多用，炭酸ガスの使用などの面で問題も多く[8]，根治性の意味からも小切開腹腔鏡補助下前立腺全摘術は現在もっとも理想的な手術フォーマットであると考える。

まとめ

小切開腹腔鏡補助下の前立腺全摘術は拡大画像下に精密な組織所見を得ながら正確に操作を行うことができ，低侵襲性のみならず根治性の面からも優れた手技であると考えられた。

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International Journal of Urology (2010) 17, 960–961 doi: 10.1111/j.1442-2042.2010.02632.x

Skills and Pitfalls

Upfront transection and subsequent ligation of the dorsal vein complex during laparoscopic radical prostatectomy

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Abstract: Laparoscopic radical prostatectomy for localized prostate cancer offers several advantages, including creation of a pneumoperitoneum that results in less blood loss than is seen with the corresponding open procedure. Transection of the deep dorsal vein complex remains among the most challenging aspects, however. Safe and secure completion of this procedure is important to minimize blood loss and maximize the chance of cure. Liberal use of coagulation for hemostasis at the dorsal vein complex (DVC) risks thermal damage to the sphincteric muscle. DVC ligation before transection, though commonly performed, can cause loss of some sphincteric fibers and potentially result in delayed recovery of urinary continence. Furthermore, ligation may at times prove difficult, especially in obese patients with a short and broad DVC, a large prostate gland, and a narrow pelvis. The presence of prominent pubic tubercles may further increase the difficulty. We have found that bleeding from the DVC is easily controlled without suture ligation through a combination of a modest pneumoperitoneum with pinpoint coagulation of one or two small arteries that are consistently found in the superficial layer of the complex. Precise, even-level transection is possible under direct vision with no more than modest blood loss. A stitch in a Z-shaped fashion is then applied to the entire transected stump of the DVC. This procedure is simple and easily performed, even by those with limited experience. Here we provide an overview of our current technique.

Key words: dorsal vein complex, laparoscopic radical prostatectomy, prostate cancer.

Technique

A five-port transperitoneal approach is used. Antegrade laparoscopic radical prostatectomy (LRP) is started, as previously described, under an ordinary pneumoperitoneal pressure of 12 mmHg and with the patient in the Trendelenburg position.[1] The dissection is advanced toward the apex until it reaches a point at which only the DVC, the urethra, and the prostate gland are connected. The pneumoperitoneal pressure is then temporarily raised to 15 mmHg. The superficial layer of the mid-portion of the DVC is cut with a pair of scissors (Microline), starting from the midline (Fig. 1). No electrocautery is applied at this stage. As cutting proceeds from the midline, pulsating bleeders from one or sometimes two small arteries that run near the midline of the DVC will be encountered (Fig. 2). The transected arterial stump or stumps are handled through pinpoint monopolar coagulation using the very tip of the scissors. The rest of the DVC is easily dissected, as venous bleeding is well controlled by the pneumoperitoneum alone (Fig. 3). A stitch in a Z-shaped fashion (V-20, 2-0 1/2. polyglactin; Tyco Healthcare group LP, Norwalk, CT, USA) is then applied to the entire transected stump of the DVC (Fig. 4). The urethra is identified with the use of a 20-Fr metal sound inserted through the urethra.

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Received 13 May 2010; accepted 13 August 2010.
Online publication 26 September 2010

Discussion

Complete hemostasis after transection of DVC constitutes an extremely important step during the course of radical prostatectomy.[1] The DVC is a complex structure that contains veins, arteries, sphincteric muscle fibers, and other components. Main arteries consistently lie superficially to this complex. One of the advantages of LRP is that the bleeding from veins can easily be controlled with modest peumoperitoneal pressure alone. This also applies to veins in the DVC. It is impressive that under the pneumoperitoneum, most of the bleeding from the DVC can be controlled with only pinpoint coagulation of arteries. The simultaneous transection of arteries and veins leads a surgeon to believe that bleeding from the DVC is often difficult to control even in LRP. The cardinal rule here is to identify and coagulate the arteries first. There was no difference in the amount of intraoperative bleeding either before or after we undertook the procedure (262 *vs* 259 mL).

In our experience, pinpoint use of monopolar scissors for electrocoagulation had no significant adverse effects on

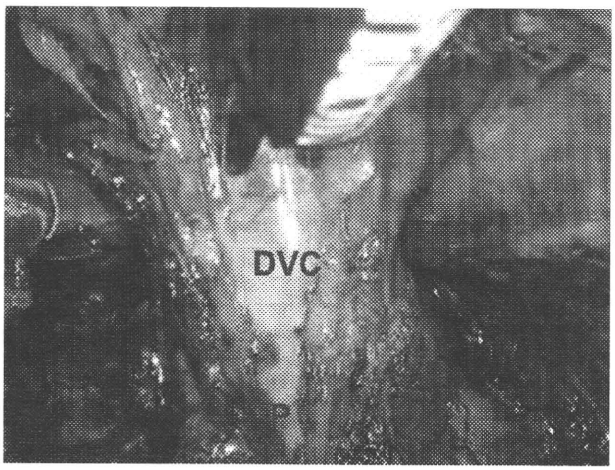

Fig. 1 The dorsal vein complex (DVC) is sectioned from the midline. P, prostate.

Fig. 2 Pulsating bleeding from the artery can be observed.

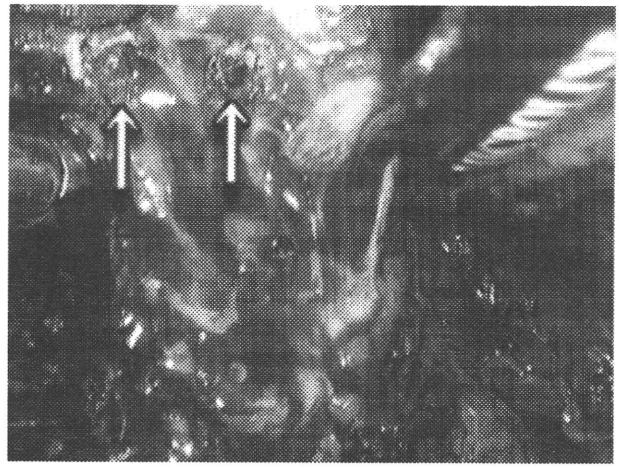

Fig. 3 The resection stump of this artery is handled through monopolar coagulation using the tips of the scissors.

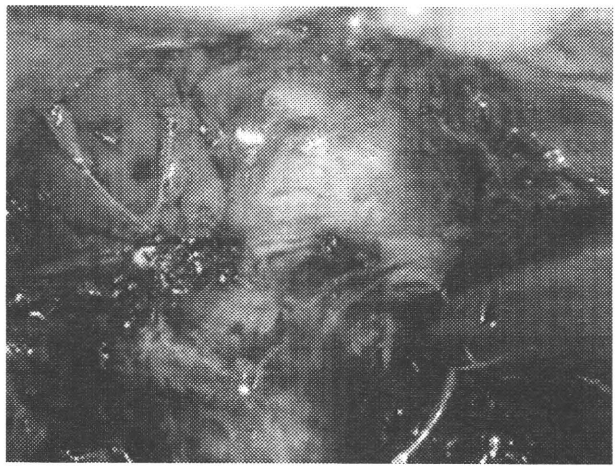

Fig. 4 A selective suture of Santorini's venous plexus is performed.

functional or pathological outcome after surgery. The recovery of urinary continence (pad-free rates) for the past 100 patients has been 71.7% by 3 months and 96.5% by 12 months.[1] The overall positive surgical margin rate in pT2 disease has been 6.5%.[1] We do not believe this procedure itself affected potency recovery.

A technique known as "selective ligature" has recently been reported.[2,3] This method involves meticulous hemostatic suturing after dissection of the DVC and is intended to improve postoperative urinary continence. Bipolar coagulation was reported as the preferred method of coagulation, but no effort was made to find arteries. At least in theory, the use of bipolar coagulation[3] may have some advantage in terms of avoiding penetrating thermal damage. However, it cannot be used in a pinpoint fashion and seems more traumatic overall than our approach. The reported continence recovery was comparable to ours: 80.0% at 3 months (24/30) and 90.0% at 12 months (27/30). Our own experience with selective ligature has not demonstrated any advantage for this technique.

The procedure we report here is simple, safe and easily applicable to any patient, even those with anatomical difficulties. Greater and more thorough understanding of DVC anatomy is important and can lead to further advances in surgical techniques.

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A Phase I Study of Personalized Peptide Vaccination Using 14 Kinds of Vaccine in Combination With Low-Dose Estramustine in HLA-A24-Positive Patients With Castration-Resistant Prostate Cancer

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BACKGROUND. To evaluate the safety, tolerability, immune response, and antitumor activity of a combination of personalized peptide vaccination (PPV) and estramustine phosphate (EMP) in patients with castration-resistant prostate cancer (CRPC).

METHODS. In a phase I dose-escalation study, four peptides showing the highest levels of peptide-specific immunoglobulin G (IgG) to 14 vaccine candidates (ITK-1) were subcutaneously injected every week in three different dose settings (1, 3, and 5 mg per peptide) for 6 weeks with a low dose of EMP, and the patients were followed by maximum 2 years extension study either weekly or bi-weekly six times PPV as one course with a low dose of EMP.

RESULTS. Fifteen patients were enrolled in the phase I study. No serious treatment-related adverse events were observed. The most common adverse events were grade 2 skin reactions at the injection sites. The maximum acceptable dose of ITK-1 was 8.643 mg. There were no treatment-related systemic adverse events of grade 3 or more, and maximum tolerated dose could not be determined. Cytotoxic T lymphocyte responses measured by interferon-γ release assay were boosted in 10 of 15 (67%) patients, and IgG responses were boosted in 7 of 15 (47%) patients. Twelve patients proceeded to the extension study, and the median survival time was 23.8 months during a median follow-up of 23.8 months.

CONCLUSIONS. PPV treatment for HLA-A24 positive patients with CRPC could be recommended for further stages of clinical trials because of its safety and the higher frequency of boosting immune responses. *Prostate 9999: 1–10, 2010.* © 2010 Wiley-Liss, Inc.

KEY WORDS: **personalized peptide vaccine; immunotherapy; phase I study; estramustine phosphate**

Conflict of interest statement: The authors indicated no potential conflict of interest with the exception of Yamada and Itoh who received a research grant from the Green Peptide Co., Ltd; Yamada and Itoh own stocks in the Green Peptide Co.; Yamada is a part-time executive of the Green Peptide Co.

Grant sponsor: Green Peptide Co., Ltd.

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Received 10 March 2010; Accepted 9 August 2010
DOI 10.1002/pros.21261
Published online in Wiley Online Library (wileyonlinelibrary.com).

INTRODUCTION

In the initial trials, peptide-based vaccine treatment of cancer patients rarely induced clinical responses and the levels of immune responses was low, indicating that the classical type of peptide vaccines did not have a promising future in the treatment of advanced cancer [1,2]. However, there have been slow but substantial advances in peptide vaccines and dendritic cell (DC)-based vaccines with regard to both clinical responses and immunological markers [3–12].

We previously reported that repeated multiple peptide vaccine regimen planned according to the pre-existing immunity (personalized peptide vaccine: PPV) could prolong the overall survival of patients with advanced cancer, and IgG specific to each peptide can frequently be detected in pre- and post-vaccination plasma [13]. In the previous trial, PPV was administered in 113 patients with advanced cancer, and the levels of peptide-specific cytotoxic T lymphocyte (CTL) precursors were measured by the interferon (IFN)-γ release assay and those of anti-peptide immunoglobulin (IgG) were estimated by enzyme-linked immunosorbent assay (ELISA). The level of anti-peptide IgG was a laboratory marker that predicted clinical responses to the PPV with a positive relationship to overall survival. Further, we showed that 58 patients with castration-resistant prostate cancer (CRPC) treated with a combination therapy of PPV and a low dose of estramustine phosphate (EMP) survived for a relatively long period of 17 months, which was comparable with the results of chemotherapy with docetaxel, and serious adverse events occurred less frequently in the study [4].

ITK-1 is a peptide set consisting of 14 kinds of peptide discovered as a HLA class I epitope, which being developed by Green Peptide Co., Ltd. All the 14 peptide candidates can induce CTLs, and each of them can induce HLA-A24-restricted and tumor-specific CTL activity in peripheral blood mononuclear cells (PBMCs) of cancer patients [14–18]. We have conducted a phase I study on PPV and low-dose EMP in HLA-A24-positive patients with CRPC in order to define the safety, tolerability, and immune and prostate-specific antigen (PSA) responses of this drug combination.

PATIENTS AND METHODS

Patients

This was a multi-center study and approved by each institutional review board (IRB) that evaluated it from the viewpoint of the science and ethics in all four hospitals in Japan before the initiation of the study. Patients who had a histological diagnosis of prostate adenocarcinoma (PC) and progressive disease (PD) by diagnostic imaging (computerized tomography; CT, magnetic resonance imaging; MRI or bone scintigraphy) or PSA after both androgen deprivation therapy either by castration or with luteinizing hormone-releasing hormone (LHRH) agonists and anti-androgen therapy, as well as oral EMP treatment were eligible. PSA progression was defined as at least three consecutive rises in serum PSA taken over 2 weeks apart, in the setting of castration levels of testosterone. Patients were required a washout period of at least 4 weeks before the first vaccination after the completion of prior hormone therapy, hormone-chemotherapy, chemotherapy, or immune therapy. Anti-androgen therapy was discontinued for at least 4 weeks before the first vaccination for patients receiving flutamide and 6 weeks for those receiving bicalutamide. All patients had an Eastern Cooperative Oncology Group performance status of 0 or 1, HLA-A24-positive type, and serum testosterone level $\leq$50 ng/dl, and were maintained on LHRH agonist therapy or castration. Adequate organ functions were required and were defined as white blood cell count $\geq$3,000/mm^3, lymphocyte count $\geq$1,200/mm^3, hemoglobin $\geq$9 g/dl, platelets $\geq$100,000/mm^3, total bilirubin $\leq$1.5 mg/dl, AST and ALT $\leq$2$\times$ (upper normal limit), and serum creatinine $\leq$1.4 mg/dl. Patients with comorbidities including serious cardiovascular, hepatic, nephritic, and hematological diseases $\geq$grade 3 of Common Terminology Criteria for Adverse Events (CTCAE), serious gastric ulcers, and infectious diseases with antibiotic treatment, were excluded. Radiation therapy or immunosuppressive treatment using a systematic steroid within the last 1 year was not permitted. All patients gave written informed consent approved by each IRB.

Study Design

This was a phase I open-labeled dose-escalation study. After a pre-vaccination measurement of peptide-specific IgG in the plasma of patients reactive to 14 kinds of vaccine candidate peptides (ITK-1) with the ability to induce CTLs, patients were treated with 6 weekly subcutaneous administration of the top four peptides showing the strongest antibody responses at three different dose settings (1, 3, and 5 mg/peptide), with daily oral EMP 313.4 mg in the phase I study. This was followed by a maximum of 2 years in an extension study of six PPVs either weekly or bi-weekly as one course. All patients were treated at the hospital during the first 1 week followed by outpatient clinic visits. ITK-1 consists of 14 kinds of peptides: $SART2_{93-101}$, $SART3_{109-118}$, $Lck_{208-216}$, $PAP_{213-221}$, $PSA_{248-257}$, $EGFR_{800-809}$, $MRP3_{503-511}$, $MRP3_{1293-1302}$, $SART2_{161-169}$,

$Lck_{486-494}$, $Lck_{488-497}$, $PSMA_{624-632}$, $EZH2_{735-743}$, and $PTHrP_{102-111}$. All peptides were prepared under Good Manufacturing Practice (GMP) compliance by American Peptide Company (San Diego, CA) and by PolyPeptide Laboratories (San Diego, CA), and were supplied in lyophilized vials; 4 mg, including inactive ingredients, under GMP compliance. Selected peptides were dissolved in 1 ml distilled water and emulsified with 1 ml of incomplete Freund's adjuvant (Montanide ISA-51VG; Seppic, Paris, France), under GMP compliance. Each of four peptides in 0.5 ml emulsion at a dose level of 1 mg/peptide (4 mg/2 ml), 1.5 ml emulsion at a dose level of 3 mg/peptide, and 2.5 mL emulsion at a dose level of 5 mg/peptide were injected subcutaneously into the thigh, the hip or the lower part of trunk area. Each peptide was independently injected nearby. EMP was administered orally as a 156.7 mg capsule, one capsule twice daily, for a total daily dose of 313.4 mg, half of the standard dose of EMP (626.8 mg/day) to avoid immunosuppression as reported in our previous study [19]. From the starting dose of 1 mg/peptide, subsequent dose levels were increased after the evaluation of the safety data by the Data and Safety Monitoring Committee (DSMC) according to the dose escalation design of the protocol. The initial cohort included six patients. If the DSMC recommended proceeding to the next level as a result of the safety evaluation of the prior level, new six patients were enrolled. The highest dose level enrolled three patients at first and was evaluated the safety data by the DSMC to include additional three patients. The maximum acceptable dose (MAD) was defined as the lowest dose level at which at least two-thirds of patients experienced grade 2 or greater injection site reactions after the sixth treatment. The maximum tolerated dose (MTD) was defined as the lowest dose level at which more than one-third of patients experienced grade 3 or greater systemic adverse events caused by ITK-1 after the sixth treatment. Adverse events were graded according to the CTCAE version 3.0 and were coded using MedDRA/J (Medical Dictionary for Regulatory Activities Terminology/Japanese) version 12.0. Patients who experienced no significant (≥CTCAE grade3) adverse events and no disease progression, and signed informed consent were eligible to extend treatment until disease progression or unacceptable adverse events occurred, or the patient met other withdrawal criteria.

Pretreatment and Follow-Up Studies

A complete history, physical examination, and routine laboratory studies, including complete blood counts, biochemical tests, ECG, relevant radiologic studies, PSA, and urinalysis were performed before treatment and repeated after every six injections.

Immune Responses

For evaluation of immune responses, peptide-specific CTL precursors in PBMCs and peptide-specific IgG levels in plasma were measured as described previously [13]. Also, peptide-specific IgG levels were measured using patient's plasma of the screening examination to select the best peptides. Briefly, 30 ml of peripheral blood samples were obtained from each patient to measure peptide specific CTL and IgG prior to vaccination, at the fourth and after the sixth vaccinations, and after every sixth vaccination in the extension study, and then the PBMCs and plasma were isolated by Ficoll–Conray density gradient centrifugation. We reported that the IgG specific to each peptide measured by Luminex system as the fluorescence intensity unit (FIU) could frequently be detected in pre- and post-vaccination plasma, and the level of peptide-specific IgG is a laboratory marker that predicts clinical responses to the PPV with a good relationship to overall survival [13,20]. Therefore, peptides were chosen on the basis of evaluation of peptide-specific IgG levels in plasma. Peptide-specific CTL precursors in PBMCs were detected using a previously reported culture method [21]. Briefly, PBMCs (1×10^5 cells/well) were incubated with 10 μM of each peptide in U-bottom-type 96-well microculture plates (Nunc, Roskilde, Denmark) in 200 μl of culture medium. The culture medium consisted of 45% RPMI-1640 medium, 45% AIM-V® medium (Invitrogen Corp., Carlsbad, CA), 10% FCS, 20 U/ml of interleukin-2 (IL-2), and 0.1 mM MEM nonessential amino acid solution (Invitrogen Corp.), 36 mg/L gentamicin sulfate (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Half of the medium was removed and replaced with new medium containing a corresponding peptide (20 μM) every 3 days for up to 12 days. On the 12th day of the culture, 24 hr after the last stimulation, these cells were harvested, washed three times, and then tested for their ability to produce IFN-γ in response to C1R-A2402 cells preloaded with either a corresponding peptide or HIV peptide (RYLRQQLLGI) as a negative control in HLA-A24. The target cells (C1R-A2402, 1×10^4/well) were pulsed with each peptide (10 μM) for 2 hr, and then effector cells (1×10^5/well) were added to each well with a final volume of 200 μl. After incubation for 18 hr, the supernatants (100 μl) were collected, and the amounts of IFN-γ were measured using an ELISA (limit of sensitivity: 10 pg/ml). All experiments were performed in quadruplicate assay.

Definition of Treatment Outcomes

Outcomes were assessed by post-therapy changes in serum PSA and immune responses. A post-therapy

TABLE I. Baseline Demographics

Characteristics	No. of patients (%)
No. of patients	15
Age, years	
Median	73
Range	63–78
ECOG PS	
0	14 (93)
1	1 (7)
Gleason score	
7	3 (20)
8	5 (33)
9	4 (27)
10	1 (7)
Unknown	2 (13)
PSA (ng/mL)	
Median	39.6
Range	0.2–354.4
Site(s) of metastasis	
None	4 (27)
Lymph node	2 (13)
Bone	6 (40)
Lymph node + bone	1 (7)
Other	2 (13)
Local therapy	
Prostatectomy	4 (27)
EBRT	3 (20)
No definitive local therapy	8 (53)
Hormone therapy	
Primary therapy only	1 (7)
≥2 therapies	14 (93)
Chemotherapy	
EMP	15 (100)
Other	2 (13)

ECOG PS, Eastern Cooperative Oncology Group performance status; PSA, prostate-specific antigen; EBRT, external-beam radiation therapy; EMP, estramustine phosphate.

decrease of PSA to a normal range was defined as a complete response (CR) and a decrease in PSA of ≥50% from baseline was defined as a partial response (PR) in the phase I study. Also, a post-therapy PSA decrease of <50% or an increase >25% from baseline were interpreted as no change (NC) [22] and PSA above 125% of the baseline PSA value was defined as PD. Positive immune responses were defined as post-IgG levels/pre-IgG levels ≥3, post-IFN-γ levels/pre- IFN-γ levels ≥3, respectively. All patients were followed up every 3 months for life. Data, except the survival data, were analyzed by November 2009 using SAS (Statistical Analysis System) software version 9.1.3. The Student's *t*-test and the chi-square test were used to compare quantitative and categorical variables, respectively. Overall survival was calculated from the study registration date to the date of the last follow-up or the death from any cause. The Kaplan–Meier method was used to estimate product-limit estimate curves with the survival data obtained in March 2010. Tests results were considered significant at a two-sided significance level of 5%. The analysis was performed by intent to treat.

RESULTS

Patient Characteristics

Fifteen patients were recruited to the study between April 2006 and September 2007. Patient characteristics are listed in Table I. All patients were HLA-A24-positive, and had hormone and EMP refractory prostate cancer. In addition, all 15 patients were evaluated for the safety and the efficacy of the PPV treatment.

Dose Escalation

The dose-escalation scheme is presented in Table II. Maximum dose escalation preplanned for each peptide of 5 mg/2.5 mL (4 peptides, 20 mg/10 mL) was achieved. There were no treatment-related grade 3 or 4 adverse events or deaths in this study. Grade 2 injection site reactions were observed in two of six patients in the first dose level of 1 mg/peptide, and five of six patients in the second dose level of 3 mg/peptide after the sixth treatment. At the 5 mg/peptide dose

TABLE II. The Results of Dose-Escalation in Phase I Study

Peptides dose level (mg/peptide)	No. of patients		No. of patients	
	Enroll	Discontinued or skipped[a]	MAD (≥grade 2 injection site reaction)	MTD (≥grade 3 systemic treatment-related AE)
1	6	0/6	2/6	0/6
3	6	0/6	5/6	0/6
5	3	3/3	3/3	0/3
Total	15	3/15	10/15	0/15

MAD, maximum acceptable dose; MTD, maximum tolerated dose; AE, adverse event.
[a]Patients were discontinued or skipped the treatment because both widespread grade 2 injection site reactions and patients' own requests.

level, three patients were treated, but the vaccination was skipped or discontinued in all three patients considering the ethical viewpoint because of patients' own requests and physical burden, caused by widespread grade 2 injection site reactions. After these treatment-related adverse events, two of three 5 mg/peptide dose level patients were entered in the extension study and then the dose level was reduced to 3 mg/peptide during treatment. The DSMC reviewed the results and recommended stopping the additional three enrollments for the dose level of 5 mg/peptide. Subsequently, the MAD for PPV was calculated to be 8.643 mg/4 peptide (2.161 mg/peptide) based on the logistic regression model.

Adverse Events

There were no treatment-related serious adverse events and no grade 3 or greater adverse events in the phase I study. In contrast, a grade 3 injection site reaction and a grade 3 pyrexia occurred in one patient each during the extension study. All treatment-related adverse events observed in whole study (phase I and extension study) are listed in Table III. The primary nonhematologic treatment-related adverse events were injection site reaction (93.3%), malaise (33.3%), edema peripheral (33.3%), and fatigue (20.0%). These adverse events were manageable with routine intervention. Hematologic adverse events were, grade 1 white blood cell count increased and grade 1–2 lymphocyte count decreased occurred in 4 of 15 (26.7%) and 3 of 15 (20.0%) patients, respectively. One patient at a dose level of 5 mg/peptide had a grade 1 blood fibrinogen increased, and another patient at a dose level of 3 mg/peptide had grade 1 blood triglycerides increased during the first course, and these changes returned to normal levels on the next course.

Immune Response

The best peptides for each patient were selected based on peptide-specific IgG levels for each peptide at the screening examination (data not shown). The results of the immune response in the first course are given in Table IV. After the sixth vaccination, IgG responses were increased in one of six patients with 1 mg/peptide, four of six patients with 3 mg/peptide, and two of three patients with 5 mg/peptide tested. CTL responses measured by IFN-γ release assay were increased in four of six patients with 1 mg/peptide, six of six patients with 3 mg/peptide, and zero of three patients with 5 mg/peptide tested.

Clinical Response

PSA response after the sixth vaccination was CR in one patient (6.7%) receiving 3 mg/peptide, PR in one patient (6.7%) receiving 1 mg/peptide, and PD in two patients (13.3%) receiving 5 mg/peptide. At the time of data analysis, nine patients had died and all deaths were attributed to prostate cancer or metastases. The median follow-up time for all patients was 23.8 months, ranging from 3.0 to 38.3 months. None of the patients was lost to follow-up during this analysis. The median overall survival was 23.8 months for all 15 patients (95% CI, lower limit was 15.6 months, upper limit was not estimated; Fig. 1).

DISCUSSION

We performed a multicenter, open-label, phase I trial to evaluate the safety, tolerability, immune response, and PSA response of a combination of escalating doses of PPV and low-dose EMP. All patients had hormone and EMP-refractory prostate cancer. The treatment regime was well tolerated at all dose levels, except the injection site reaction at the highest dose level of 5 mg/peptide observed in all three patients enrolled, and no MTD was established in this trial. The most common adverse event was injection site reaction. The concept of dose escalation in a phase I trial to identify an MTD may not be applicable to most therapeutic cancer vaccines [23]. Peptide vaccines based on non-mutated melanoma antigens such as MART-1/Melan A and gp100 were initially evaluated in a phase I setting, at doses ranging from 0.1 to 10 mg [24,25]. However, no toxicity was observed even at the highest doses, and in vitro analysis did not reveal any correlation between the peptide dose and the generation of specific T-cell reactivity from the PBMCs of the vaccinated patients. Neither the safety nor efficacy of the vaccine can be assessed in patients with a blunted immune response since both safety and efficacy depend on the immune response. In contrast, our initial trial for colorectal cancer patients with 0.3, 1, and 3 mg/injections of SART3 peptide showed that a dose of 3 mg/injection was better than that of 0.3 and 1 mg/injection based on the induction of cellular immune responses to both tumor cells and peptides [26]. The current phase I study also showed that a dose of 3 mg/injection was better than those of 1 and 5 mg/injection based on the induction of cellular immune responses to peptides, although total doses of four peptides were 4 mg/2 mL, 12 mg/6 mL, and 20 mg/10 mL. Under these conditions, there were no serious adverse events caused by ITK-1; however, grade 2 injection site reactions were observed in two of six patients receiving 1 mg/0.5 mL/peptide, five of six patients receiving 3 mg/1.5 mL/peptide, and three of three patients receiving 5 mg/2.5 mL/peptide in the phase I study. The vaccination was skipped or discontinued in three of three patients receiving 5 mg/2.5 mL/peptide

TABLE III. Treatment-Related Adverse Events for Castration-Resistant Prostate Cancer

	No. of patients experienced treatment-related adverse events during phase I study/whole study[a] by grade									Total (15 patients)	
	1 mg/peptide group (6 patients)			3 mg/peptide group (6 patients)			5 mg/peptide group (3 patients)			All grade	
MedDRA/J ver12.0 symptom: preferred Trem(PT)	G1 (PI/ Whole)	G2 (PI/ Whole)	G3 (PI/ Whole)	G1 (PI/ Whole)	G2 (PI/ Whole)	G3 (PI/ Whole)	G1 (PI/ Whole)	G2 (PI/ Whole)	G3 (PI/ Whole)	P I	Whole
Vomiting	1/1									1 (6.7%)	1 (6.7%)
Ventricular extrasystoles	0/1										1 (6.7%)
Fatigue	0/1	0/1		1/0	0/1					1 (6.7%)	3 (20.0%)
Injection site reaction	2/2	2/3		1/1	5/4	0/1		3/3		13 (86.7%)	14 (93.3%)
Malaise	1/2			0/1	0/1		0/1			1 (6.7%)	5 (33.3%)
Oedema peripheral	1/2	0/1			0/1		0/1			1 (6.7%)	5 (33.3%)
Pyrexia						0/1					1 (6.7%)
Aspartate aminotransferase increased	0/1										1 (6.7%)
Blood fibrinogen increased							1/1			1 (6.7%)	1 (6.7%)
Blood triglycerides increased				1/1						1 (6.7%)	1 (6.7%)
Crystal urine present	0/1										1 (6.7%)
Blood urine present				0/1							1 (6.7%)
Lymphocyte count decreased	1/1	1/1			1/1					3 (20.0%)	3 (20.0%)
Neutrophil count increased	0/1										1 (6.7%)
Urinary casts	0/1										1 (6.7%)
White blood cell count increased	0/1			1/2			1/1			2 (13.3%)	4 (26.7%)
White blood cells urine positive	0/1			0/1							2 (13.3%)
Bacteria urine identified				0/1							1 (6.7%)
Dizziness				0/1							1 (6.7%)
Dizziness postural				0/1							1 (6.7%)
Headache				1/0	0/1					1 (6.7%)	1 (6.7%)
Insomnia		0/1									1 (6.7%)
Cough	0/1										1 (6.7%)
Rash generalized					0/1						1 (6.7%)

[a]Whole study means phase I and extension study.

TABLE IV. Immunorogical Responses During the Personalized Peptide Vaccination

			Anti-peptide IgG response (FIU)[a]				Anti-peptide cellular response (pg/ml)[b]			
Dose of peptide	Pts No.	Peptide	Pre	Post (fourth)	Post (after sixth)	Increased response (after sixth)	Pre	Post (fourth)	Post (after sixth)	Increased response (after sixth)
1 mg	1	Lck-486	94	90	81	—	ND	ND	ND	—
		PSMA-624	<5	<5	<5	—	ND	ND	ND	—
		PTHrP-102	42	30	23	—	113	ND	ND	—
		SART3-109	31	24	21	—	ND	ND	ND	—
	2	Lck-486	310	206	976	Positive	667	ND	204	—
		MRP3-1293	38	21	28	—	ND	ND	186	Positive
		SART2-93	20	11	9	—	ND	ND	656	Positive
		SART3-109	27	13	18	—	899	ND	ND	—
	3	Lck-486	102	102	114	—	ND	78	ND	—
		Lck-488	45	46	52	—	462	ND	ND	—
		MRP3-1293	52	45	50	—	ND	ND	ND	—
		PAP-213	252	210	215	—	ND	ND	ND	—
	4	Lck-486	200	199	247	—	ND	ND	1,393	Positive
		Lck-488	<5	<5	<5	—	ND	ND	472	Positive
		PSA-248	117	99	109	—	ND	ND	ND	—
		PTHrP-102	171	138	142	—	564	ND	ND	—
	5	Lck-486	575	364	396	—	ND	117	57	—
		Lck-488	144	102	92	—	ND	ND	439	Positive
		MRP3-1293	91	64	51	—	133	160	ND	—
		PAP-213	90	70	77	—	3,764	ND	114	—
	6	MRP3-1293	779	586	411	—	ND	477	ND	—
		PSA-248	804	756	1,825	—	ND	ND	ND	—
		PTHrP-102	502	414	310	—	ND	93	753	Positive
		SART3-109	142	152	83	—	ND	ND	3,276	Positive
3 mg	7	Lck-486	202	216	9,028	Positive	ND	1,636	ND	—
		MRP3-1293	29	21	22	—	ND	ND	ND	—
		PAP-213	<5	<5	5	—	274	ND	1,494	Positive
		PSA-248	11	12	1,902	Positive	173	ND	ND	—
	8	Lck-486	298	261	287	—	2,543	ND	ND	—
		Lck-488	10	9	11	—	ND	ND	598	Positive
		MRP3-1293	23	21	23	—	ND	ND	ND	—
		PAP-213	8	5	9	—	ND	ND	2,613	Positive
	9	Lck-486	329	290	308	—	ND	ND	72	—
		Lck-488	128	103	106	—	ND	119	627	Positive
		MRP3-1293	53	36	40	—	ND	1,706	ND	—
		PAP-213	<5	<5	10,992	Positive	ND	683	ND	—

(Continued)

TABLE IV. (*Continued*)

Dose of peptide	Pts No.	Peptide	Anti-peptide IgG response (FIU)[a] Pre	Post (fourth)	Post (after sixth)	Increased response (after sixth)	Anti-peptide cellular response (pg/ml)[b] Pre	Post (fourth)	Post (after sixth)	Increased response (after sixth)
	10	Lck-486	826	1,632	16,376	Positive	127	ND	7,014	Positive
		Lck-488	21	22	48	—	117	227	115	—
		MRP3-1,293	21	22	24	—	ND	109	ND	—
		PAP-213	15	15	60	Positive	189	ND	285	—
	11	Lck-208	19	18	21	—	211	54	ND	—
		Lck-486	434	349	105	—	ND	ND	ND	—
		Lck-488	12	12	12	—	ND	ND	5,258	Positive
		PTHrP-102	102	99	135	—	ND	2,991	2,934	Positive
	12	Lck-486	392	549	348	—	ND	ND	1,136	Positive
		Lck-488	87	96	64	—	ND	ND	ND	—
		PSA-248	157	2,653	18,163	Positive	ND	ND	ND	—
		SART3-109	76	87	58	—	ND	ND	794	Positive
5 mg	13	Lck-486	183	231	861	Positive	184	103	104	—
		PAP-213	39	35	8,490	Positive	232	ND	ND	—
		SART2-93	56	49	51	—	59	215	ND	—
		SART3-109	31	31	38	—	391	ND	165	—
	14	Lck-486	162	120	2,950	Positive	185	348	126	—
		MRP3-1293	29	27	149	Positive	97	104	ND	—
		SART2-161	16	17	27	—	178	200	263	—
		SART3-109	23	20	108	Positive	1,285	117	1,024	—
	15	Lck-486	809	837	916	—	1,339	ND	ND	—
		MRP3-1293	710	543	550	—	251	ND	ND	—
		SART2-161	72	46	57	—	ND	ND	55	—
		SART3-109	311	248	236	—	100	ND	110	—

[a]Values indicate fluorescence intensity unit (FIU) of IgG antibodies reactive to each peptide.
[b]Values indicate the mean of specific interferon-γ production in positive wells reactive to each peptide.

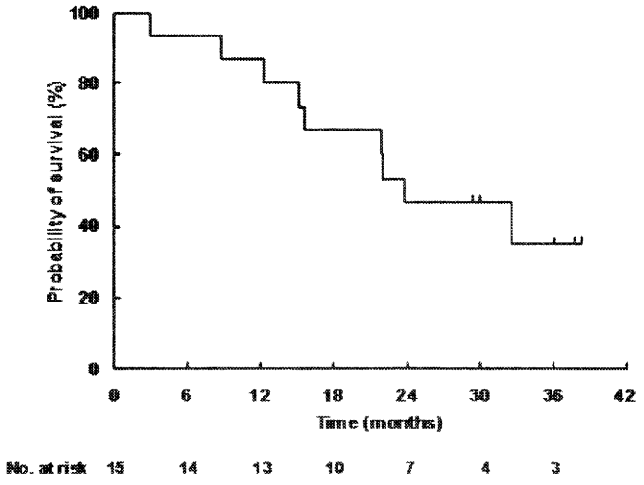

Fig. 1. Kaplan–Meier estimates of overall survival for 15 patients treated by personalized peptide vaccination with low-dose estramustine. Median overall survival is 23.8 months.

because of both widespread grade 2 skin reactions and patients' own requests. Subsequently, we calculated MAD as 8.643 mg/4 peptides in this study. Therefore, considering the adverse events, tolerability, and immune responses, the 3 mg/1.5 mL/peptide dose of PPV will be recommended for further clinical trials.

In the present study, CTL responses measured by IFN-γ release assay and IgG responses were enhanced in 10/15 (66.7%) and 7/15 (46.7%) of the examined patients, respectively, and in the PSA response, CR and PR was one patient each (6.7%) and PD was two patients (13.3%) after the sixth vaccination. In addition, the long-term (23.8 months) median survival time after combination therapy with PPV and low-dose EMP observed in the extension study indicated that this treatment suppresses tumor growth. However, the exact mechanism of this interaction is unclear and further studies are needed.

In conclusion, the results of safety, immune responses, and improved overall survival without MTD, as well as the consistency between these results and the data from our previous trials [4,19,27], could lead to us to the next phase of randomized clinical trial wherein we can confirm the survival benefit of such personalized immunotherapy in HLA-A24 positive patients with CRPC.

ACKNOWLEDGMENTS

We thank Tadao Kakizoe (medical advisor).

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Implications of Serum Bone Turnover Markers in Prostate Cancer Patients With Bone Metastasis

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OBJECTIVES To assess the diagnostic accuracy of serum bone turnover markers for detection of bone metastasis in patients with prostate cancer (PCa) and to assess the usefulness of these markers as predictors of mortality from PCa.

METHODS Serum total alkaline phosphatase, bone-specific alkaline phosphatase, carboxy-terminal pyridinoline cross-linked telopeptide parts of type-I collagen (1CTP), tartrate-resistant acid phosphatase type 5 b, and prostate-specific antigen (PSA) levels were measured in 222 patients (58 with bone metastasis, 57 with T2M0 PCa, 55 with T3M0 PCa, and 52 without PCa). Multivariate stepwise logistic regression analysis was used to identify independent predictors of bone metastasis. Correlation of serum marker levels with bone metastasis was assessed using receiver operating characteristics analysis. Multivariate Cox proportional hazards analysis was used to predict cause-specific survival in PCa patients with bone metastasis.

RESULTS Serum total alkaline phosphatase, bone-specific alkaline phosphatase, 1CTP, tartrate-resistant acid phosphatase type 5 b, and PSA levels were significantly elevated in patients with bone metastasis, and correlated significantly with the extent of disease on bone scintigraphy. Multivariate stepwise logistic regression analysis demonstrated that serum PSA and 1CTP were significant predictors of bone metastasis. Receiver operating characteristics analyses showed that serum 1CTP level was the most reliable predictor of bone metastasis (area under the curve = 0.85). Multivariate Cox proportional hazards analysis revealed that only serum 1CTP was an independent prognostic factor for PCa-related death.

CONCLUSIONS Serum 1CTP level was a more reliable marker than the others to detect bone metastatic spread and to predict survival probability in PCa patients with bone metastasis. UROLOGY 75: 1446–1451, 2010. © 2010 Elsevier Inc.

The incidence of prostate cancer (PCa) is rapidly increasing in Japan. PCa metastasizes to bone in approximately 70% of patients with advanced disease.[1] These bone metastases are associated with many complications and much morbidity including severe bone pain, prolonged hospital stay, reduced mobility, hypercalcemia, and pathologic fractures. Furthermore, skeletal-related events (SRE) have been correlated with reduced overall and median survival and quality of life of patients with PCa.[2,3] The assessment of bone metastases relies primarily on imaging techniques, and ^{99m}Tc-based bone scintigraphy is routinely used for the detection of bone metastases.[4-6] Although bone scintigraphy has high sensitivity, it lacks specificity in the detection of skeletal metastases, and the value of bone scanning for detecting disease progression has been questioned on the basis of cost-effectiveness.[7] When serum prostate-specific antigen (PSA) levels is <20 ng/mL, the likelihood of a positive bone scan in asymptomatic patients was estimated to be only 0.8%.[8] Therefore, inexpensive, repeatable, convenient, noninvasive, and rapid laboratory tests are needed.

Bone metastases cause osteoclastogenesis and bone resorption, disrupting the balance between osteoblast and osteoclast activity. Bone formation markers are direct or indirect products of osteoblast activity, whereas bone resorption markers are derived from skeletal collagen degradation. Several studies have assessed the diagnostic efficacy of both bone formation and resorption markers for the detection of bone metastases in PCa.[9-15]

This work was supported in part by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology (Contract grant numbers: 19591834, 19791101), Japan Osteoporosis Foundation and Young Researcher Promotion Grant from the Japanese Urological Association (2008).

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Submitted: September 18, 2009, accepted (with revisions): November 26, 2009

0090-4295/10/$34.00
doi:10.1016/j.urology.2009.11.049

This study of Japanese patients with PCa aimed to assess the accuracy of serum bone turnover markers for detection of bone metastasis and their usefulness as predictors of PCa mortality. We measured the bone formation markers total alkaline phosphatase (tALP) and bone-specific alkaline phosphatase (BAP) and the bone resorption markers carboxy-terminal pyridinoline cross-linked telopeptide parts of type-I collagen (1CTP) and tartrate-resistant acid phosphatase type 5 b (TRAP 5b). We aimed to ascertain whether serum levels of these markers paralleled clinicopathological factors in monitoring bone metastasis of PCa.

MATERIAL AND METHODS

Study Population

A total of 222 men treated at Chiba University between 2002 and 2008 were enrolled in this study. All cases of PCa were histologically diagnosed by prostatic needle biopsy. Specimens were fixed immediately in 10% buffered formalin for 24 hours and embedded in paraffin. Routine sections stained with hematoxylin and eosin were reviewed and tumor grade was established. Cancer stage was assigned according to the tumor-node-metastasis classification, using digital rectal examination, transrectal ultrasonography, computed tomography, magnetic resonance imaging, or bone scintigraphy. Bone scintigraphy with ^{99m}Tc-methylene-diphosphonate and, occasionally, x-ray, computed tomography, or magnetic resonance imaging was used to diagnose bone metastases. The extent of bone metastasis was classified by extent of disease (EOD) grade according to the method of Soloway et al.[16] SRE were defined according to the method of Saad et al[17] as pathologic bone fractures (vertebral or nonvertebral), spinal cord compression, surgery or radiation therapy to bone (including the use of radioisotopes), hypercalcemia of malignancy, or a change of antineoplastic therapy to treat bone pain. Patients were divided into 4 groups: bone metastasis (n = 58), T2M0 PCa (n = 57), T3M0 PCa (n = 55), and benign prostatic disease (BPD, n = 52). Age ranged from 49 to 89 years, with an average of 66.4 years. Patients receiving medication known to interfere with bone metabolism (eg, calcium supplements, bisphosphonates) were excluded from the analysis, as were those with a history of liver disease or renal failure.

Blood Samples and Basic Laboratory Data

Blood samples were taken with informed consent. All serum samples were taken at the first examination, and were immediately frozen and stored at −20°C until analysis. Serum PSA (ARCHITECT, Abbott Laboratories, Abbott Park, IL), hemoglobin (SULFOLYSER, Sysmex Corporation, Hyogo, Japan), and serum calcium (Autosera CA, Sekisui Medical Corporation, Tokyo, Japan) were measured.

Bone Formation Markers

Serum tALP levels were measured with IATROLQ ALP (Mitsubishi Chemical Medience Corporation, Tokyo, Japan). Serum BAP levels were determined with Osteolinks-BAP (Quidel Corporation, San Diego, CA), which specifically quantifies skeletal ALP with low immunoreactivity for the liver/kidney isoforms.

Bone Resorption Markers

Serum 1CTP levels were measured by radioimmunoassay (Immundiagnostik, Bensheim, Germany) to avoid the instability of radioiodinated reagents. Serum TRAP-5b levels were determined with Osteolinks-TRAP b (Nitto Boseki Corporation, Tokyo, Japan).

Statistical Analysis

Statistical significance was examined using Mann–Whitney U test, Student's t test, Kruskal–Wallis test, the log-rank test, and simple regression. Survival curves were created using the Kaplan–Meier method with log-rank test. Multivariate stepwise logistic regression analysis according to a Cox proportional hazards model was used to identify significant independent predictors for bone metastasis and factors predicting cause-specific mortality. Furthermore, receiver operating characteristics analysis was also performed and area under the curve was calculated as measure of each test's performance, with a perfect test having an area of 1.0. Sensitivity, specificity, accuracy, positive predictive value, and negative predictive value were calculated. All statistical tests were 2-sided and a P value less than.05 was considered to indicate significance. All statistical analyses were performed using SPSS version 11.0 (SPSS Inc, Chicago, IL).

RESULTS

Table 1 shows clinical and laboratory data in the patient groups. PCa patients were subdivided into 3 groups: M0 (T2), M0 (T3), and M1b. BPD patients were younger on average than PCa patients. Serum tALP, BAP, 1CTP, TRAP 5b, and PSA levels were significantly higher ($P < .05$) in PCa patients with bone metastasis than in patients with BPD and PCa with no bone metastasis.

Table 2 shows clinicopathologic factors among PCa patients with bone metastasis (M1b). Histologic grade and Gleason sum were unknown in 1 case. Breakdown of SRE was as follows: radiation therapy to bone (n = 7), hypercalcemia (n = 4), and spinal cord compression (n = 2). We performed univariate and multivariate analysis for factors predictive of SRE in PCa patients with bone metastasis. Only serum levels of BAP were correlated significantly with incidence of SRE ($P < .05$). As EOD score increased, the serum levels of tALP, BAP, 1CTP, TRAP 5b, and PSA increased significantly ($P < .05$). By contrast, serum levels of PSA and bone turnover markers showed no significant relationship with clinical classification, lymph node status, histologic grade, Gleason sum, or SRE (data not shown).

We performed univariate and multivariate analysis of predictors of bone metastasis (M0 vs M1b) according to the logistic regression model. In univariate analysis, PSA, 1CTP, tALP, TRAP 5b, and BAP were significant predictors of bone metastasis ($P < .0001$). In particular, PSA level (OR, 1.045, 95% CI, 1.028-1.063) and 1CTP level (OR, 3.211, 95% CI, 3.211-4.789) were both independently significant predictors on multivariate analysis (data not shown). Subsequently, receiver operating characteristics analysis was also performed (Fig. 1). The area under the curve was 0.76, 0.73, 0.85, and 0.64 for tALP,

Table 1. Clinical characteristics of the study groups

	Non-PCa	PCa				
	(BPD)	T2M0	T3M0	M1b	Total	*P*
No. patients	52	57	55	58	222	
Age (y)						
Mean + SD	63 + 6.3	64.6 − 5.4	68.6 + 6.7	69 + 8.2	66.4 + 7.2	<.0001
Range	49-78	49-72	57-84	52-89	49-89	
PSA (ng/mL)						
Mean + SD	6.4 + 1.6	11.8 − 7.4	42.3 + 29.8	1402.4 + 2055.3	381.4 + 1208.4	<.0001
Range	4.1-9.5	3.6-34.4	5.0-143.0	26.7-9033	3.6-9033.0	
1CTP (ng/mL)						
Mean + SD	2.7 + 1	2.8 − 0.8	2.8 + 0.6	6.4 + 5	3.4 + 3.1	<.0001
Range	1.6-7.9	1.3-6.3	1.5-4.2	1.7-32.3	1.3-32.3	
tALP (mIU/mL)						
Mean + SD	211.9 + 55.4	216.5 − 54.3	217.4 + 56.0	651 + 579	381.4 + 1208.4	<.0001
Range	113-330	131-437	108-338.0	117-2192	108-2192	
No. patients	52	54	48	50	204	
TRAP-5b (mU/dL)						
Mean + SD	45.2 + 37.8	43.9 − 50.5	70.5 + 49.3	144.3 + 168.5	75.1 + 100.6	.0003
Range	10-138	10-254.0	10-170	10-645	10-645	
No. patients	45	48	46	50	189	
BAP (ng/mL)						
Mean + SD	15.7 + 4.1	16.7 + 4.9	15.7 + 4	60.4 + 77.2	27.8 + 44.1	<.0001
Range	10.2-28.4	10-33.9	10.2-28	9.8-357	9.8-357	

PCa = prostate cancer; BPD = benign prostatic disease; PSA = prostate-specific antigen; BAP = bone-specific alkaline phosphatase.

Table 2. Clinicopathologic characteristics among M1b PCa patients

Characteristic	No. Patients
Clinical T stage	
T3	35
T4	23
Lymph node status	
N0	16
N1	42
Histological grade*	
Moderate	12
Poor	45
Gleason sum†	
<8	45
≥8	12
EOD score	
1,2	30
3,4	28
SRE	
−	45
+	13
Serum Ca (mg/dL)	
Mean ± 1SD	8.8 + 0.5
Range	6.7-9.8
Hb (g/dL)	
Mean ± 1SD	13.3 + 2.1
Range	7.4-18.3
Cause-specific survival (%)	
2 years	82
5 years	48

SRE = skeletal-related events.
* Histological grade was unknown in 1 patient.
† Gleason sum was unknown in 1 patient.

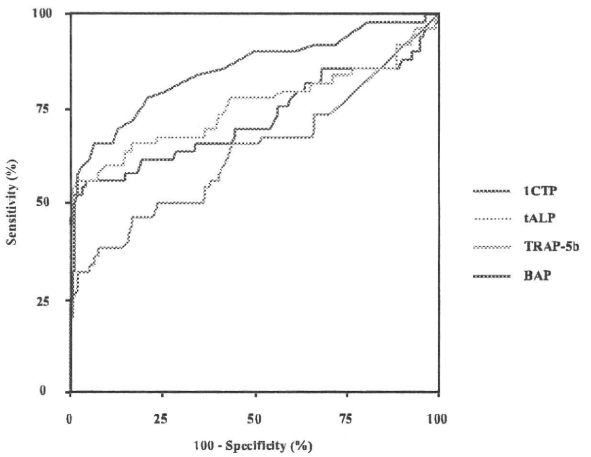

Figure 1. ROC curves for markers to detect bone metastasis in PCa patients. 1CTP = carboxy-terminal telopeptide of type-I collagen, tALP = alkaline phosphatase, TRAP-5b = tartrate-resistant acid phosphatase isoenzyme 5 b, BAP = bone-specific alkaline phosphatase, PCa = prostate cancer; ROC, receiver operating characteristics analysis.

BAP, 1CTP, and TRAP 5b, respectively. Cut-off values of 275 mIU/mL for tALP, 24.2 ng/mL for BAP, 3.3 ng/mL for 1CTP, and 105.5 mU/dL for TRAP 5b provided the best prediction of bone metastasis. Using these tentative cut-off values, the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy were 69, 84, 61%, 89%, and 80%, respectively, for tALP; 56, 95, 82%, 85%, and 84% for BAP; 78, 81, 58%, 91%, and 80% for 1CTP; 46, 84, 49%, 83%, and 75%, for TRAP 5b.

Cause-specific survival was estimated using the Kaplan–Meier method in M1b PCa patients (data not shown). Mean follow-up was 35.0 ± 24.6 months (range, 1.8-97.2). Twenty-one patients died of PCa. We divided M1b PCa patients into a high serum levels group and a low serum levels group, using the "mean+1SD" for serum tALP (683.4 mIU/mL), BAP (71.9 ng/mL), 1CTP (6.7

Table 3. Cox proportional hazards analysis of factors predicting time to cause-specific death in PCa patients with bone metastasis

	Univariate HR			Multivariate HR		
	Relative HR	95% CI	*P*	Relative HR	95% CI	*P*
Gleason score (<8/≥8)	4.820	1.117-20.808	.0350	5.951	1.143-30.989	.0341
Histological grade (moderate/poor)	3.112	0.723-13.398	.1274			
EOD score (1-3/4)	11.695	2.105-64.975	.0053	1.614	0.241-10.809	.6215
T stage (3/4)	1.083	0.408-2.618	.9454			
N stage (N0/N1)	2.525	0.698-9.129	.2392			
PSA (<1592.5/>1592.5)	1.062	0.386-2.918	.9073			
1CTP (<6.7/>6.7)	2.708	1.144-6.412	.0235	3.151	1.336-11.909	.0406
tALP (<683.4/>683.4)	2.494	1.055-5.898	.0374	5.550	0.919-33.513	.0617
TRAP-5b (<175.7/>175.7)	2.184	0.681-7.001	.1886			
BAP (<71.9/>71.9)	3.930	1.407-10.976	.0090	0.931	0.154-5.625	.9375
Hb (<11.2/>11.2)	0.292	0.103-0.828	.0206	0.528	0.139-2.001	.3474
Ca (<9.3/>9.3)	1.295	0.156-10.634	.8098			

tALP = total alkaline phosphatase; TRAP = tartrate-resistant acid phosphatase type 5 b.

ng/mL), TRAP 5b (175.7 mU/dL), PSA (1592.5 ng/mL), and calcium (9.3 ng/mL), and using the "mean−1SD" for hemoglobin (11.2 g/dL). Patients with a Gleason sum < 8 (n = 14) or EOD 1-3 (n = 54) had a significantly better outcome than those with a Gleason sum ≥ 8 (n = 43) or EOD 4 (n = 3) ($P < .05$). Similarly, patients with low serum tALP (n = 39), BAP (n = 38), or 1CTP (n = 40) levels had a significantly better outcome than those with high serum tALP (n = 19), BAP (n = 12), or 1CTP (n = 18) levels ($P < .05$). Patients with high hemoglobin levels (n = 50) had a significantly better outcome than those with low hemoglobin levels (n = 9) ($P < .05$) (data not shown).

Table 3 shows the factors that were predictive of disease-free survival in both univariate and multivariate analysis in PCa patients with bone metastasis. In univariate analysis, Gleason sum, EOD score, tALP, BAP, 1CTP, and hemoglobin were significant predictors of cause-specific survival ($P < .05$). In multivariate analysis, Gleason sum (HR = 5.951, 95% CI, 1.143-30.989) and 1CTP (HR = 3.151, 95% CI, 1.336-11.909) were significant independent predictors of cause-specific survival ($P < .05$).

COMMENT

Most patients with advanced PCa have bone metastasis, and this greatly affects prognosis and quality of life. Bone scintigraphy is considered the gold standard for diagnosis and monitoring of metastatic bone lesions. Moreover, the extent of bone metastasis as evaluated by bone scintigraphy is an important prognostic indicator.[9] The present study supports this in that patients with EOD 1-3 (n = 54) had a significantly better outcome than those with EOD 4 (n = 3) ($P < .05$). Bone scintigraphy has high sensitivity, but lacks specificity in the detection of bone metastasis, and the value of bone scanning for detecting disease progression has been questioned on the basis of cost-effectiveness.[7] Previous studies suggest that pretreatment serum PSA levels do not provide significant prognostic information,[9,18] and the present study had similar findings.

Several biochemical markers of bone formation and resorption with varying specificity and sensitivity are used to predict bone turnover, to diagnose metabolic bone disease, and to monitor antiresorptive treatment and its effectiveness. Several studies have assessed the diagnostic efficacy of both bone formation and resorption markers for detection of bone metastases in PCa. There is worldwide consensus that serum markers are more appropriate than urine markers (ie, C- and N-terminal telopeptide cross-linked collagen type I), because use of the former eliminates possible errors associated with creatinine measurement and is associated with much lower analytic and biological variation.[19]

Metastases in PCa are generally osteoblastic rather than osteolytic, and therefore involve both bone formation and bone resorption. 1CTP is a bone degradation marker that may be useful in the assessment of bone metastasis in PCa. Koopmans et al[20] reported a significantly greater increase in serum 1CTP levels in PCa patients with bone metastasis than those without bone metastasis. Koga et al[21] reported that serum 1CTP levels in PCa patients with bone metastasis were significantly higher than in PCa patients without bone metastasis or patients with benign prostate hyperplasia. Moreover, serum 1CTP levels correlated significantly with EOD score, showing a significant downward trend in response to hormonal therapy in PCa patients with bone metastasis. In the present study, serum 1CTP level was an independent predictor of bone metastasis according to univariate and multivariate analysis. Furthermore, as EOD score increased, serum levels of 1CTP increased significantly. Serum 1CTP level was also a significant independent predictor of cause-specific survival according to univariate and multivariate analysis. In other words, our study suggests that serum 1CTP level is a reliable marker to detect metastatic spread and to predict survival probability in PCa patients with bone metastasis. However, cli-

nicians should be aware that 1CTP accumulates in the circulation in renal failure.

tALP has been used as a nonspecific marker of bone metastasis from PCa since 1936, when Gutman et al[22] showed that its serum level increased with osteoblastosis. It has stood the test of time and remains a reliable indicator of osteoblastic activity, as in bone metastases.[23] Chybowski et al[24] reported that normal or minimal elevations in serum PSA (<20 ng/mL) might identify a subgroup of patients at low risk for positive bone scintigraphy. Wymenga et al[25] commented that patients with newly diagnosed and untreated PCa should undergo bone scintigraphy if there is bone pain or if ALP levels are >90 U/L. However, it must be recognized that ALP accumulates in the circulation in hepatobiliary disease, blood dyscrasias, and heart failure.

The first immunoradiometric assay for BAP incorporating 2 specific monoclonal antibodies was developed in 1990.[26] Under normal conditions, BAP, an indicator of osteoblast metabolism, represents <40% of the tALP. Lorente et al[27] reported that serum BAP levels could play a complementary role in diagnosing bone metastases of PCa. BAP could provide useful clinical information on the extent of skeletal metastasis and represent an easy way of enhancing the clinical utility of PSA. The addition of BAP to PSA in the initial evaluation could permit a staging bone scan to be avoided at a PSA range of 10-20 ng/mL, with significant implications for cost saving. In the present study, serum BAP level was a significant predictor of bone metastasis on univariate analysis.

As EOD score increased, serum levels of BAP increased significantly. Also, serum BAP levels were significant independent predictors of cause-specific survival according to univariate analysis. In particular, serum BAP levels were significantly correlated with incidence of SRE.

Halleen et al[28,29] were the first to purify TRAP 5b from human osteoclasts, and they also detected specific osteoclast-derived TRAP 5b activity from human serum. Serum TRAP 5b has 2 important advantages over other bone turnover markers. First, all other known serum bone turnover markers accumulate in the circulation in renal and hepatic failure, leading to false-positives.[30] Second, serum TRAP 5b activity has less diurnal variability than other serum bone turnover markers,[28] probably because the half-life of other markers is shorter. This makes TRAP 5b less sensitive to rapid fluctuations in bone resorption rate. Ozu et al[11] measured serum TRAP 5b, PACP, ALP, and PSA levels in 215 patients with PCa, including 160 without and 55 with bone metastasis. They reported that levels of these markers were significantly elevated in patients with bone metastases compared with those without. Moreover, serum TRAP-5b levels correlated significantly with the extent of disease on bone scintigraphy. They commented that predicted and observed risks of bone metastasis were well correlated when TRAP 5b, ALP, and PSA were combined, and that bone scan could have been omitted in 70% of patients by assessing these 3 markers. Hegele et al[13] reported that PCa patients without bone metastasis had the lowest marker levels, whereas those with bone metastasis had the highest levels, with significance for ALP, osteocalcin, and TRAP 5b. Patients with lymph node-positive PCa had significantly increased serum levels of TRAP 5b and ALP but not osteocalcin and terminal telopeptide cross-linked collagen type I. They commented that both bone resorption and bone formation markers are crucial for detecting bone metastasis in PCa. In the present study, univariate analysis showed that serum TRAP-5b levels predicted bone metastasis. Moreover, as EOD score increased, serum levels of BAP increased significantly. However, serum TRAP-5b levels were not significant independent predictors of cause-specific survival according to univariate analysis.

Serum bone turnover markers can be determined frequently and easily, with negligible disturbance to the patient. The present results suggest that a combination of tALP, BAP, 1CTP, and TRAP 5b appears to be useful for predicting bone metastasis and may provide important information for counseling patients regarding their clinical classification and the need for imaging.

CONCLUSIONS

Our present study suggested that both bone formation markers (tALP, BAP) and bone resorption markers (1CTP, TRAP 5b) might be useful in detecting bone metastasis from PCa. In particular, serum 1CTP was a more reliable marker than the others to detect bone metastatic spread and to predict survival probability in PCa patients with bone metastasis but without renal failure.

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Ets-1 and Hypoxia Inducible Factor-1α Inhibition by Angiotensin II Type-1 Receptor Blockade in Hormone-Refractory Prostate Cancer

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BACKGROUND. Accumulating evidences have suggested that the renin-angiotensin system (RAS) participates in the regulation of tumor angiogenesis. We previously demonstrated that hormone-refractory prostate cancer (HRPC) showed significantly higher angiotensin II (Ang II) type-1 receptor (AT1R) expression, and that the AT1R blocker (ARB) exerted protective effects by inhibiting angiogenesis. However, the downstream transcriptional factors induced by Ang II in prostate cancer cells have not been fully elucidated yet.

METHODS. Three human prostate cancer cell lines: LNCap, C4-2 and C4-2AT6 were used and analyzed. C4-2AT6 cells were established by culture in androgen-ablated conditioned medium for 6 months.

RESULTS. C4-2AT6 cells showed significantly higher AT1R expression, accompanied by higher HIF-1α and Ets-1 expression in the nucleus. In C4-2AT6 cells, VEGF production was significantly higher than in C4-2 cells and LNCaP cells. These results suggested that HRPC exhibited aggressive angiogenic properties, accompanied by up-regulated HIF-1α and Ets-1. Ang II stimulated VEGF production in C4-2 cells and C4-2AT6 cells but not in LNCaP cells. ARB significantly inhibited VEGF production. Western blot analysis demonstrated that AngII induced nuclear expression of HIF-1α and Ets-1 in C4-2 and C4-2AT6 cells, but not in LNCaP cells. ARB significantly inhibited HIF-1α and Ets-1 induction in C4-2 and C4-2AT6 cells.

CONCLUSIONS. This study suggests that AT1R blockade may have a significant impact on HRPC through the inhibition of HIF-1α and Ets-1 and the resulting suppression of angiogenesis. Our results provide the molecular basis of the clinical benefit of ARB as an angiogenic inhibitor in HRPC. *Prostate 70: 162–169, 2010.* © 2009 Wiley-Liss, Inc.

KEY WORDS: **hormone-refractory prostate cancer; angiogenesis; angiotensin II; renin-angiotensin system; angiotensin II type-1 receptor; VEGF; Ets-1; hypoxia inducible factor 1α**

INTRODUCTION

Prostate cancer (PCa) is the most frequently diagnosed malignant tumor and the second leading cause of cancer-related deaths in the United States [1,2]. One of the most troublesome aspects of PCa is that androgen-dependant PCa inevitably progresses to an androgen independent and hormone-refractory state after androgen ablation therapy. Death is the result of metastatic hormone-refractory disease in the majority of patients. No effective treatment for HRPC has been developed yet. Hence, the development of a novel and effective therapeutic strategy for hormone-refractory prostate cancer (HRPC) is urgently needed.

Although the renin-angiotensin system (RAS) has a central role in blood pressure control and renal

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Received 14 July 2009; Accepted 5 August 2009
DOI 10.1002/pros.21049
Published online 16 September 2009 in Wiley InterScience (www.interscience.wiley.com).