of the BEP regimen.

Results: Thirty patients were treated with BEP alone, because the tumor marker(s) declined to normal range. Twenty-five patients received BEP and HD-CT. One patient died of rhabdomyolysis due to HD-CT. Three and six (13% and 25%) out of 24 patients treated with BEP and HD-CT achieved marker-negative and marker-positive partial responses, respectively. The other patients achieved no change. Fifteen (63%) are alive and 14 (58%) are free of disease at a median follow-up time of 54 months. Severe toxicity included treatment-related death (4%).

Conclusions: HD-CT with peripheral stem cell support can be successfully applied in a multicenter setting. HD-CT demonstrated modest anticancer activity for Japanese patients with advanced testicular GCT and was well tolerated. This regimen might be examined for further investigation in randomized trials in first-line chemotherapy for patients with poor-risk testicular GCT.

Key words: chemotherapy, germ cell tumor, high-dose, peripheral blood stem cell transplantation (PBSCT), testis.

Introduction

Cisplatin-based combination chemotherapy has improved the prognosis of patients with metastatic germ cell tumor (GCT), and the long-term cure rate is approximately 80%.[1,2] However, patients with the 'advanced disease' criteria according to the Indiana University classification or the 'poor prognosis' criteria of the International Germ Cell Cancer Collaborative Group classification show survival rates of only 50–60% following standard-dose cisplatin-based chemotherapy.[3–5] Several attempts have been undertaken to improve the outcome of this patient group, including the use of double-dose cisplatin regimens or alternating dose-dense chemotherapy sequences.[6–8] However, doubling the dose of cisplatin did not lead to an improved outcome as compared with a standard cisplatin-dose regimen. Recently, high-dose chemotherapy (HD-CT) followed by autologous peripheral stem cell support or autologous bone marrow support has also been studied in these patients.[9–11] The rationale for the use of HD-CT in patients with GCT is based on preclinical and clinical data suggesting a dose–response relationship for certain drugs used in the treatment of GCT, particularly for etoposide and ifosfamide.[12,13] Dose finding studies in the high-dose setting, usually using a combination of carboplatin, etoposide and cyclophosphamide, ifosfamide, or thiotepa, have been conducted in heavily pretreated patients with relapsed or refractory disease.[14,15]

Although single center phase II trials have reported 2-year survival rates of 70–80% using first-line HD-CT approaches in poor prognosis patients, results of large phase II trials or phase III trials are lacking.[9–11] In addition, it is unclear at present whether the reported survival rates of 70–80% are maintained with longer follow up. The present study investigated the long-term results of first-line HD-CT with autologous stem cell support in Japanese patients with advanced testicular GCT in a multicenter setting. Patients with relapsed testicular GCT were excluded in this study.

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Methods

Patients

Fifty-five patients with advanced testicular GCT were entered onto this institutional review board-approved prospective trial between March 1996 and December 1999. All patients gave informed consent before they were enrolled onto the study.

Eligibility included 'advanced disease' criteria according to the Indiana classification.[3] According to the International Germ Cell Cancer Collaborative Group (IGCCCG) criteria, the numbers of good, intermediate and poor prognosis were 7, 26 and 22, respectively. All patients had histologically confirmed testicular GCT and no prior chemotherapy. Patients were also required to have a pretreatment leukocyte count greater than 3000/μL, pretreatment platelet count greater than 100 000/μL, glomerular filtration rate of 60 mL/min or higher, and serum creatinine level less than or equal to 1.5 times the upper level of the institutional norm.

Before chemotherapy, each patient was evaluated with a history and physical examination, chest radiography, computed tomography (CT) of the abdomen/pelvis, and screening chemistries, which included serum tumor markers (alpha-fetoprotein [AFP], human chorionic gonadotropin-β [HCG-β] and lactate dehydrogenase [LDH]). CT of chest or brain and bone scintigraphy were performed as indicated. All patients had chest CT, if chest X-ray suggested metastases.

Mobilization and harvest of peripheral blood stem cells

A dose of 5 μg/kg of recombinant human granulocyte colony-stimulating factor (G-CSF) was given to each patients s.c. once daily from the day of the nadir of neutrophil count after bleomycin, etoposide and cisplatin (BEP) combination chemotherapy. After white blood cells (WBC) recovered to 5000/μL, leukapheresis collections of peripheral blood stem cells were carried out for 2–3 consecutive days using a CS 3000 blood cell separator (Baxter Limited, Deerfields, IL, USA). A total volume of 10–15 L of blood was processed in each patient. Mononuclear cells containing hematopoietic stem/progenitor cells were cryopreserved in liquid nitrogen. The target harvest was more than 2.0×10^6 CD34-positive nucleated cells/kg patient bodyweight.

Conventional-dose chemotherapy

All patients were treated with three cycles of BEP as induction chemotherapy. The doses of anticancer agents, treatment schedule and treatment-related toxicity have been described previously (bleomycin 30 mg, i.v., days 2, 9, 16; etoposide 100 mg/m^2, i.v., days 1–5; cisplatin 20 mg/m^2, i.v., days 1–5).[16] After three cycles of BEP therapy, patients whose tumor marker(s) were still elevated received one cycle of HD-CT with peripheral blood stem cell transplantation (PBSCT). If the serum tumor markers declined to normal range, the patients did not receive HD-CT.

High-dose chemotherapy

For treatment with HD-CT and autologous PBSCT, performance status 0 or 1 was required. HD-CT consisted of 1250 mg/m^2 of carboplatin, 1500 mg/m^2 of etoposide, and 7.5 g/m^2 of ifosphamide followed by 300 mg/m^2 of Mesna (bolus i.v. every 8 h, days 1–5). HD-CT was administered in five divided doses from day −7 to day −3. PBSCT was given i.v. on day 0. All patients received 5 μg/kg of G-CSF s.c., beginning the day following PBSCT and continuing until neutrophil count recovery. If all abnormally elevated serum tumor marker values returned to normal, surgery was performed when it was necessary to resect residual tumors.

Evaluation procedures

Serum tumor markers were determined before each treatment cycle and 4 weeks after the end of therapy. Evaluation of measurable disease by radiographic means was performed after HD-CT cycle and 4–6 weeks after the end of treatment. Subsequent follow-up tests including CT scans, serum tumor marker values and routine blood tests were performed at 3-month intervals during the first 2 years and then every 6 months up to 5 years of follow up.

Response to first-line HD-CT was defined according to World Health Organization (WHO) criteria.[17] Complete response (CR) was defined as the disappearance of all evidence of disease for at least 6 weeks when documented by imaging and all tumor markers. Partial response (PR) was defined as at least 50% reduction in the product of perpendicular diameters of each indicator lesion. PR was divided into two categories, partial response with tumor marker normalization (PR^{m-}) and marker positive partial response (PR^{m+}). Progressive disease (PD) was defined as 25% increase in the product from any lesion or the appearance of any new lesion. No change (NC) was defined as that which did not meet any of the above criteria. NC was also divided to two categories, no change with tumor marker normalization (NC^{m-}) and marker positive no change (NC^{m+}), Response and duration of survival were measured from the date of initiation of HD-CT.

Statistical analysis

Disease-specific survival was determined by the Kaplan–Meier method. For statistical analysis, a χ^2 test was used.

Results

Patient characteristics

Fifty-five patients, ranging in age 16–51 years (median, 27 years), were entered into this trial. Patient characteristics are summarized in Table 1a. Approximately 75% of all patients had lung metastasis and involvement of abdominal lymph nodes. Liver metastasis was present in 20% of patients. Four percent of patients had bone metastasis and 7% had central nervous system metastasis at diagnosis. Their histological types of GCT were four pure seminomas and 51 non-seminomas.

Response and survival

Five of 55 patients treated with three cycles of BEP achieved CR. Twenty-five of 50 patients who achieved PR or NC by induction BEP had normal concentrations of serum tumor markers. These 30 patients received another cycle of BEP therapy

Thus, following three cycles of induction BEP therapy, the remaining 25 patients whose tumor marker(s) (AFP, HCG-β and/or lactate dehydrogenase [LDH]) were still elevated when treated with one cycle of HD-CT with PBSCT. The patient characteristics are summarized in Table 1b. According to the IGCCCG criteria, the numbers of good, intermediate and poor prognoses were 1, 11 and 13, respectively. Because one patient died of rhabdomyolysis due to HD-CT, 24 patients were available to evaluate. Table 2 and Figure 1 summarize the outcome data. No patient achieved CR after one cycle of HD-CT. In all,

Table 1 Patient characteristics

Characteristics	No. of patients
(a)	
Patients age (years)	55
Median	27
Range	16–51
Histology	
Seminoma	4
Non-seminoma	51
Number of metastatic sites	
1	8
2	36
3 or more	11
Sites of metastasis	
Lung	48
Retroperitoneal lymph node	45
Mediastinal lymph node	12
Supraclavicular lymph node	3
Liver	11
Brain	4
Bone	2
Serum tumor markers	
HCG-β (ng/mL)	
Median elevated value	42
Range	0.1–120 000
AFP (ng/mL)	
Median elevated value	205
Range	10–62 274
LDH (IU/L)	
Median elevated value	1 550
Range	213–7 479
(b)	
Patients age (years)	25
Median	27
Range	16–45
Histology	
Seminoma	1
Non-seminoma	24
Number of metastatic sites	
1	5
2	13
3 or more	7
Sites of metastasis	
Lung	24
Retroperitoneal lymph node	19
Mediastinal lymph node	5
Liver	3
Brain	2
Serum tumor markers	
HCG-β (ng/mL)	
Median elevated value	1.35
Range	0.2–9.59
AFP (ng/mL)	
Median elevated value	52
Range	9–2 383
LDH (IU/L)	
Median elevated value	548
Range	419–781
Follow-up (months)	
Median	54
Range	10–80

AFP, alpha-fetoprotein; HCG-β, human chorionic gonadotropin-β; LDH, lactate dehydrogenase.

Table 2 Outcome data

Outcome	No. of patients (%)
Partial response	9 (37.5)
Marker-negative	3 (12.5)
Marker-positive	6 (25.0)
No change	15 (62.5)
Marker-negative	4 (16.7)
Marker-positive	11 (45.8)
Overall response rate	37.5

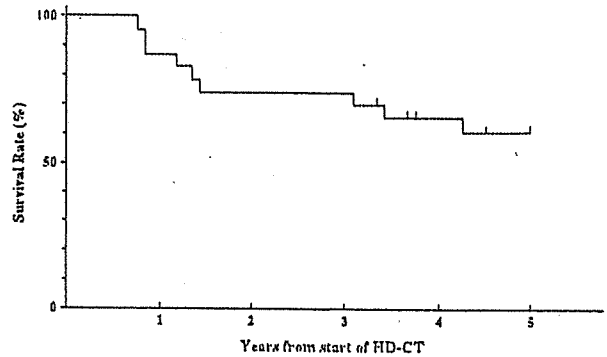

Fig. 1 Disease-specific survival of testicular germ cell tumor (GCT) patients treated with high-dose chemotherapy (HD-CT). Disease-specific survival rate was determined by the Kaplan–Meier method.

nine (38%) of 24 patients achieved PR. Marker-negative PR were achieved in only three patients. Fifteen NC and no PD were observed.

Seven patients showed high serum levels of AFP before HD-CT. The serum levels of AFP in six patients decreased after chemotherapy, but the levels in three patients were higher than normal range. In addition, serum AFP levels in one patient increased after HD-CT. Nineteen patients had high levels of serum HCG-β. The high serum HCG-β levels in five patients became less than the sensitivity of examination after HD-CT. Although the levels of serum HCG-β in 11 patients decreased after HD-CT, the serum levels were higher than normal range. The increased and same serum HCG-β levels in one and two patients were observed after HD-CT. Five patients demonstrated high serum LDH levels. The serum LDH level in three patients decreased to normal range after HD-CT, and the levels in the other two patients decreased, but to more than normal range.

Seventeen of 24 patients underwent operations for residual tumors. The patients included eight PR (marker-negative PR, three; marker-positive PR, five) and nine NC (marker-negative NC, four; marker-positive NC, five). Salvage surgery was performed for 10 patients (five PR and five NC) with positive marker(s). The pathological examinations revealed nine necrosis (marker-negative, three; marker-positive, six), one mature teratoma (marker-negative, one) and seven viable malignant tumors (marker-negative, three; marker-positive, four).

Twenty-one patients received additional therapy after first-line HD-CT and/or surgical resection of residual masses. Three patients received additional HD-CT, and nine patients received salvage chemotherapy with VIP (etoposide, ifosfamide and cisplatin), taxol or camptothecin, and radiation.

Table 3 Relationship between survival and various characteristics

Characteristics	Outcome (patient no.)	
	Alive (n = 15)	Dead (n = 9)
Serum tumor marker after HD-CT		
Positive	10	7
Negative	5	2
IGCCCG criteria		
Good	1	0
Intermediate	5	6
Poor	9	3
Histology: choricarcinoma		
(+)	7	3
(−)	8	6
Histology: yolk sac tumor		
(+)	5	4
(−)	10	5

There was no statistical significance by χ^2 test. HD-CT, high-dose chemotherapy; IGCCCG, International Germ Cell Cancer Collaborative Group.

Table 4 Adverse events

Adverse events	Grade				
	0	1	2	3	4
Neutropenia	0	0	0	1	22
Thrombocytopenia	0	0	3	4	16
Anemia	0	0	11	6	6
Fever	19	2	2	0	0
Mucositis	7	6	9	1	0
Nausea/vomiting	1	6	8	8	0
Diarrhea	11	5	6	1	0
Liver toxicity	13	4	4	1	1
Renal toxicity	21	1	0	1	0
Skin	20	2	1	0	0

The median duration of follow up is 54 months with a range of 9–80 months. Fourteen patients are currently alive and free of GCT and one patient remains alive with disease. All six patients with marker-positive status after HD-CT, whose pathological examinations of salvage surgery revealed necrosis, are alive. Nine have died of disease. The 3-year and 5-year survival rates were approximately 75% and 63%, respectively (Fig. 1). There was no correlation between survival and various characteristics (Table 3).

Adverse effects

Table 4 describes the toxicity in this study according to the WHO scale. There was a toxic death caused by rhabdomyolysis due to HD-CT.

Neutropenia was significant in all patients, and all patients except one experienced WHO grade 4 neutropenia. Nine patients had neutropenia with fever. The median duration of neutropenia less than 500/μL was 9 days (8–15 days). All patients received G-CSF, and the median duration of use of G-CSF was 11 days (8–21 days). Similarly, grade 2–4 thrombocytopenia/anemia were reported in all patients. The median duration of thrombocytopenia less than 20 000/μL was 9 days (0–18 days). All patients received platelet transfusions during HD-CT. The median amount of platelet transfusion was 55 units (20–200 units). Twenty patients received red blood cell transfusions during the chemotherapy. The median amount of transfusion was 4 units (0–11 units). Discontinuity of chemotherapy was not necessary for this hematological toxicity.

As expected, the other most common non-hematological side-effects were mucositis and nausea/vomiting. Diarrhea was also common. The most frequent grade 3/4 complications were nausea/vomiting, which were sufficiently controlled with anti-emetic therapy. Severe neurotoxicity was rare.

No specific investigations regarding late toxicity have yet been performed. At present, no patient developed therapy-related secondary leukemia.

Discussion

This study on first-line chemotherapy with HD-CT/PBSCT consisting of carboplatin, etoposide and ifosfamide was carried out in cooperation with 30 centers within the Japan Blood Cell Transplantation Study Group. The objectives of this trial were to determine the outcome, feasibility and toxicity of HD-CT/PBSCT in a multicenter setting. The rationale for HD-CT is based on the assumption that the front-line use of HD-CT may induce cell death in a higher fraction of sensitive and intermediate-sensitive GCT before drug resistance develops. This assumption is based on the observation in several solid tumor types including lymphomas and testicular cancer, that applying chemotherapy with a higher dose-intensity may lead to improved outcome.[18,19] Several phase II studies on the use of first-line HD-CT in testicular GCT have investigated schedules consisting of two to three standard-dose cycles followed by high-dose cycles.[9–11] These phase II studies have reported 2-year survival rates of 70–80% following first-line HD-CT, indicating that a 15–20% survival advantage may be achievable with first-line HD-CT as compared with standard BEP therapy.[9–11] In the phase II study, Motzer *et al.* demonstrated that first-line high-dose chemotherapy is well tolerated, and suggested a survival advantage following this approach compared to a historical control group treated with vinblastine, actinomycin-D, cyclophosphamide, cisplatin and bleomycin.[9] In a subsequent trial by the same investigators, poor prognosis patients with insufficient marker decline following two cycles of standard-dose VIP therapy received two cycles of high-dose carboplatin, etoposide and cyclophosphamide therapy followed by autologous stem cell support. Among 58 patients treated with this approach, 50% remained disease-free as compared to 25% of control patients who only received standard-dose therapy.[10] The present study demonstrated that first-line HD-CT with PBSCT achieved a 37.5% response rate, with NC in another 62.5% of patients, and that the 2-year survival rate was 75% following this chemotherapy. This result is comparable to those phase II studies.

The only randomized study investigating HD-CT as part of the first line chemotherapy for poor-risk GCT applied HD-CT with autologous bone marrow transplantation as consolidation after three cycles at standard doses was by Chevreau *et al.*[20] In this study, patients received four cycles of cisplatin, etoposide, vinblastine and bleomycin (PVeVB) at standard doses or three cycles of PVeVB followed by one cycle of high-dose cisplatin, etoposide and cyclophosphamide. This study failed to demonstrate a survival advantage for the high-dose group. The results of this trial are difficult to interpret, because the standard regimen contained double dose cisplatin, with approximately 30% of

the patients randomized to the high-dose treatment arm not completing high-dose therapy because of toxicity or early death. Another study has conducted a matched pair analysis including 456 patients in which first-line HD-CT was compared with standard-dose chemotherapy.[21] An 11% improvement in the 2-year overall survival rate was demonstrated in the HD-CT group and a multivariate analysis revealed the use of first-line HD-CT to be an independent positive predictive factor for improved survival. One recent phase III randomized controlled trial failed to show improvement of three cycles of standard-dose VIP chemotherapy followed by one cycle of HD-CT (carboplatin, etoposide and cyclophosphamide) compared with four cycles of VIP standard-dose chemotherapy for advanced GCT.[22] These findings suggest that first-line HD-CT might be more effective against poor prognosis testicular GCT than standard-dose chemotherapy. However, these data including the present study are limited, and large randomized clinical trials are necessary. There are two ongoing randomized clinical studies comparing multicycles of HD-CT with standard-dose chemotherapy (four cycles of BEP) for patients with poor prognostic GCT. HD-CT arms are two cycles of BEP followed by HD-CT (carboplatin, etoposide and cyclophosphamide), and one cycle of VIP followed by three cycles of HD-CT (VIP), respectively. These studies will clarify the role of HD-CT for GCT.

Overall toxicity was acceptable and the feasibility of this HD-CT regimen was demonstrated. As expected, all patients except one developed grade 4 neutropenia, but all of them recovered fully due to the stem cell support and G-CSF administration. Other hematological toxicity was also universal, but was quite manageable. Although platelet transfusions were required in all patients, there was no evidence of cumulative thrombocytopenia. No patient was removed from this study because of hematological adverse effects. Apart from hematological toxicity, side-effects consisted mainly of gastrointestinal events. Gastrointestinal side-effects were mostly manageable by supportive treatments such as anti-emetic therapy. Rhabdomyolysis was fatal only in one patient (4%). No septic death occurred during this study. Symptomatic acute severe ototoxicity, nephrotoxicity or peripheral neuropathy, which are common cisplatin-related toxicities, were rare.

Considering the high cure rate of GCT patients after first-line HD-CT, late toxicity is of particular interest. The previous report showed that 10% of patients suffered from late effects, mainly compensated renal failure and peripheral neuropathy.[23] In this study, no specific investigations regarding persistent late complications have yet been performed and therefore the incidence of late complications is unclear. However, no patient developed a therapy-related leukemia, which is an already-described serious late complication following high cumulative etoposide doses.

The results observed for the 24 poor prognosis GCT patients with a median follow up of nearly 4 years, demonstrate a 5-year disease-specific survival rate of 63%. Following standard-dose therapy, it has been known that relapses occurring more than 2 years after therapy are rare. This appears to be similar for patients receiving first-line HD-CT with only 12.5% of relapses occurring beyond 2 years.

The major goal of investigation for patients with poor-risk testicular GCT is identification of more effective chemotherapy. The results conducted at multiple centers in this study suggest that first-line HD-CT plus stem cell support for poor-risk testicular GCT might have a modest improved treatment outcome. In addition, this dose-intense chemotherapy is associated with relatively high but acceptable toxicity. Furthermore, there is only a minimal risk for severe late toxicity or secondary chemotherapy-induced cancer. However, it is necessary to define the optimal regimen for further studies. Moreover, the identification of prognostic factors for first-line HD-CT is needed and may be applied to select patients for a favorable treatment outcome, although HD-CT is effective for relapsed testicular GCT.[24]

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Clinical efficacy of alternative antiandrogen therapy in Japanese men with relapsed prostate cancer after first-line hormonal therapy

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Background: To confirm the effectiveness of alternative antiandrogen therapy (AAT) in Japanese patients with prostate cancer relapse after first-line hormonal therapy.

Methods: A total of 80 patients who had successive serum prostate-specific antigen (PSA) progression after first-line hormonal therapy (luteinizing hormone-releasing hormone agonist alone: 21 cases; combined antiandrogen blockade therapy: 59 cases) were enrolled. We evaluated the positive ratio of antiandrogen withdrawal syndrome (AWS), the PSA responses with second- and third-line AAT, and cause-specific survival in terms of the effectiveness of AAT.

Results: The overall positive AWS ratio after first-line therapy was 33%, while that after second-line therapy was 7%. There was no correlation between the first-line PSA response and the positive AWS. Of the 10 positive and the 20 negative AWS cases, secondary antiandrogen administration was effective in 50% and 60% of cases, respectively. The positive PSA responders at second- and third-line therapy were 51% and 13%, respectively. For second-line therapy, the effective rates from steroidal to non-steroidal, from non-steroidal to non-steroidal antiandrogen, and from non-steroidal to steroidal were 83%, 43%, and 14%, respectively. The cause-specific survival of the second-line responders was significantly better than that of the non-responders.

Conclusion: There was a substantial number of patients who found second-line AAT to be modestly effective. Flutamide was effective as an alternative antiandrogen for the patients' relapse treatment with bicalutamide in Japanese men.

Key words: alternative antiandrogen, antiandrogen withdrawal syndrome, prostate cancer, relapse.

Introduction

The incidence, as well as the mortality of prostate cancer in Japan, is still lower than those in Western countries.[1] However, prostate cancer is becoming a major public health concern in Japan because the age-adjusted incidence of this malignancy rapidly increased 6.5-fold between 1975 and 1998.[2] The age-adjusted mortality rate also increased 4.3-fold between 1980 and 2000. In addition to the increasing incidence and mortality rate, 40% of all registered Japanese prostate cancer cases ($n = 4529$) in 2000[3] were diagnosed at >75-years-old and ≈ 20% of the men were newly diagnosed with metastatic disease. Considering the high incidence and mortality specifically in elderly Japanese men and the substantial number with metastatic disease, antiandrogen therapy still plays a major role in treating prostate cancer. However, most patients with locally advanced or metastatic disease relapse after initial treatment with castration or combined androgen blockade (CAB) therapy. In 1997, Scher *et al.* reported that 38.5% of patients with progressive disease who relapsed after treatment with flutamide responded to alternative antiandrogen therapy (AAT).[4] Thereafter, Kojima *et al.* reported the clinical efficacy of AAT in 70 Japanese patients with prostate cancer.[5]

The aim of this study is to confirm the efficacy of AAT and to compare effectiveness in terms of steroidal and non-steroidal antiandrogen administration.

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Materials and methods

A total of 80 Japanese patients with histologically proven prostate cancer were enrolled from January 1999 to December 2004. The patients' age ranged from 52–86 years (mean ± SD: 71.7 ± 8.4 years). The median prostate-specific antigen (PSA) ranged from 7.7–8710 ng/mL (mean ± SD: 868 ± 1741 ng/mL). The follow-up time was 21–150 months (median: 42 months). All the patients were treated with hormonal therapy and had disease progression after first-line hormonal therapy (luteinizing hormone-releasing hormone [LH-RH] agonist alone: 21 cases, CAB therapy: 59 cases). Of the 59 cases with CAB, 53 cases received non-steroidal antiandrogen (flutamide [FLT], 375 mg daily: 22 cases; bicalutamide [BCL], 80 mg daily: 31 cases). The remaining six cases received steroidal antiandrogen (chlormadinone acetate [CMA], 100 mg daily).

We obtained institutional review board approval with the aim of retrospectively reviewing the patients' medical records. No patient had received prior therapy, including irradiation and cytotoxic therapies. The relevant patient characteristics are listed in Table 1. To analyse the responses to AAT in comparison with an equivalent group of Japanese men, we applied similar evaluative criteria to those in the report by Kojima *et al.*[5]

Definition of serum prostate-specific antigen responses at 3 months after first-line androgen deprivation therapy

1 A complete response (CR): normalization of PSA level (<4.0 ng/mL).
2 A partial response (PR): >50% decrease in the PSA level compared to the initial PSA level but >4.0 ng/mL.

Table 1 Clinical characteristics of the enrolled patients

Characteristic	N (%)
Gleason score:	
5–7	45 (56)
8–10	35 (44)
T category:	
T2	6 (8)
T3	54 (67)
T4	20 (25)
N category:	
N0	49 (61)
N1	31 (39)
M category:	
M0	30 (38)
M1	50 (62)
Clinical stage:	
C	26 (32)
D1	8 (10)
D2	46 (58)

3 Progression of disease (PD): >25% increase in the PSA level compared with the initial level.
4 No change: defined as between PR and PD.

Definition of prostate-specific antigen progression and antiandrogen withdrawal syndrome

Prostate-specific antigen progression was defined as three successive PSA level increases. After PSA progression, primary antiandrogen administration was discontinued in all the 59 cases with CAB (>4 weeks for FLT and CMA, >8 weeks for BCL), while the LH-RH analog agonist continued. The positive antiandrogen withdrawal syndrome (AWS) was defined as >50% decrease of the PSA level compared with that at the time of discontinuing the primary antiandrogen.

Prostate-specific antigen evaluation after alternative antiandrogen therapy

The PSA responders at second- and third-line therapy were classified as those who had a 50% decrease of the PSA level at the end of first- and second-line antiandrogen therapy, respectively.

Definition of response duration

Response duration was defined as the time from the start of hormonal therapy until progression.

Statistical analyses

For the statistical analysis of data, the Student's *t*-test, χ^2 test or Fisher's exact test were applied using StatView software (SAS Institute, Cary, NC, USA). The statistical significance was defined as $P < 0.05$. To compare the cause-specific survival rates, Kaplan-Meier curves were constructed.

Results

Of the enrolled 80 patients, 35 cases (43.7%) had CR of the PSA level at 3 months after first-line therapy (47.6%, 10/21) with the LH-RH agonist alone, compared with 42.3% (25/59) with CAB therapy ($P = 0.79$). Of the 21 cases with the LH-RH agonist alone and the 59 cases with CAB therapy, 95.2% (20/21) and 94.9% (56/59) reached CR or PR, respectively. Of the 76 patients with CR or PR of the PSA level, there was no significant difference in the duration of response for first-line hormonal therapy between the LH-RH agonist-alone group (22.3 ± 23.0 months) and the CAB therapy group (10.7 ± 11.9 months). In addition, there was no significant difference in the duration of the response based on steroidal or non-steroidal antiandrogen administration (CAB using BCL: 7.6 ± 7.8 months, CAB using FLT: 13.9 ± 14.7 months, CAB using CMA: 17.0 ± 17.5 months).

Relationship between first-line prostate-specific antigen response and positive antiandrogen withdrawal syndrome rate after primary antiandrogen and second alternative therapy

A total of 30 cases (51%, 30/59) could be evaluated for the AWS rate after primary antiandrogen therapy. The remaining 29 cases were excluded because of the shortage of observation for the AWS. Secondary antiandrogen (FLT or CMA) was started <8 weeks after the discontinuing of BCL in 27 (93%) of the 29 cases.

We compared the positive AWS rate after primary antiandrogen and the second alternative therapy in terms of the PSA response. Of the 30 cases that were evaluated for the AWS rate, the PSA decreased to PR or CR (>50%) in 10 cases (33%, response of duration mean ± SD: 6.7 ± 4.0 months). Of the 15 patients, one patient (7%) responded to the second-line antiandrogen withdrawal and the positive AWS was not observed in any of the three cases after the third-line hormonal therapy was discontinued. Comparing the CR cases with the PR cases, there were no significant differences in the positive AWS rate (CR: 32%, 6/19; PR: 36%, 4/11) or in the duration of the antiandrogen withdrawal response (CR: 6.6 ± 4.7 months; PR: 8.5 ± 4.4 months). The positive AWS rates in men treated with CMA, FLT, and BCL were 40% (2/5), 33% (2/6), and 32% (6/19), respectively. There were no significant differences between the AWS responses and the antiandrogens.

Of the 10 positive and the 20 negative AWS cases, secondary antiandrogen was effective in five (50%) and 12 (60%) cases, respectively. There was no significant difference between the AWS response and the effect of subsequent hormonal therapy. In our series, the AWS response could not predict the effect of subsequent hormonal therapy.

Efficacy of second-line and third-line antiandrogen therapy

To compare our series with the previous report by Kojima *et al.*[5] simultaneously, we set the two figures (Fig. 1a,b) based on the efficacy of AAT against relapsed prostate cancer. The effective rate in men who were given additional steroidal or non-steroidal antiandrogen after androgen suppression monotherapy was 71% in our series. In our series, the effective rates from CMA to non-steroidal antiandrogen (FLT or BCL) and from non-steroidal antiandrogen to CMA were 83% (5/6) and 14% (1/7), respectively. The rates from FLT to BCL and from BCL to FLT were 53% (9/17) and 38% (11/29), respectively. The change in antiandrogen from second-line to third-line is shown in Figure 1b. Of the 15 cases, the effective rate was 13% in our series,

a)

First-line		Second-Line	Effectiveness (%)	Duration of response* (mos.)
AS	→	MAB with CMA	6/10 (60%)	15.8±5.6
	↗	MAB with FLT	4/5 (80%)	10.7±6.0
	↘	MAB with BCL	5/6 (83%)	29.2±6.4
MAB with CMA	→	MAB with FLT	3/3 (100%)	12.0±7.1
	↘	MAB with BCL	2/3 (67%)	4.3±2.1
MAB with FLT	→	MAB with CMA	1/5 (20%)	4.5
	↘	MAB with BCL	9/17 (53%)	5.5±4.9
MAB with BCL	→	MAB with FLT	11/29 (38%)	5.1±5.2
		MAB with CMA	0/2 (0%)	
			41/80 (51%)	8.6±4.6

b)

Second-Line		Third-Line	Effectiveness (%)	Duration of response** (mos.)
MAB with CMA	→	MAB with FLT	0/1 (0%)	
	↘	MAB with BCL	1/3 (33%)	5.2
MAB with FLT	→	MAB with BCL	1/3 (33%)	4.8
	↘	MAB with CMA	0/6 (0%)	
MAB with BCL	→	MAB with CMA	0/1 (0%)	
	↘	MAB with FLT	0/1 (0%)	
		Total	2/15 (13%)	5.0

Fig. 1 Efficacy of alternative antiandrogen therapy in the enrolled patients. (a) Change of antiandrogen between first- and second-line therapy. *, duration of the response to second-line therapy (months). (b) Change of antiandrogen between second- and third-line therapy. **, duration of response to third-line therapy (months).

while that in Kojima *et al.* was 29.4% (5/17).[5] In our series, no responder was treated with CMA as third-line therapy (0/7).

Cause-specific survival in terms of the response to second-line therapy

Similar to the results in the previous report,[5] the survival of second-line responders in all cases (stages C, D1, and D2) was significantly better than that of the non-responders (5-year survival rates = 91.7% and 62.2%, respectively; $P = 0.002$) (Fig. 2a). In the cases with stages D1 and D2 alone, there was also a significant difference between the responders and non-responders (5-year survival rates = 80.0% and 53.0%, respectively; $P = 0.012$) (Fig. 2b). Comparing the PSA response during first-line therapy between the responders and non-responders, the proportion of responders in second-line therapy who had achieved PSA CR (PSA response at 3 months starting after first-line therapy; 18/26) was statistically higher than those without CR (12/40, $P = 0.004$).

Discussion

In the previous studies, a substantial amount of AWS responses has been reported in men with advanced prostate cancer prior to the starting of AAT.[5,6] However, the positive response rates were widely ranged based on the dosage of the primary or secondary antiandrogen administration. Kojima *et al.* speculated that a low daily dose of FLT in Japan induced a lower positive AWS response.[5] As described, all the subjects in this study were Japanese and daily doses for FLT, BCL, and CMA were very similar to those in the previous study.[5] Furthermore, in terms of the characteristics of the enrolled patients, there were no significant

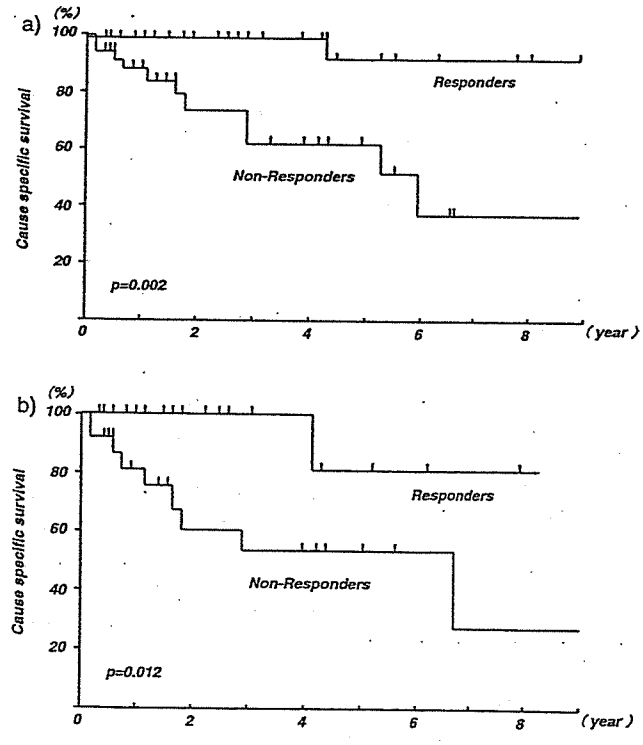

Fig. 2 Cause-specific survival in terms of the response to second-line therapy. (a) All patients whose stages were C, D1, and D2. The survival was evaluated from the time of progression of the first-line therapy. (b) Patients with stage D1 and D2 alone. The survival was evaluated from the time of progression of the first-line therapy.