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Efficacy of Combined Androgen Blockade with Zoledronic Acid Treatment in Prostate Cancer with Bone Metastasis: The ZABTON-PC (Zoledronic Acid/Androgen Blockade Trial on Prostate Cancer) Study

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Abstract. *Aim: Zoledronic acid (ZA) reduces the risk of skeletal-related events (SREs) in castration-resistant prostate cancer (CRPC) with bone metastasis and improves quality of life. It remains unclear when clinicians should initiate ZA treatment. Patients and Methods: Hormone-naïve patients were randomized to a combined androgen blockade (CAB) group or CAB with ZA group (CAB-ZA) based on Gleason score (GS) or extent of disease. The primary end-point of the study was progression-free survival (PFS) and the secondary end-point was incidence of SREs and bone pain. Results: Thirty-one and 29 patients among 60 enrolled patients were assigned to the CAB group and the CAB-ZA group, respectively. There was no significant difference in PFS between the two groups. Subgroup analyses revealed better PFS in the CAB-ZA group with GS ≥8 (p=0.021). Moreover, incidence of SREs, including bone pain, was lower in the CAB-ZA group (p=0.019). Conclusion: CAB-ZA treatment was found to improve PFS for patients with prostate cancer with high GS. CAB-ZA treatment could be recommended for treatment of patients with prostate cancer.*

The standard treatment strategy against prostate cancer (PC) with bone metastasis is androgen ablation. Currently, combined androgen blockade (CAB) using bicalutamide in combination with luteinizing hormone-releasing hormone (LH-RH) agonist as castration means is performed widely in Japan, since a significant overall survival advantage has been recognized in favor of CAB over LH-RH agonist monotherapy (1). Furthermore, an alternative anti-androgen therapy in which bicalutamide is switched to flutamide after relapse is often adopted in Japan (2, 3). Since more than 90% of PC patients with bone metastasis exhibit a response to CAB, prolonged survival is anticipated with CAB. It is, therefore, extremely important to manage bone metastasis, since it causes not only bone pain but also skeletal-related events (SREs) that worsen, often markedly, the quality of life (QOL) of the patients (4, 5).

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Saad demonstrated that zoledronic acid (ZA) is the first-generation bisphosphonate enabling for better control of bone pain and reducing the incidence of SREs predominantly associated with the osteoblastic bone lesions characteristic of PC (6). In particular, it was reported that ZA administered every three weeks prolonged the median time to the first onset of SREs by 167 days compared with a placebo in patients with castration-resistant prostate cancer (CRPC) (7). In addition, in patients with renal cell carcinoma, ZA significantly prolonged the time to the first onset of SREs and significantly reduced the total risk of onset of SREs (8). Moreover, ZA tended to prolong the overall survival in patients with CRPC, although no significant difference was observed (7). Lipton *et al.* also demonstrated that bone resorption marker normalization by ZA was correlated with survival improvement in patients with solid cancer with bone metastases (9).

However, all the prospective clinical studies conducted using ZA, thus far, targeted CRPC or prevention of bone metastases of PC (10); when should clinicians actually start ZA treatment in patients with advanced PC with bone metastasis, or which type of patients with PC can benefit from the ZA treatment in terms of progression is still controversial. Since our previous *in vitro* studies demonstrated that a third-generation bisphosphonate, minodronate, not only reduced the number of osteoclasts in the tibia of a severe combined immunodeficiency mouse inoculated with human PC cells, but also had an antitumor effect and inhibited bone invasion (11, 12), it was expected that early administration of ZA to patients with PC with bone metastases might prevent relapse of PC and improve progression-free survival (PFS). Thus, in this study, we investigated whether the ZA treatment added to CAB could improve PFS in patients with advanced PC with bone metastases and whether ZA treatment could delay the onset of SREs.

Patients and Methods

Study design. This study was under a still ongoing randomized multicenter collaborative open-labeled project of CAB alone compared with CAB plus ZA in patients with stage D2 prostate cancer and registered as a clinical trial in University hospital Medical information Network (UMIN) Center in Japan (UMIN000001137). A total of 12 domestic medical institutions, including those related to Kanazawa University Hospital, participated in this study. Patients were screened at each institution after verification of eligibility criteria, and they were randomly assigned. In this prospective study, written consent to participate was obtained from all the target patients, and all the tests and examinations were performed under the approval of the Institutional Review Board (IRB) of each medical institution. Medical records of each patient were collected and analyzed statistically at the central institution.

The enrolled patients were untreated patients with PC of stage D2 in whom PC was diagnosed pathologically by prostate gland needle biopsy in the period from July 2006 to June 2011 and the presence of bone metastasis was confirmed by bone scintigraphy. Those patients who consulted the Department of Dentistry or Oral Surgery and received or were to receive an invasive dental treatment such as tooth extraction or implant within six months before participating in this study were excluded. Before participating in this study, it was confirmed that each patient maintained sufficient functions of the liver, kidney and bone marrow and had a favorable Eastern Cooperative Oncology Group (ECOG) performance status (PS) in systemic evaluations, including hematological examinations. As the first hormonal therapy, CAB was adopted, and 80 mg of bicalutamide were orally administered once a day in addition to administration of an LH-RH agonist as internal castration. The enrolled patients were randomly assigned to two groups: one group of CAB alone in which patients were treated with LH-RH agonist and bicalutamide; and one group of CAB-ZA in which patients were treated with ZA in combination with CAB, based on the baseline Gleason score (less than 7 or not less than 7), or extent of disease (EOD) score (less than 2 or not less than 2) (13). In the CAB-ZA group, 4 mg of ZA were administered by intravenous infusion within one month after starting the CAB therapy and thereafter the intravenous infusion was repeated every four weeks. In this study, 31 patients were finally registered in the group of CAB-alone and 29 in the group of CAB-ZA.

Clinical and pathological evaluations. The clinical stage was assessed by digital rectal examination (DRE), transrectal ultrasonography (TRUS), computed tomography (CT) and bone scintigraphy. The expanse of bone lesion was evaluated with the findings in bone scintigraphy according to the EOD classification. It is desirable that a central pathologist performs the histopathological evaluation by prostate gland needle biopsy (Gleason score) at a single medical institution, since the Gleason score is one of the most important factors for the evaluation of the therapeutic effect and prediction of prognosis, and the evaluation results may differ from pathologist to pathologist. In this study, however, the Gleason score was determined at each medical institution by a pathologist specialized in urology.

Definition of progression. When the prostate-specific antigen (PSA) level was elevated, the presence/absence of anti-androgen withdrawal effect (AWE) was first confirmed. The change of the anti-androgen therapy (including the change of the drug type) to 375 mg/day flutamide was decided upon discretion of the physician in charge at each medical institution. Progression of the disease was defined as a case where the elevation of PSA level was confirmed at three consecutive time points (in three consecutive months). However, the elevation of PSA level after a transient decrease of PSA level in the following cases was not to be judged as progression: (i) cases where bicalutamide was withdrawn and AWE was confirmed with a decrease of the PSA level, and (ii) cases where bicalutamide was switched to 375 mg/day flutamide and the PSA level decreased once. In other words, the non-progression period was defined as the period from the day of treatment start to the time point of initiation of elevation of PSA level, before the treatment with estramustine phosphate or docetaxel.

End-points. In this study, the primary end-point was PSA progression-free survival (PFS), and the secondary end-points were incidence of SREs and bone pain and causal relationship between the ZA treatment and the change of bone turnover markers. In

Table I. *Baseline patient characteristics.*

Characteristic	CAB alone (n=31)	(%)	CAB-ZA (n=29)	(%)	*p*-Value
Observation (months)					
Average	27.4		32.1		
Age (years)					
Median	71.8		71.7		0.99
Range	50.2-83.1		46.7-86.4		
PS					
0	19	(61)	19	(66)	
1	10	(32)	8	(28)	0.77
2	2	(6)	2	(7)	
EOD score					
1	14	(45)	13	(45)	
2	8	(26)	5	(17)	0.75
3	7	(23)	9	(31)	
4	2	(6)	2	(7)	
Initial PSA (ng/ml)					
Median	366.9		399		0.59
Range	46.4-4526.8		34.2-7410.0		
Gleason sum					
7	7	(23)	3	(10)	0.37
8	9	(29)	10	(35)	
9	13	(42)	15	(52)	
10	2	(6)	1	(3)	

Performance status (PS), extent of disease (EOD), and prostate-specific antigen (PSA).

general, SREs are often assessed with four items as follows: (i) pathological fracture, (ii) spinal cord compression, (iii) radiotherapy to the bone lesion, and (iv) surgical operation of bone lesions, or with five items including additionally (v) hypercalcemia. Moreover, the appearance of bone pain was also included in the assessment in this study.

Statistical analyses. The differences in variables related to patient background and bone turnover markers were evaluated using the Mann-Whitney *U*-test. The rate of PFS was estimated by the Kaplan-Meier method. Two-sided *p*-values were calculated in all tests, and the differences were evaluated by log-rank test. In this study, differences of $p \leq 0.05$ were considered significant. For statistical analyses, the GraphPad Prism® version 5.0 (GraphPad Software Inc., San Diego, CA) was used.

Results

Table I shows the patients' background. The mean age was 71.8 years in the CAB-alone group and 71.7 years in the CAB-ZA group. The mean observation period was 27.4 months in the CAB-alone group and 32.1 months in the CAB-ZA group. There were no statistically significant differences between the two groups in age, PS, EOD score at the start of study, PSA level at biopsy, and Gleason score obtained from the biopsy sample. Out of the 60 included patients, 31 were randomly assigned to the CAB-alone arm, and 29 to the CAB-ZA arm (Table I). In patients, disease was controlled with CAB alone, while in the CAB-ZA group, disease in 18 patients was controlled. In total, 14 patients had died from PC during the observation period, eight in the CAB-alone group and 6 in the CAB-ZA group, respectively (Figure 1). Figure 2A shows the total rate of PFS in each group. In the observation period up to the present time point, no significant difference was recognized between the two groups. However, a tendency for better PFS of the CAB-ZA group than that of the CAB-alone group was found. Figure 2B shows the results of subgroup analysis in the patients whose baseline EOD score was at least 2. No statistically significant difference was seen again between the two groups ($p=0.158$), but the time to 50% PFS was prolonged in the CAB-ZA group. Furthermore, Figure 1C shows the results of subgroup analysis in the patients whose baseline Gleason score was at least 8. The time to 50% PFS was significantly prolonged in the CAB-ZA group ($p=0.021$), and the prolonged time reached 11 months compared with the CAB-alone group.

Next, the SREs and bone pain were also evaluated (Figure 3). In the observation period up to the present time point, 11 patients experienced bone pain in the CAB-alone group and seven in the CAB-ZA group. The time to the first appearance of bone pain was 11.7 months in the CAB-alone group and 17.2 months in the CAB-ZA group, suggesting that co-administration of ZA might be able to delay the appearance of

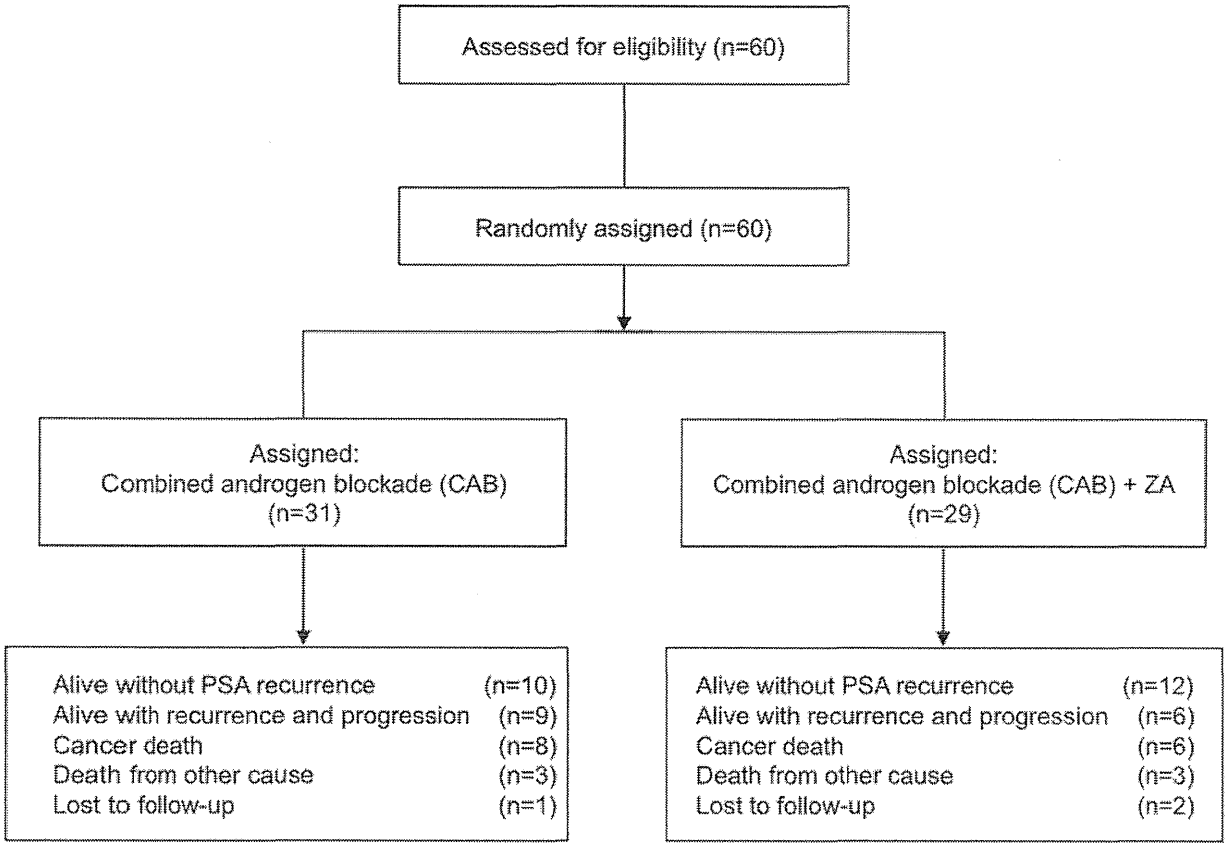


Figure 1. *Study design and patient allocation. Prostate-specific antigen (PSA), Zoledronic acid (ZA).*

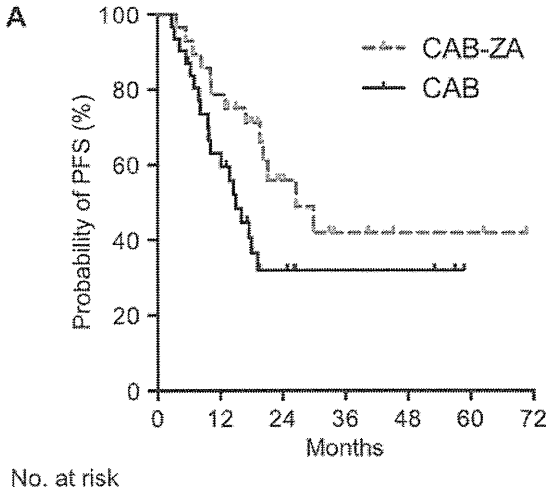
bone pain (Figure 3A). In the CAB-ZA group, the patients experiencing bone pain were generally well-controlled with oral Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) or opioids. In the CAB-alone group, pathological fractures and spinal cord compression were observed in three patients. The affected patients received external-beam radiation therapy (EBRT) or surgical treatment. Figure 3B shows the incidence of SREs. Statistically significant differences were recognized between the two groups, and it was shown that the occurrence of SREs, including bone pain was delayed by continued co-administration of ZA.

During the observation period, there were no serious adverse events, such as osteonecrosis of the jaw and serum hypocalcium. But for two patients in the CAB group, ZA was additionally used for reduction of bone mineral density after long-term hormonal treatment.

In terms of the baseline level of the bone resorption marker, C-terminal crosslinking telopeptide of type I collagen (1-CTP), and the bone formation marker, bone alkaline phosphatase (BAP), the patients of each group were divided into two groups, for subgroup analysis of PFS between the CAB-alone group and the CAB-ZA group. The subgroup analysis was performed only with these baseline values (Kaplan–Meier plots not shown). There were no significant differences in subgroup analysis. However, it was shown that early coadministration of ZA would be more useful in patients with more advanced disease or higher risk at the start of CAB treatment, such as patients with a higher PSA level, a higher EOD score, a higher Gleason score (not less than 8) or a higher value of bone turnover marker (Figure 4).

Discussion

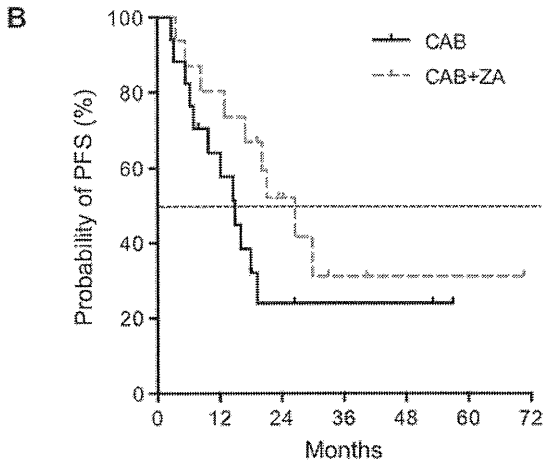
ZA is said to produce a significant delay in the occurrence of SREs and to improve bone pain in patients with CRPC with bone metastasis, and it has been also reported that co-administration of docetaxel and ZA could be promising against CRPC with bone metastasis (14). However, previous studies on when clinicians should start the treatment with ZA or which type of patients should be administered ZA earlier



	p-Value (log-rank)	HR (95% CI)
CAB alone		1.907
CAB-ZA	0.073	(0.941 – 3.865)

No. at risk

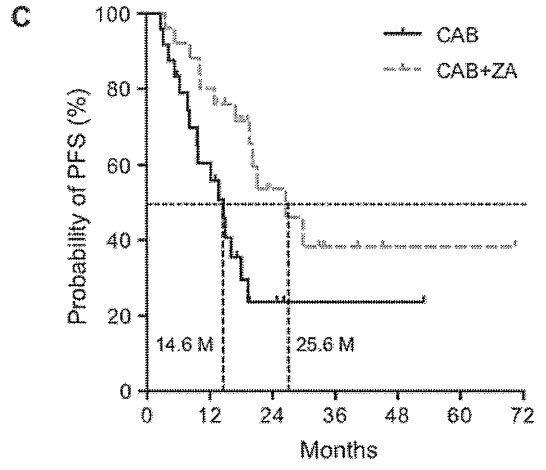
CAB alone	31	19	7	3	3	
CAB + ZA	29	23	10	4	2	2



No. at risk

CAB alone	17	10	4	2	2
CAB + ZA	16	13	7	2	1

	N	*p*-Value (log-rank)	HR (95% CI)
CAB alone	17		0.564
CAB-ZA	16	0.158	(0.143-0.98)



No. at risk

CAB alone	24	14	4	1
CAB + ZA	26	21	8	3

	N	*p*-Value (log-rank)	HR (95% CI)
CAB alone	24		0.548
CAB-ZA	26	0.021	(0.076 – 1.022)

Figure 2. *Kaplan–Meier plots of rate of progression-free survival. (A) All the patients. (B) Subgroup analysis of the patients whose baseline EOD score was ≥2. (C) Subgroup analysis of the patients whose baseline Gleason score was ≥8. Combined androgen blockade (CAB), prostatic specific antigen (PSA), zoledronic acid (ZA).*

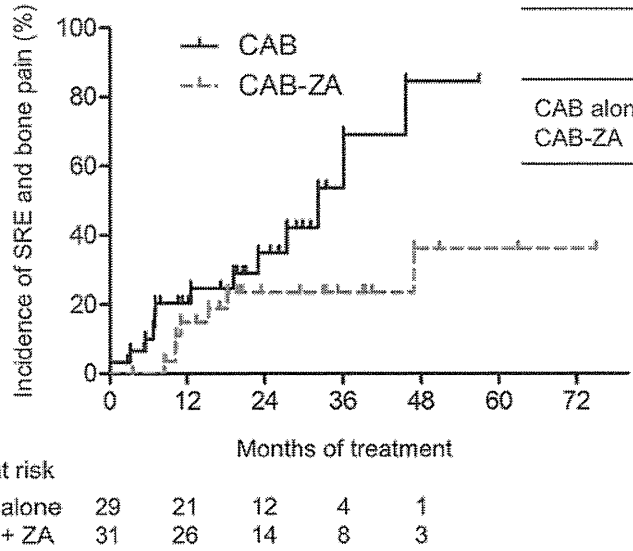
were limited to retrospective analyses, involving comparisons with the historical control, and no prospective studies have been conducted so far (15, 16). The results of the present study may help to resolve the issue of when clinicians should start the treatment with ZA in patients with advanced PC with bone metastasis.

In the present study, all the patients administered ZA from the beginning of CAB therapy exhibited prolongation of PFS,

A

	CAB alone (n=31)	CAB + ZA (n=29)				CAB alone	CAB +ZA
Event			Duration (average, month)		Treatment		
Bone pain	11	7	11.7	17.2	Medicine	10	6
					EBRT	1	3
					Strontium	2	
					Zoledronic acid	4	
Pathologic fracture	1		6.8		EBRT	2	
Spinal cord compression	2		9.2		Bone surgery + EBRT	1	

B



		p-Value (log-rank test)	HR (95% CI)
CAB alone	31	0.019	0.3812
CAB-ZA	29		(0.154 – 0.943)

No. at risk

CAB alone	29	21	12	4	1
CAB + ZA	31	26	14	8	3

Figure 3. *Summary of SREs and bone pain (A) and Kaplan–Meier plots of incidence of SREs and bone pain (B). Prostatic specific antigen (PSA), Zoledronic acid (ZA), external-beam radiation therapy (EBRT).*

although no significant difference was recognized. However, PFS was significantly improved in the patients with high malignancy (baseline Gleason score of ≥8). This fact suggests that ZA might not only have an apoptosis-inducing effect on osteoclasts but an antitumor effect as well (12, 17, 18). In addition, clinically, we reported that the bisphosphonate, suppressed serum PSA level in patients with CRPC with bone metastasis and that ZA improved not only bone metastasis but also lung metastasis and liver metastasis of kidney cancer (19, 20). Furthermore, in patients with breast cancer without bone metastasis, an antitumor effect of ZA was reported (21, 22).

The reason why no significant differences in PFS of the patients overall were not significant but the differences in PFS of the patients with GS ≥8 between two groups may be that CAB monotherapy can control the cancer to a certain extent in patients with tumor of low malignancy but additional ZA treatment is necessary in those with highly malignant tumors. As a result, co-administration of ZA might improve the PFS of patients with GS ≥8.

One of the most prominent effects seen upon ZA treatment in patients with cancer is improvement of bone pain. It was reported that ZA also improves bone pain in

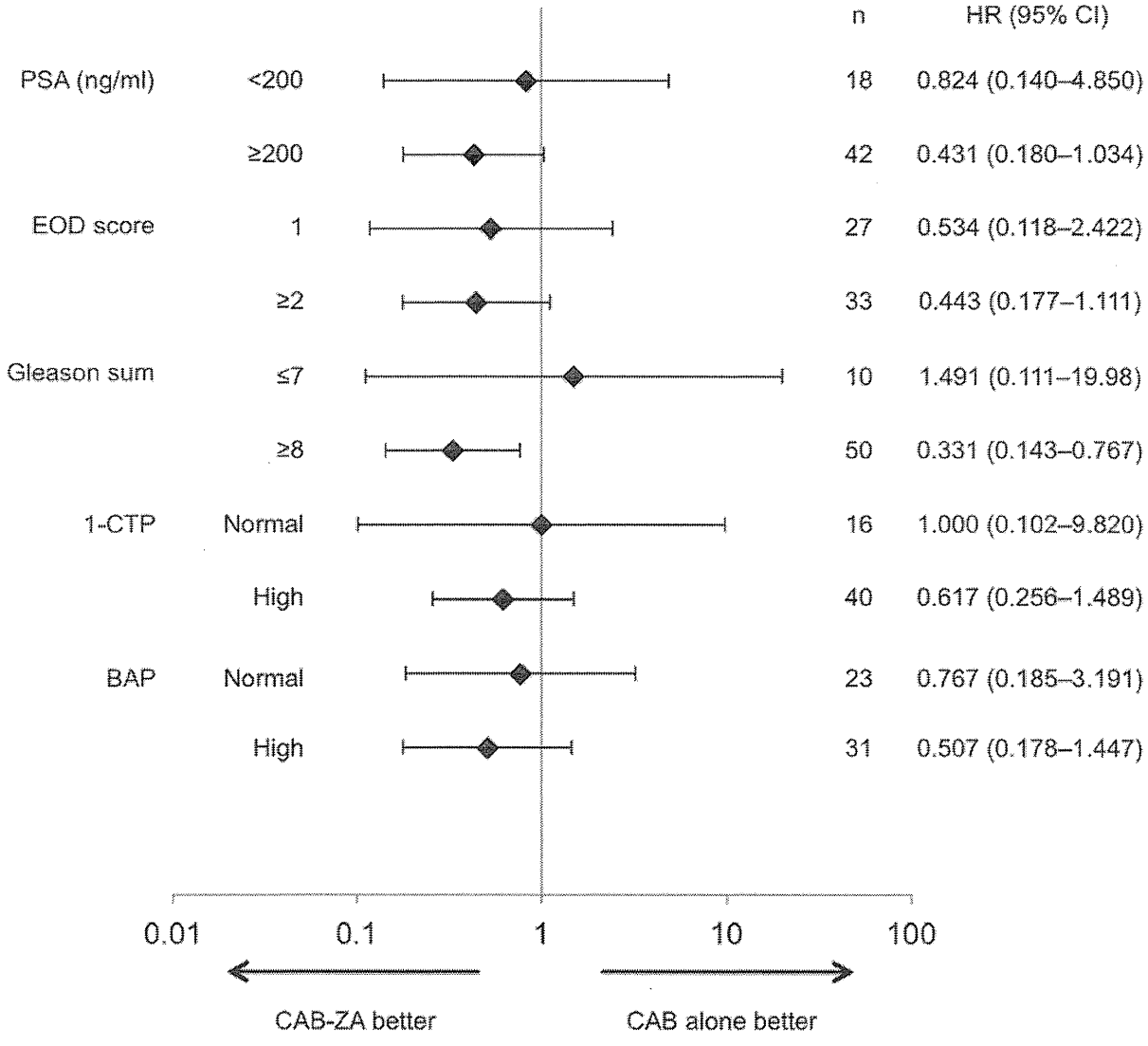


Figure 4. *Subgroup analyses of hazard ratio for PFS regarding PSA, EOD score, GS, 1-CTP, and BAP.*

patients with PC, breast cancer, and multiple myeloma with bone metastasis (23). Continuous co-administration of ZA from an early stage of treatment delayed the occurrence of bone pain and generally prevented severe SREs, such as pathological fractures and spinal cord compression in this study. Furthermore, statistically significant differences were recognized in the incidence of SREs, including bone pain. This study also showed that early co-administration of ZA would be more beneficial in patients with more advanced disease or a higher risk at the start of CAB treatment, such as patients with a higher PSA level, a higher EOD score, a higher Gleason score or a higher value of bone turnover marker, in terms of the SRE-free rate.

In conclusion, since ZA had not only a preventive effect on the occurrence of SREs but also a progression-delaying effect, probably due to a direct antitumor effect, in the treatment of PC with bone metastasis, the use of ZA from the beginning of hormonal therapy is recommended, even in the absence of bone pain, at least in patients with a high Gleason score. However, careful observation of the patient's progress is essential, since the long-term use of ZA may increase the incidence of adverse effects, such as osteonecrosis of the jaw, renal dysfunction, and hypocalcemia.

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ORIGINAL ARTICLE

Combination of hemoglobin, alkaline phosphatase, and age predicts optimal docetaxel regimen for patients with castration-resistant prostate cancer

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Abstract
Background We aimed to find the prognostic factors predicting overall survival (OS) in patients with castration-resistant prostate cancer (CRPC) who had docetaxel (DTX) chemotherapy, and to construct a model predicting the optimum number of cycles of DTX.
Methods A total of 279 CRPC patients who received DTX ($\geq$50 mg/m^2) every 3–4 weeks were studied retrospectively. Prognostic factors predicting treatment cycles as well as OS were analyzed, and a risk table for predicting treatment cycles was constructed.
Results The longer treatment group (>10 cycles) had a significantly longer OS than the standard treatment group ($p < 0.0001$). Multivariate analysis demonstrated that a decrease of $\geq$50 % in prostate-specific antigen (PSA), serum markers at the start of DTX therapy [PSA, alkaline phosphatase (ALP), and C-reactive protein (CRP)], and the number of DTX courses were independent predictors of OS. The risk table employing the combination of three factors [ALP (cut-off 189 IU/L), hemoglobin (11.3 g/dL), and age (65 years) at the start of DTX therapy], and scoring based on the hazard ratio of each risk factor (ALP 4, hemoglobin 2, age 3) could effectively predict the probability of the length of DTX therapy, with lower score (0–6) predicting >10 cycles, and higher score (7–9) predicting $\leq$5 cycles ($p < 0.0001$). No significant difference was found regarding grade 3/4 adverse events between the two groups.
Conclusion A model using three factors prior to chemotherapy may be beneficial for deciding the duration of DTX therapy in patients with CRPC.

Keywords CRPC · Docetaxel · Risk model · ALP · Hemoglobin · Age

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Introduction

Prostate cancer is the most commonly diagnosed malignancy in men. Both the prevalence and mortality of this disease have shown a marked increase in Japan over recent years, with an estimated threefold increase of its incidence by 2020 compared with 2000 and a 23 % increase in death from 2000 to 2005 [1]. Accumulating evidence has suggested that the docetaxel (DTX: 75 mg/m^2 over 3 weeks) plus prednisolone regimen has become the standard therapy for patients with castration-resistant prostate cancer (CRPC), with a median time to disease progression and median overall survival (OS) of approximately 6 and 19 months, respectively [2–4]. Although new androgen receptor (AR)-targeting agents and anticancer agents have been approved for CRPC patients [5, 6], all such treatments are coordinated with either pre-DTX or post-DTX therapy. At least four cycles of DTX therapy were recommended to evaluate the response, and the number of cycles is likely to have an impact on survival [7]. Since the number of cycles has been limited to 10 based on the protocol [2, 3], the optimum number of cycles of the regimen remains unclear. Several authors have argued the benefits and safety of longer DTX therapy, but the effect is controversial [8–11]. A model for predicting OS based on several factors (visceral disease, bone scan progression, and baseline anemia) was proposed for patients enrolled in the clinical trial [12], and a few risk models have reportedly been well adapted for patients receiving 10 or more cycles of DTX therapy [9, 11]. To our knowledge, however, no investigation has focused on the adequate number of treatment cycles of the DTX regimen. Accordingly, this study was performed to clarify prognostic factors predicting OS in patients with CRPC who had DTX chemotherapy, and to construct a model for predicting the optimal number of treatment cycles of the DTX regimen.

Patients and methods

Patients

A total of 279 CRPC patients who received DTX ($\geq$ 50 mg/m^2) every 3–4 weeks with prednisolone (10 mg) were retrospectively enrolled in this study (Table 1). In principle, the standard DTX dose was set to 70–75 mg/m^2. The dose, however, was modified in each institution based on the individual patient conditions. DTX dose grouping ($\geq$ 50 mg/m^2) in this study was based on preliminary data showing significantly longer OS than the low dose group ($<$ 50 mg/m^2) [13]. Data from the medical records were analyzed with consent. Inclusion criteria for this study were

Table 1 Patient backgrounds in the study

Factors[a]	Variables
Age (years), median (range)	71 (48–91)
≤65 years, no. (%)	65 (23.4)
>65 years, no. (%)	213 (76.6)
ECOG PS, no.	
0	200
1	53
≥2	13
Gleason score, no.	
≤6	13
7	54
≥8	186
Clinical stage, no. (%)	
II	0
III	60 (21.5)
IV	219 (78.5)
Laboratory data[b], median (range)	
PSA (ng/mL)	35.2 (0.05–3134)
CRP (mg/dL)	0.3 (0–22)
Corrected Ca (mg/dL)	8.7 (6.9–10.2)
LDH (IU/L)	192 (92–1160)
<215	143 (61.1)
>215	91 (38.9)
ALP (IU/L)	271 (102–6130)
<189	50 (20.1)
>189	198 (79.8)
Hemoglobin (g/dL)	11.8 (6–15.8)
≤11.2	87 (34.7)
>11.3	164 (65.3)
Metastases, no. (%)	
Bone	169 (60.5)
Lymph node	89 (31.9)
Visceral organs	22 (7.9)
Lung	12
Other organs	10
Other treatment, no. (%)	
Prior therapies	279 (100)
ADT	279 (100)
Steroid	137 (49.1)
EMP	109 (39.1)
Estrogen	58 (20.8)
Radiation	54 (19.4)
Zol	29 (10.4)
Chemo	11 (3.9)
RP	11 (3.9)
Concurrent treatment	10 (3.6)
Zol	6
EMP	2
Estrogen	2
FT	2

Table 1 continued

Factors[a]	Variables
Docetaxel treatment, median (range)	
Initial dose (mg/m^2)	70 (50–85)
Interval (weeks)	4 (3–8)
Total dose (mg/m^2)	525 (60–3720)
Relative dose intensity	0.8 (0.4–1.1)
DTX course	8 (1–62)
≤10, no. (%)	178 (63.7)
>10, no. (%)	101 (36.2)

ADT androgen deprivation therapy, *Steroid* dexamethasone or prednisolone, *EMP* estramustine, *Zol* zoledronic acid, *Chemo* chemotherapy other than EMP or DTX, *RP* radical prostatectomy, *FT* futraful (5-fluorouracil prodrug)

[a] Values are at the initiation of docetaxel treatment, except Gleason score

[b] Missing data: age 1, ECOG PS 13, Gleason score 26, PSA 15, LDH 45, ALP 31, hemoglobin 28

(1) histologically confirmed prostate cancer, (2) failure of combined androgen blockade (CAB) therapy followed by confirmation of androgen withdrawal syndrome, and (3) simultaneous LHRH agonist treatment with DTX therapy.

Methods

All the treating urologists continued the regimen indefinitely until disease progression or adverse events (AE) (grade 3/4 toxicity) or patient choice. Demographic factors were compared between 178 patients who received ≤10 cycles of DTX (standard treatment group) and 101 patients who received >10 cycles (longer treatment group). When laboratory data were analyzed, variables were dichotomized by the cut-off value determined from the area under the receiver operating characteristics (ROC) curve for predicting a longer treatment (>10 cycles) group. Response to treatment was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) for measurable lesions or from the decrease in prostate-specific antigen (PSA) after the initiation of DTX therapy. A Cox proportional hazards model was applied to identify prognostic factors for OS. A table that predicted the probability of longer treatment was constructed by using the significant prognostic factors for longer treatment group, and a risk score was also developed based on the hazard ratio of each prognostic factor. All patients were followed by performing biochemistry tests, complete blood counts, and PSA measurement immediately before each treatment cycle, while radiographic examinations including computed tomography were done every 2–3 months. PSA response was defined as a decrease of ≥50 % from the pretreatment concentration to the second measurement of PSA after at least 4 weeks, with no clinical or radiographic evidence of disease progression during this period. Disease progression was defined as an increase in serum PSA concentration of ≥25 % from the lowest value, or progressive disease (PD) based on the RESICT criteria of measurable lesions or detection of new metastases on radiographic examination.

Adverse events, including hematopoietic, cardiovascular, pulmonary, and gastrointestinal events, bone pain, and infections, were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

Statistical analysis

Primary analysis was performed to identify factors showing a significant association with a longer duration of DTX therapy and to construct a model for predicting longer DTX therapy. The secondary analysis involved assessment of the safety and feasibility of treatment with more than 10 cycles of DTX therapy. Kaplan–Meier analysis with the log-rank test was applied to compare the time until death or final follow-up. Multivariate logistic regression analysis with stepwise selection of variables was performed to devise the risk score and to construct a table for predicting longer DTX therapy. Predictive accuracy was quantified using the area under the curve (AUC) values determined by ROC analysis. All statistical analyses were performed with JMP software (ver. 9), and $p < 0.05$ was regarded as significant.

Table 2 Response to treatment and prognosis

	All patients	≤10 cycles	>10 cycles	*p* value
Best response, no. (%)				
CR	4 (2.3)	1 (1)	3 (4.2)	0.3787
PR	25 (14.6)	13 (13.1)	12 (16.7)	
SD	61 (35.7)	39 (39.4)	22 (30.6)	
PD	81 (47.4)	46 (46.5)	35 (48.6)	
Total	171			
PSA response[a], no. (%)				
≥50 %	157 (57.5)	92 (52.3)	65 (67.1)	0.0214
Patient prognosis, median months (95 % CI)				
Overall survival				
From DTX	26 (20.7–32)	18 (14–22)	40 (32–49.3)	<0.0001
From ADT	94 (81–101)	85 (70.8–96)	102 (85–158)	0.0113
Time to progression	11 (8–16)	6 (5–9)	28 (16–32)	<0.0001
Time to CRPC	39 (30–57)	39 (30–57)	34 (24–99)	0.6483
Follow up, months (range)	94 (81–101)			
Death, no. (%)	124 (44.6)			
Disease-specific	115			
Other causes	9			

[a] Missing data: 6

CR complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease

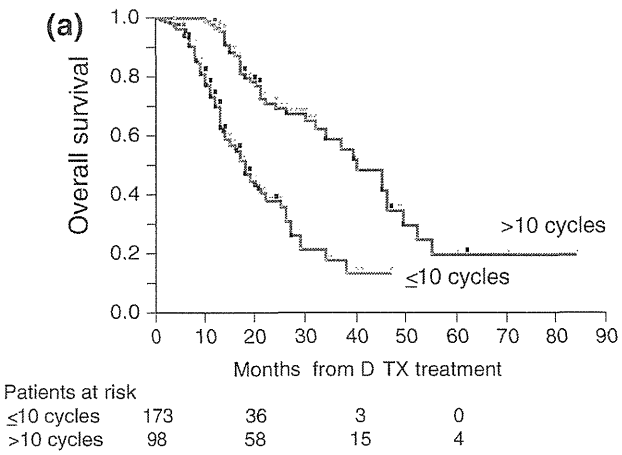
Results

Overview of the study

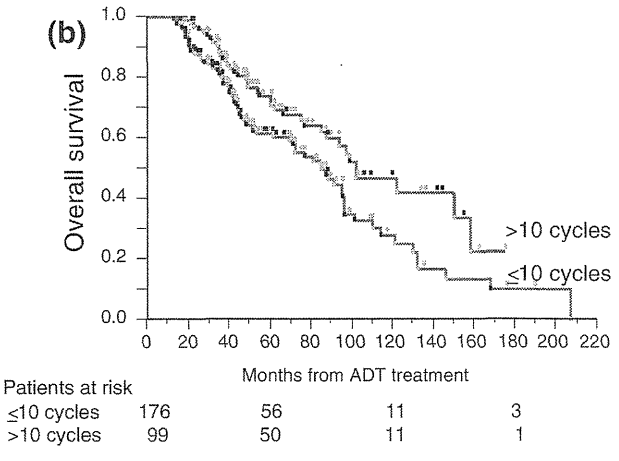
The patient demographic profile is shown in Table 1. The median age at the initiation of DTX therapy was 71 years and the median number of courses was 8, ranging from 1 to 62 [lower quartile 5 (87 patients), interquartile 6–12 (120), upper quartile 13 (72)]. Reasons for the discontinuation of the DTX therapy were PD including cancer death, death from other causes, AE, and patient refusal in 86.8, 9.6, 2.4, and 1.2 %, respectively (data not shown). The median serum PSA level at the start of DTX therapy was 35.2 ng/mL. The median initial DTX dose, course interval, and relative dose intensity for patients were 70 mg/m^2 (range 50–85), 4 weeks (range 3–8), and 0.8, respectively (Table 1). Median time from CRPC to initiation of DTX was 8 months, ranging from 0 to 142. Treatment response to DTX therapy and the outcome are shown in Table 2. The objective response rate for evaluable lesions was 16.9 %, including a complete response in 4 patients (2.3 %) and a partial response in 25 patients (14.6 %). There was a significant difference in the PSA response in favor of the longer treatment group ($p = 0.0214$), but there was no significant difference in the response rate ($p = 0.3787$) between the two groups. Kaplan–Meier analysis demonstrated significantly longer OS in patients in the longer treatment group than in the standard group from the start of DTX treatment ($p < 0.0001$, Fig. 1a), as well as from the initiation of androgen deprivation therapy (ADT) ($p = 0.0113$, Fig. 1b).

Factors predicting OS and prolonged DTX therapy

The results of univariate and multivariate analyses of the predictors of OS from the start of DTX therapy are shown in Table 3. PSA (cut-off 35 ng/mL, $p = 0.0245$), alkaline phosphatase (ALP) (cut-off 189 IU/L, $p = 0.0406$), C-reactive protein (CRP) (cut-off 0.32 mg/dL, $p = 0.0268$), PSA decline by ≥50 % ($p = 0.0165$), and the number of courses of DTX therapy [$p < 0.0001$, hazard ratio (HR) 0.91, 95 % confidence interval (CI) 0.87–0.95] were independent predictors of OS in multivariate-1 analysis. Multivariate-2 analysis demonstrated that the HR of DTX/cycle was 0.91, meaning a 9 % risk reduction per cycle. Table 4 shows the result of predictive factors predicting more than 10 courses of DTX chemotherapy. ALP [< 189 vs. ≥ 189 IU/L, HR 0.26 (inverse number 3.84), $p = 0.0003$], age [≤65 vs. >65 years, HR 0.34 (2.93), $p = 0.0028$], and Hb (<11.3 vs. ≥11.3 g/dL, HR 1.96, $p = 0.0455$) were significant factors for predicting longer treatment group. Subdivided DTX dose (50–69 vs. >70 mg/m^2), course interval, or relative dose intensity was not related to longer treatment group (data not shown). Based on the hazard ratio, an ALP > 189 IU/L, age > 65 years and Hb < 11.3 g/dL were assigned a score



No. Cycles	No. Pts.	Median OS(M)	95% CI	P vale
≤10	173	18	14-22	<0.0001
>10	98	40	32-49.3	
All	271	26	20.7-32	



No. Cycles	No. Pts.	Median OS(M)	95% CI	P vale
≤10	176	85	70.8-96	0.0113
>10	99	102	85-158	
All	275	94	81-101	

Fig. 1 Kaplan–Meier plot of overall survival from the start of docetaxel treatment (**a**) and from the start of androgen-deprivation therapy (**b**) for the standard (≤10 cycles) and longer treatment (>10 cycles) groups. Median OS calculated from the initiation of DTX (**a**) and from the initiation of ADT (**b**) was significantly shorter in the standard treatment group than in the longer treatment group (**a** $p < 0.0001$, 18 vs. 40 months, **b** $p = 0.0113$, 85 vs. 102 months)

Table 3 Univariate and multivariate Cox hazard model predicting overall survival from docetaxel treatment

Variables	Univariate			Multivariate-1[b]			Multivariate-2[b]		
	HR	95 % CI	*p* value	HR	95 % CI	*p* value	HR	95 % CI	*p* value
Age[a]									
≤65 vs. >65 (years)	1.08	0.72–1.65	0.7124						
PSA[a]									
Continuous	4.29	0.99–12.7	0.055						
≤35 vs. >35 (ng/mL)	2.33	1.60–3.44	<0.0001	2.07	1.10–4.07	0.0245	1.89	1.02–3.67	0.044
Gleason score									
≤7 vs. ≥8	1.09	0.73–1.70	0.6697						
Visceral metastasis[a]									
Yes vs. no	1.81	0.91–3.22	0.0849						
Hb[a]									
Continuous	0.83	0.72–0.96	0.0114						
<11.3 vs. ≥11.3 (g/dL)	0.68	0.47–0.99	0.0498	0.69	0.41–1.18	0.17	0.67	0.39–1.15	0.1466
PSA ≥50 % decline									
Yes vs. no	1.53	1.06–2.21	0.0249	1.91	1.13–3.26	0.0165	1.71	1.01–2.92	0.0478
ALP[a]									
Continuous	1	1.00–1.00	<0.0001						
<189 vs. ≥189 (IU/L)	2.79	1.66–5.04	<0.0001	2.64	1.04–7.71	0.0406	2.95	1.15–8.85	0.0231
LDH[a]									
Continuous	1	1.00–1.00	<0.0001						
<215 vs. ≥215 (IU/L)	2.09	1.41–3.09	0.0003	1.25	0.68–2.27	0.468	1.15	0.65–2.02	0.6359
CRP									
Continuous	7.26	1.61–22.76	0.0131						
<0.32 vs. ≥0.32 (mg/dL)	2.44	1.52–4.01	0.0002	1.94	1.08–3.55	0.0268	2.38	1.30–4.46	0.0045
DTX course									
Continuous	0.94	0.92–0.96	<0.0001				0.91	0.87–0.95	<0.0001
<10 vs. ≥10 (cycles)	0.31	0.20–0.46	<0.0001	0.24	0.12–0.46	<0.0001			

[a] At the time of starting DTX

[b] -1: DTX course as categorical variable, -2: DTX course as continuous variable

of 4, 3, and 2, respectively. The AUC obtained by ROC analysis for predicting patients with more than 10 cycles of DTX treatment was 0.7274 ($p < 0.0001$) when the total risk score was applied. The probability of longer treatment stratified by each combination of the three parameters and the total score of each combination are shown in Table 5. The best combination (ALP < 189 IU/L, Hb ≥ 11.3 g/dL, and age ≤ 65 years) predicted longer treatment for 4 of the 5 patients (80 %) in this category with a total score of 0, while the worst combination (ALP ≥ 189 IU/L, Hb < 11.3 g/dL, and age > 65 years) predicted longer treatment in only 7 of the 58 patients (12.1 %) with a total score of 9. When the number of DTX courses was divided into a lower quartile (≤ 5 cycles), interquartile (6–12), and upper quartile (≥ 13), the total score was significantly different between these groups (Fig. 2). A lower total score (0–6) was significantly associated with longer DTX therapy, showing a sensitivity, specificity, and overall accuracy of 61.6, 75, and 70.3 %, respectively (Table 5b, $p < 0.0001$). In contrast, a higher total score (7–9) was significantly associated with the lower quartile group, having a sensitivity, specificity, and positive predictive value of 83.1, 48.5, and 45.1 %, respectively ($p < 0.0001$).

Feasibility of longer DTX therapy

Concerning AEs, 114 of the 178 patients (64 %) from the standard treatment group and 59 of the 101 patients (58.4 %) from the longer treatment group had any type of AE, with no significant difference between the two groups (Table 6, $p = 0.371$). Hematological events were the most frequent type of AE in this study, occurring in 57.4 % of the longer treatment group and 60.7 % of the standard treatment group, respectively ($p = 0.6136$). DTX therapy was withdrawn in three patients due to AEs (infection, interstitial pneumonia, and patient refusal due to unknown

Table 4 Univariate and multivariate regression analysis predicting longer (>10) DTX courses

Variables	Univariate			Multivariate		
	HR	95 % CI	*p* value	HR	95 % CI	*p* value
Age[a]						
≤65 vs. >65 (years)	0.35	0.20–0.62	0.0003	0.34	0.17–0.69	0.0028
PSA[a]						
≤35 vs. >35 (ng/mL)	0.94	0.57–1.55	0.798			
Gleason score						
≤7 vs. ≥8	0.85	0.48–1.52	0.5737			
Visceral metastasis[a]						
Yes vs. no	0.48	0.15–1.27	0.1446			
Hb[a]						
<11.3 vs. ≥11.3 (g/dL)	2.17	1.22–3.95	0.0073	1.96	1.01–3.89	0.0455
CRP						
<0.32 vs. ≥0.32 (mg/dL)	0.52	0.27–0.99	0.0475	0.83	0.35–1.90	0.6629
PSA ≥50 % decline						
Yes vs. no	1.85	1.11–3.13	0.0176	1.75	0.94–3.30	0.0769
ALP[a]						
<189 vs. ≥189 (IU/L)	0.24	0.12–0.46	<0.0001	0.26	0.12–0.54	0.0003
LDH[a]						
<215 vs. ≥215 (IU/L)	0.43	0.24–0.76	0.0035	0.62	0.31–1.18	0.1456

[a] At the time of starting DTX, except Gleason score

AE each in one). Most of the AEs were tolerable and manageable, and no significant differences were found between the two groups, including other toxicities.

Discussion

Despite establishment of the DTX regimen, several issues remain to be resolved. Does the regimen actually prolong OS from the initiation of ADT? Does extended DTX therapy (>10 cycles) prolong OS without compromising quality of life? To address the former query, we showed that DTX therapy produced significant improvement of OS from the initiation of ADT, which excluded the possibility of lead-time bias in relation to starting DTX therapy in the prolonged treatment group [13]. Concerning the influence of additional courses (>10 cycles) on OS, Pond et al. [8] compared two clinical trials (up to 10 cycles vs. a median of 15 cycles) and found that the 6- and 12-month estimated survival after cycle number 10 were similar. They concluded that there was no survival benefit conferred by >10 cycles of DTX therapy. The lack of survival benefit by prolonged DTX therapy may be attributable to the small cohort (37.4 %) receiving longer treatment and different characteristics of the two groups at cycle 10. Interestingly, they also found that patients who received 10 courses were more likely to have a lower median ALP value at baseline. Kawahara et al. [9] performed a retrospective analysis of 52 CRPC patients receiving DTX therapy (55 mg/m^2 every 3–4 weeks), and reported significantly shorter OS for the group receiving (≤9 cycles than the group receiving ≥10 cycles (11.2 vs. 28.5 months). Nishimura performed extended DTX therapy in patients who responded to the initial 10 cycles of the regimen (median dose 60 mg/m^2) and delivered a median of 18 cycles without compromising quality of life or increasing grade 3/4 toxicity [10]. Miyake et al. [11] performed a retrospective review of 257 consecutive Japanese CRPC patients who received DTX therapy (75 % of the patients had 3-weekly courses of 70–75 mg/m^2) and concluded that performance status, significant pain, prior treatment with estramustine, the number of cycles, and the PSA response were independently related to OS (median 25.4 months). They emphasized that the number of treatment cycles (≥11) was the strongest predictor of OS. Our hypothesis in this study was that OS may be improved by continuing DTX therapy for more than 10 cycles. The hypothesis may be justified by the data on DTX regimen as a prognostic factor for OS with a 9 % risk reduction per course. Care, however, must be taken in the interpretation of the data, since the DTX regimen had been continued until patient death or withdrawal due to AEs. In that instance, patients with good performance status or lesser tumor burden may survive

Table 5 Risk table predicting probability of longer (>10) courses of docetaxel treatment (a) and relation of risk score with number of docetaxel cycles (b)

(a)

	Age			
	≤65 years		>65 years	
	% (no. patients)	Risk score (95 % CI)	% (no. patients)	Risk score (95 % CI)
ALP < 189 IU/L				
Hb ≥11.3 g/dL	80 (5)	0 (−0.1 to 0.1)	61.3 (31)	3 (2.96–3.04)
Hb < 11.3 g/dL	60 (5)	2 (1.8 to 2.2)	55.6 (9)	5 (4.92–5.08)
ALP ≥189 IU/L				
Hb ≥11.3 g/dL	56.7 (30)	4 (3.96 to 4.04)	27.4 (95)	7 (6.98–7.02)
Hb < 11.3 g/dL	38.5 (13)	6 (5.94 to 6.06)	12.1 (58)	9 (8.97–9.03)

(b)

Risk score	DTX cycles		*p* value	DTX cycles[a]		*p* value
	≤10	>10		≤5	≥6	
0–6	40	53	<0.0001	14	79	<0.0001
7–10	120	33		69	84	
	160	86		83	163	

[a] ≤5: lower quartile cycles, ≥6: interquartile to higher quartile cycles

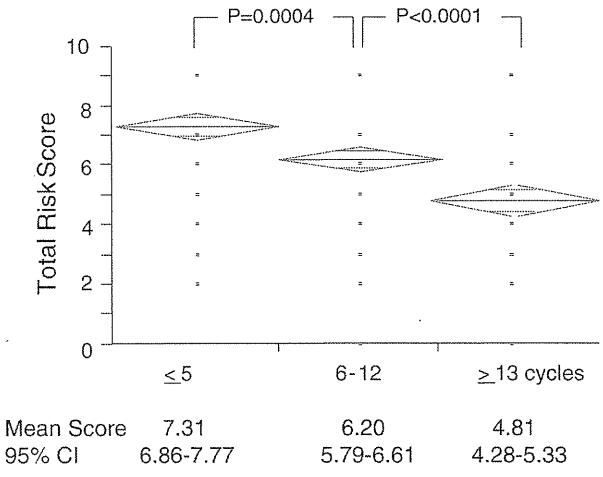


Fig. 2 Relationship between the total risk score and the number of DTX courses. The cut-off value of the total risk score was set on the basis of the ROC curve predicting longer (>10 cycles) DTX therapy

longer irrespective of the DTX regimen. This is the limitation of our retrospective analysis.

As for the influence of age, a recent phase 3 randomized trial comparing DTX every 2 weeks versus every 3 weeks at the same dose intensity showed that the time to treatment failure was significantly longer in the 2-week group than in the 3-week group for patients younger than 65 years [14]. This report is in good agreement with our finding that an age under 65 years was one of the factors predicting a longer duration of DTX therapy. There is increasing evidence that ALP and Hb are prognostic markers in patients with CRPC [15–19]. Hb may be a relevant marker that reflects inflammatory cytokines like CRP, which is strongly correlated with the prognosis of CRPC patients [20–22]. Since ALP reflects the activity of bone metastases, patients with lower ALP levels are likely to have less aggressive cancer and may be expected to achieve better OS [19].

How should our risk model be employed in the era of new second-line agents, such as abiraterone, enzalutamide or cabazitaxel? An option may be to establish a treatment strategy that involves discriminating between patients who should withdraw DTX within five cycles and those who could receive more than 10 cycles. In our series, 87 patients (31.2 %) received ≤5 cycles of DTX therapy (lower quartile group), and this group was discriminated from the other patients with a sensitivity of 83 % by a higher risk score. Such patients may select the new AR-targeting agents instead, because DTX down-regulates AR expression [23] and attenuates the efficacy of AR-targeting therapy.

The limitations of this study were that it was a retrospective study with heterogeneous patient backgrounds. Missing data for laboratory tests may influence the results. The risk table with scoring was not validated by other cohorts. A prospective study should be done to validate our risk model.

In conclusion, more than one-third of CRPC patients could safely receive over 10 cycles of DTX therapy with a

Table 6 Adverse events

	DTX cycles						
	≤10 (n = 178)			>10 (n = 101)			
	Grade 1–2	≥Grade 3	%	Grade 1–2	≥Grade 3	%	p value[a]
Blood/bone marrow (%)	41	108	60.7	27	58	57.4	0.6136
Cardiovascular	6	0	0	4	0	0	–
Gastrointestinal	36	2	1.1	20	3	3.0	0.356
Hepatic	7	2	1.1	6	0	0	0.5364
Pulmonary	8	4	2.2	8	1	1.0	0.7566
Bone pain	8	1	5.6	7	1	1.0	1.0000
Infection	39	8	4.5	24	2	2.0	0.5561
Others	6	7	3.9	5	6	5.9	0.3375
Any event	–	114	64	–	59	58.4	0.3710

[a] Comparison of ≥ grade 3 events between standard and longer treatment groups

9 % reduction in the risk of OS per cycle. The combination of ALP, Hb, and age could be used to predict optimal cycles of DTX therapy in patients with CRPC.

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Adenovirus-mediated REIC/Dkk-3 gene therapy: Development of an autologous cancer vaccination therapy (Review)

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Abstract. Reduced expression in immortalized cells (REIC)/Dickkopf (Dkk)-3 is a tumor suppressor and therapeutic gene and has been studied with respect to the application of cancer gene therapy. Our previous studies demonstrated that the intratumoral injection of an adenovirus vector carrying the human REIC/Dkk-3 gene (Ad-REIC) suppresses tumor growth in mouse models of prostate, breast and testicular cancer and malignant mesothelioma. The mechanisms underlying these antitumor therapeutic effects have only been clarified recently. It has been demonstrated that Ad-REIC treatment inhibits cancer progression via the upregulation of systemic anticancer immunity. Under experimental conditions, autologous cancer vaccination via cancer-specific apoptosis and anticancer immune activation is a possible therapeutic mechanism. The robust anticancer effects observed in previous preclinical studies support the clinical utility of Ad-REIC. At present, a phase I-IIa study of Ad-REIC gene therapy in prostate cancer patients is ongoing. The current study reviews the observations of previous fundamental studies and summarizes the anticancer mechanisms of intratumoral Ad-REIC treatment in terms of cancer vaccination.

Contents

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1. Introduction

A number of therapeutic cancer vaccines have been previously developed and evaluated in phase II/III clinical trials (1-3). The strategies for immunotherapy include the injection of peptides or proteins in adjuvant treatment and recombinant viruses and plasmids encoding immune factors, as well as the delivery of killed tumor cells and protein- or peptide-activated dendritic cells (DCs) to patients. With respect to the concept of cancer vaccination, controlling tumor-associated antigen (TAA) and systemic immune activation against TAA is essential. A number of previous clinical studies have been conducted focusing on single specific TAA molecules and designing a protocol to target TAA. However, previous trials of cancer vaccines have been unable to demonstrate robust therapeutic effects in spite of the activation of specific cytotoxic T lymphocytes against TAA (4). One possible reason for this is that targeting a single specific TAA is not sufficient to achieve substantial tumor reduction, since not all cancer cells express TAA and the cells without TAA escape the acquired immunity. In addition, there is a possibility that each immunological design of a cancer vaccine, such as specific peptides, is unlikely to cover the range of individual immune systems and may therefore, be ineffective. Hence, it is important to overcome the issues derived from the limited abilities of selected TAA molecules and differences in the immunological characteristics of patients.

A good strategy to address these issues is to kill the cancer cells at the tumor site via the direct injection of anticancer agents. In this way, the various TAAs released from the dead cancer cells are exposed to the individual immune system. The released TAAs are taken up by antigen-presenting cells (APC) and activated APCs upregulate the anticancer

immune response by presenting the TAAs to immune effector cells (1,2). This type of therapy aims to use and vaccinate autologous tumors for anticancer immune activation. This autologous cancer vaccination strategy is predicted to activate individual immunity against the broad range of TAAs present in the cancer cells of the individual. This strategy is also attractive since systemic anticancer immunity may be activated simultaneously with substantial tumor reduction and these two therapeutic effects are predicted to be synergistic.

Gene therapy has been utilized in a number of previous clinical trials of human cancer and exhibits an innovative and attractive therapeutic potential. Adenovirus-mediated gene delivery continues to be the preferred treatment for cancer as the vectors indicate the high transduction efficacy of the therapeutic gene and the safety of the procedure when used for direct local injection (5-7). A number of previous clinical trials have demonstrated the utility and safety of the intratumoral injection of adenoviral vectors in cancer lesions (7). Adenoviral vectors are suitable for use in autologous cancer vaccination strategies as they succeed in robustly killing cancer cells and upregulating specific anticancer immunity pathways (6,8).

The reduced expression in immortalized cells (REIC) gene is identical to Dickkopf (DKK)-3 and REIC/Dkk-3 expression is significantly downregulated in a broad range of human cancer cells (9-17). In our previous study, the therapeutic effects of the REIC/Dkk-3 gene as a tumor suppressor gene were determined by the development of an adenovirus vector carrying the human REIC/Dkk-3 gene (Ad-REIC). The agent was found to significantly induce apoptosis in various cancer cells (13,18-20). Recently, the mechanisms of action of Ad-REIC agents in cancer gene therapy have been clarified *in vitro* and *in vivo* using several mouse tumor models. Under these experimental conditions, autologous cancer vaccination via cancer-specific apoptosis and anticancer immune upregulation is a potential therapeutic mechanism. We herein review the previously reported observations of fundamental studies and summarize the anticancer mechanisms of intratumoral Ad-REIC treatment in terms of cancer vaccination.

2. Characteristics of REIC/Dkk-3

The REIC gene was originally identified at Okayama University (Okayama, Japan) and reported in 2000 (9) as a gene whose expression is decreased via the immortalization of normal human fibroblasts. The authors performed mRNA expression profiling using subtractive hybridization of two types of cell lines, cobalt-irradiated normal fibroblasts, which stop proliferating and immortalized fibroblasts, which continue to proliferate. Subsequently, REIC was identified since expression of the gene was significantly reduced in the immortalized fibroblast cells. The sequence of the REIC gene was found to be consistent with that of the human Dkk-3 gene, a member of the Dkk family that encodes secreted proteins and consists of four primary members in vertebrates (Dkk-1, -2, -3 and -4). The expression of this gene was found to be markedly decreased in a variety of human immortalized cells and was therefore, named REIC. Previously, significant downregulation of the REIC/Dkk-3 expression has been reported in a broad range of human malignant tissues and REIC/Dkk-3 is hypothesized to function as a tumor suppressor gene (9-17).

The REIC/Dkk-3 gene is located on human chromosome 11p15.1 and contains 9 exons spanning >50 kbp (21). The REIC/Dkk-3 gene product is a secretory protein, while the gene itself encodes a deduced 38.3 kDa protein with 350 aa that is detected as two major bands of 60-68 kDa in size, according to variable glycosylation levels. The cDNA possess an N-terminal signal peptide, two cysteine-rich domains and two coiled-coil domains. REIC/Dkk-3 is an N-glycosylated protein, the majority of which intracellularly localizes to the endoplasmic reticulum (ER) (22). REIC/Dkk-3 is expressed in the majority of normal tissues in humans and mice, including the brain, heart, lungs, liver, colon and kidneys and is significantly downregulated in a broad range of human cancer cell lines (9,22). REIC/Dkk-3 has also been found to be downregulated in a variety of cancer tissues compared with surrounding normal tissue, including those of colorectal, lung, gastric, pancreatic, prostate, breast and bladder cancer, hepatocellular and renal cell carcinoma and malignant mesothelioma (10-17). Consistently, the REIC/Dkk-3 expression in cancer specimens is downregulated at the critical transition from low- to high-level malignant disease (10,13,16). Therefore, the lack of REIC/Dkk-3 expression has been found to positively correlate with the malignant grade and progression of cancer in several cancer types. Hypermethylation in the REIC/Dkk-3 promoter region has been previously reported in cancer cells with an absent or reduced expression (14,15,17,23).

3. Physiological functions of REIC/Dkk-3

Previously, the physiological functions of the REIC/Dkk-3 protein have been intensively investigated using knockout or overexpression of intracellular proteins. Previous studies have demonstrated that Dkk-3 modulates fibroblast growth factor and activin/nodal signaling to regulate the mesoderm induction of Xenopus. This suggests that physiological Dkk-3 is required for transforming growth factor β (TGF-β) signaling during early Xenopus development (24). Previously, the Dkk-3 protein has been found to also play an essential role in amphioxus head formation by inhibiting Wnt/β-catenin and nodal signaling (25). The authors identified that the Dkk-3 protein inhibits Wnt/β-catenin signaling in specific mammalian cells and cancer cell lines. However, Wnt/β-catenin signaling is positively regulated by the Dkk-3 protein in the murine retina and in several types of cell lines, including HEK293 cells (25). As for mammalian prostate glands, it has been previously reported that Dkk-3 is involved in prostate acinar morphogenesis and maintains the structural integrity of the prostate gland by limiting TGF-β/Smad signaling (26,27). Consistent with these observations, exogenous REIC/Dkk-3 protein promotes prostate acinar morphogenesis, suggesting that secreted REIC/Dkk-3 protein is also involved in prostate gland differentiation (22). In addition, the increased proliferation of human prostate epithelial cells has been previously confirmed in acini cells formed by epithelial cells stably silenced for Dkk-3 (27). Finally, the Dkk-3 gene has been found to be involved in the mechanisms underlying the differentiation of partially induced pluripotent stem cells to smooth muscle cells, thereby, transcriptionally regulating SM22 via the potentiation of Wnt signaling (28).