[^{11}C]Methionine Positron Emission Tomography and Survival in Patients with Bone and Soft Tissue Sarcomas Treated by Carbon Ion Radiotherapy

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ABSTRACT

Purpose: **The development of the novel carbon ion radiotherapy (CIRT) in the treatment of refractory cancers has resulted in the need for a way to accurately evaluate patient prognosis. We evaluated whether L-[methyl-^{11}C]-methionine (MET) uptake and its change after CIRT were the early survival predictors in patients with unresectable bone and soft tissue sarcomas.**

Experimental Design: **MET positron emission tomography was prospectively performed in 62 patients with unresectable bone and soft tissue sarcomas before and within 1 month after CIRT. Tumor MET uptake was measured with the semiquantitative tumor:nontumor ratio (T/N ratio). The MET uptake in the tumor and relevant clinical parameters were entered into univariate and multivariate survival analysis.**

Results: **The overall median survival time was 20 months. Patients with a baseline T/N ratio of ≤6 had a significant better survival than patients with a baseline T/N ratio >6 (2-year survival rate: 69.4% *versus* 32.3%; $P = 0.01$). Patients with a post-CIRT ratio of ≤4.4 had a better survival than that with a post-CIRT ratio >4.4 (2-year survival rate: 63.7% *versus* 41.3%; $P = 0.01$). A significant higher survival rate was observed in patients with post-therapeutic MET uptake change of >30% than patients in lower change group (2-year survival rate: 74.6% *versus* 41.6%; $P = 0.049$). The multivariate analysis showed that both baseline and post-CIRT T/N ratio were statistically significant independent predictors of patient survival. Tumors with larger T/N ratio had a significantly poorer prognosis.**

Conclusions: **MET uptake as measured by either baseline or post-CIRT T/N ratio was an independent predictor of survival in patients with bone and soft tissue sarcomas treated by carbon ion radiotherapy, whereas post-therapeutic MET uptake change might have potential value for the same purpose.**

INTRODUCTION

Over the last three decades, treatment strategies of bone and soft tissue sarcomas have changed greatly (1). Currently, surgery still remains the vital modality for treating primary tumors, whereas adjuvant chemotherapy plays an essential role in the control of subclinical metastatic disease, and the addition of radiation therapy may allow local tumor control in the patients for whom complete surgical excision is impossible (2). On the other hand, for the treatment of bone and soft tissue sarcomas considered to be unresectable, radioresistant and/or located near critical organs, various particle therapies have been used and have offered promising results (3).

The heavy ion irradiation technique has been introduced recently in our institute, of which carbon ion beam owns unique physical and biological properties as a high linear energy transfer charged particle beam (4–7). Carbon ion beam has a precise range and travels straight forward when penetrating tissues, and its energy release is enormous at the end of its range. This well-localized energy deposit (high-dose peak) at the beam end, called the "Bragg peak," is a unique physical characteristic of the charged particle beam, which enables to safely give effective dose of radiation to bulky primary cancer without causing damage to normal tissues. In our institute, a Phase I/II carbon ion radiotherapy (CIRT) trial has been conducted in patients with unresectable bone and soft tissue sarcomas to investigate the efficacy of carbon ion radiotherapy (8). As part of this trial, positron emission tomography (PET) has been used for the evaluation of response to carbon ion radiotherapy, because PET has opened a totally new approach in evaluating metabolical changes in cancer tissue caused by chemotherapy or radiotherapy *in vivo* (9, 10). It has been shown that PET, a noninvasive imaging technique, is helpful in various clinical situations (11). PET essentially depicts metabolic maps of tumors based on their need for constituents necessary to maintain growth and increased metabolism. Amino acid metabolism of cancer is associated with numerous catabolic processes favoring tumor growth (12). As an essential amino acid, L-methionine plays a central role in the altered metabolism of cancer cells (13).

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Preclinical studies validating the possible use of L-[methyl-^{11}C]-methionine (MET) in the evaluation of chemo- or radiotherapy generally show that MET uptake is reduced rapidly, more rapidly than fluorine-18 fluorodexyglucose (FDG; Refs. 14–16), and MET uptake correlated better than FDG with tumor proliferative activity in squamous cell head and neck cancer cell lines (17). Clinical studies showed that FDG uptake increased frequently in inflammation and may limit the accuracy of FDG PET in cancer immediately after radiotherapy (18, 19). Specially, increased uptake of MET as measured by PET has been suggested to reflect increased transport, transmethylation rate, and protein synthesis of malignant tissue (20–22), and has less influence of radiation-induced inflammatory reaction after radiotherapy (16). So in this trial, we used the MET PET for the evaluation of response to carbon ion radiotherapy.

Our basic hypothesis was that responding tumors show a decrease of amino acid metabolism within a few days after carbon ion radiotherapy, whereas amino acid metabolism remains unchanged in nonresponding tumors; thus, it could be used as the early predictor for the patient prognosis. To test this hypothesis we prospectively studied patients with unresectable bone and soft tissue sarcomas during carbon ion radiotherapy. To our knowledge, no study has been published regarding MET PET and survival for patients with unresectable bone and soft tissue sarcomas treated by carbon ion radiotherapy. So the aim of this study was to investigate whether baseline MET uptake and its change after carbon ion radiotherapy are the prognostic predictors in patients with unresectable bone and soft tissue sarcomas.

PATIENTS AND METHODS

Patient Eligibility. Patients eligible for the study had histologically confirmed bone or soft tissue sarcomas without distant metastases. Tumors were judged to be unresectable by the referring surgeon, or the patients were medically inoperable or declined surgery. Patients who had undergone chemotherapy within 4 weeks before carbon ion radiotherapy or those who had prior radiation therapy at the same site were excluded from the study. The tumor had to be grossly measurable, but size could not exceed 15 cm. Eligibility criteria included Karnofsky performance status score ≥60, age ≥13 years, and estimated life expectancy of at least 6 months. Exclusion criteria were having other active cancer, infection at the tumor site, and tumors arising in the head and neck or the regions above the level of the second cervical spine. All of the patients had a complete history and physical examination, including X-ray radiography, computed tomography (CT), and magnetic resonance imaging before initiating the treatment program. To confirm the pathological diagnosis of bone or soft tissue sarcomas, and to determine the histological subtype and grade, a pathological review of the tumor specimen by the working group pathologists was carried out. After carbon ion radiotherapy, invasive biopsy was not performed to get a noninterfered follow-up for the therapeutic response to carbon ion radiotherapy. Patients without either pre- or post-CIRT MET PET were excluded from this study. All of the patients signed the informed consent form approved by the Institutional Review Board for both the treatment and PET imaging studies.

Table 1 Patient characteristics (n = 62 patients)

Characteristic	No. of patients (%)
Gender	
Female	21 (33.9)
Male	41 (66.1)
Age	
<20	6 (9.7)
20–29	7 (11.3)
30–39	3 (4.8)
40–49	8 (12.9)
50–59	18 (29.0)
≥60	20 (32.3)
KPS[a]	
60	4 (6.5)
70	22 (35.5)
80	19 (30.6)
90	17 (27.4)
Tumor site	
Extremity	6 (9.7)
Pelvis	47 (75.8)
Spine/para-spine	9 (14.5)
Tumor size in cm (median: 10 cm)	
≤10	37 (59.7)
>10	25 (40.3)
Grade	
2	4 (6.5)
3	16 (25.8)
4	5 (8.1)
Gx	13 (21.0)
Histology	
Chordoma	15 (24.2)
Chondrosarcoma	5 (8.1)
Ewing's sarcoma	2 (3.2)
Leiomyosarcoma	2 (3.2)
Liposarcoma	3 (4.8)
Melanoma	1 (1.6)
MFH	5 (8.1)
MPNST	6 (9.7)
Osteosarcoma	18 (29.0)
PNET	4 (6.5)
Synovial sarcoma	1 (1.6)
Presentation	
Primary	39 (62.9)
Recurrence	17 (27.4)
Metastases	6 (9.7)
Stage at presentation	
IB	2 (3.2)
IIB	23 (37.1)
III	12 (19.4)
IV	8 (12.9)

[a] KPS, Karnofsky performance status; MFH, malignant fibrous histiocytoma; MPNST, malignant peripheral nerve sheath tumor; PNET, primitive neuroectodermal tumor.

Patient and Tumor Characteristics. The clinical data from the study are presented in Table 1. A total of 62 patients with 62 lesions were identified with a median follow-up of 20 months (range, 5–60 months). At the end of the study, 21 patients had died. There were 21 women and 41 men. The median age was 50 years (range, 13–85 years) and the median Karnofsky Performance Status was 80. The median tumor size was 10 cm (range, 2–15 cm) and tumors were located at mobile spine or para-spine in 9 patients (14.5%), pelvis in 47 patients (75.8%), and extremities in 6 patients (9.7%). Osteosarcoma and chordoma were the most common types in the bone sarcomas

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and malignant peripheral nerve sheath tumor in soft tissue sarcomas. There were 54 unresectable lesions and 7 lesions for which surgery was declined by the patients. One patient had postoperative remnant. The presentations of lesion were primary tumor in 39 patients (62.9%), recurrent lesion in 17 patients (27.4%), and metastatic lesion in 6 patients (9.7%). Twenty-five patients (40.3%) received chemotherapy before carbon ion radiotherapy with >4 weeks interval. The patients underwent carbon ion radiotherapy as shown in Table 2.

Carbon Ion Radiotherapy Protocol. The Heavy Ion Medical Accelerator in Chiba is the first heavy ion accelerator complex dedicated to medical use in a hospital environment in the world. The heavy ion medical accelerator system and biophysical characteristics of carbon ion beam have been described previously (6). The carbon ion radiotherapy protocol has been described in details in our previous study (8). Briefly, there are three treatment rooms with fixed vertical and/or horizontal beam line. The accelerated energies of the vertical carbon ion beam are 290 MeV or 350 MeV, and those of the horizontal beam 290 MeV or 400 MeV. The range of the 290 MeV carbon ion beam is ~15 cm in water, and that of the 350 MeV beam is 20 cm. The 400 MeV carbon ion beam reaches a depth up to 25 cm in water. For modulation of the Bragg peak of carbon ion beam to confirm to a target volume, the beam lines in the treatment room are equipped with a pair of wobbler magnets, beam scatters, ridge filters, multileaf collimators, and a compensation bolus. The most appropriate size ridge filter, which corresponds to, and determines the size of the spread-out of the Bragg peak, is selected to avoid an unnecessary dose to the beam range across normal tissues in each port. Table 2 lists the carbon ion radiotherapy regiments used in this study. Carbon ion radiotherapy was given once daily, 4 days per week, for fixed 16 fractions in 4 weeks. 52.8GyE in 16 fractions, 3.3 GyE/fraction, for spine and pelvis lesions was used as the starting dose, as well as 57.6 GyE in 16 fractions, 3.6 GyE/fraction, for limb and other sites lesions.

PET Imaging. MET of high specific activity was produced with a standard technique using a method modified from the synthesis of Langstrom *et al.* (23). Whole-body scanners (ECAT EXACT HR+ and ECAT EXACT 47; Siemens CTI, Knoxville, TN) were used, providing an axial field of view of 15.5 and 16.2 cm, resulting in 63 and 47 transverse slices with a thickness of 2.5 and 3.4 mm, respectively. The spatial resolution of the reconstructed images is 4.2–6.0 mm at full width half maximum. Transmission scans were performed with germanium-68 rod sources. Emission data corrected for random events, dead time, and attenuation were reconstructed by filtered backprojection using a Ramp filter with a cutoff frequency of 0.4, followed by the decay correction.

Table 2 Carbon ion radiotherapy regiments[a]

Site	No. of patients	Total dose (GyE)	Fraction dose (GyE)
Extremity	6	57.6–64.0	3.6–4.0
Pelvis	47	52.8–73.6	3.3–4.6
Spine	9	52.8–70.4	3.3–4.4

[a] Carbon ion radiotherapy given once daily, 4 days per week, for a fixed 16 fractions in 4 weeks.

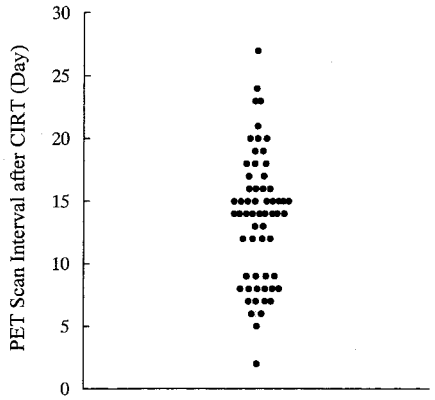

Fig. 1 Positron emission tomography (*PET*) scan interval after carbon ion radiotherapy (*CIRT*) in 62 patients.

A baseline MET-PET was performed before initiation of carbon ion radiotherapy, and MET PET was repeated within 1 month (mean, 14 ± 5 days; Fig. 1) after the completion of carbon ion radiotherapy. Patients fasted for at least 4 h before PET imaging. Before MET injection, all of the patients underwent transmission scan for one (ECAT EXACT HR+) or two bed positions (ECAT EXACT 47) including lesion site, each bed position for 20 and 10 min for ECAT EXACT HR+ and ECAT EXACT 47, respectively. Static emission data for the same positions were obtained from 23 min after the i.v. administration of ~740 MBq of MET. For the difference of sensitivity in PET scanners, static emission scans were performed for 30 min in ECAT EXACT HR+ and 15 min in ECAT EXACT 47 for each bed position, respectively.

Analysis of the PET Images. The PET images were interpreted by two nuclear medicine physicians (H. Z. and K. Y.). For quantitative evaluation, regions of interest with a diameter of 1 cm were manually drawn over the tumor in the transaxial slice with maximum MET uptake in the baseline scan, with the understanding that in these heterogeneous tumors, this scan reflects the most metabolically active area of the tumor. The most metabolically active areas are thought to reflect tumor regions with more aggressive tumor. The inherent assumption was made that the overall behavior of the tumor is predicted by the activity of the most aggressive regions. The regions of interest s for the background radioactivity measurement were drawn on the homologous contralateral or surrounding normal tissue. Tumor-to-nontumor ratios (T/N ratio) were calculated using the following formula: T/N ratio = mean counts per pixel of tumor regions of interest/mean counts per pixel of normal tissue regions of interest.

Statistical Analysis. Survival time was defined as the time interval from the initiation of carbon ion radiotherapy until death or the end of this study. Univariate analysis was performed to determine the association among clinical, imaging, and pathological parameters using the log-rank test to compare survival curves for dichotomous variables. Multivariate analysis was performed using the Cox proportional hazards model. The Cox model was used in the multivariate survival regression analysis, adjusting survival comparisons for various factors that

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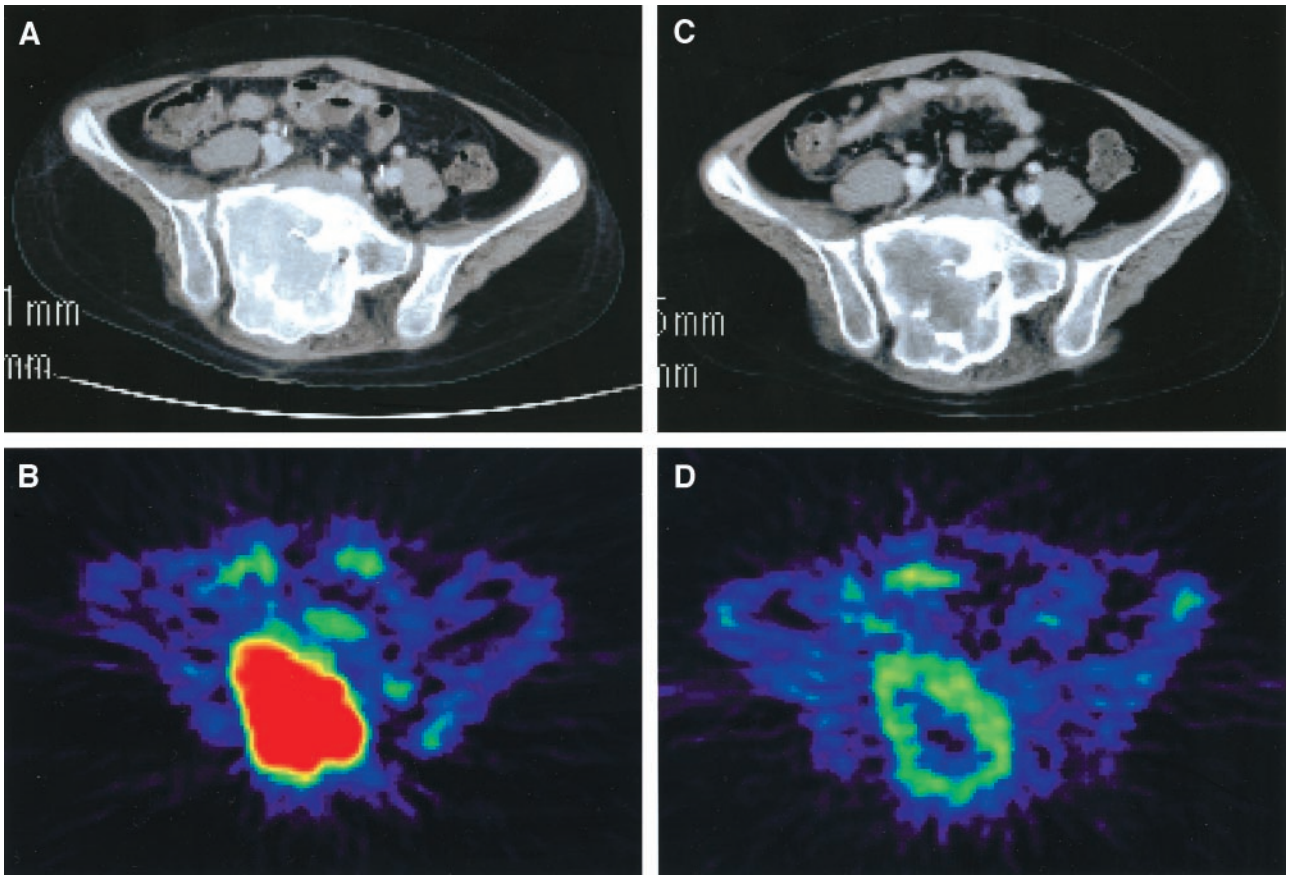

Fig. 2 A 13-year-old male who received 70.4 GyE to the whole pelvis for sacral ostaesarcoma. *A,* computed tomography (CT) image showed a large mass in the sacrum. *B,* baseline whether L-[methyl-^{11}C]-methionine uptake demonstrated a high homogeneous mass in the region as shown in CT image. *C,* CT image still revealed a large mass after CIRT. *D,* after CIRT, a moderately large region of absent whether L-[methyl-^{11}C]-methionine uptake was seen within the tumor, indicative of necrosis.

otherwise influenced survival. Hazards ratio estimates were computed from the model. This analysis permits the examination of the influence of the PET-measured T/N ratio information, whereas controlling for the impact of tumor type, stage, and presentation, as well as other potentially relevant patient information such as chemotherapy before carbon ion radiotherapy, metastases, and recurrence after carbon ion radiotherapy, age, and gender. A difference with $P < 0.05$ was considered significant. The statistical calculations were performed in Statview software (Version 5.0; SAS Institute Inc., Cary, NC).

RESULTS

MET PET Imaging. Almost all of the tumors were clearly visible in the baseline MET PET study (Fig. 2). Delineation of several tumors was somewhat difficult to interpret because of the low MET uptake and the possible influence of previous chemotherapy. However, the tumors could still be delineated from the image when the anatomical location of the tumor was known by the authors before image interpretation. Overall, the tumor baseline MET PET uptake showed a mean T/N ratio of 4.58 ± 2.57. After CIRT, the mean T/N ratio decreased to 3.11 ± 2.04 significantly ($P = 0.00029$).

Univariate Survival Analysis. The univariate survival analysis results are presented in Tables 3–5. In all 12 of the variables, 5 variables of metastases post-CIRT, recurrence post-CIRT, baseline uptake, post-CIRT uptake, and percentage of change uptake were proved to be significant at the 0.05 level. Patients with metastases after CIRT had a significantly lower survival than those without metastases ($P = 0.0014$), and patients with recurrence after CIRT showed a significantly lower survival than those without recurrence after CIRT ($P = 0.0014$).

The influence of tumor baseline uptake, and post-CIRT uptake of MET and its percentage of change after CIRT (%change) were explored for various baseline T/N ratios (Fig. 3*A*), post-CIRT uptake (Fig. 4*A*), and percentage of change cutoff values (Fig. 5*A*). The most discriminative cutoff point for prognosis proved to be at a baseline T/N ratio of 6, post-CIRT T/N ratio of 4.4, and a percentage of change of 30%, respectively.

The overall median survival time was 20 months (range, 5–60 months). Patients with a baseline T/N ratio of ≤6 had a much better survival (2-year survival rate of 69.4%) than patients with a baseline T/N ratio >6 (2-year survival rate of 32.3%; $P = 0.010$; Fig. 3*B*). Patients with a post-CIRT ratio of

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Table 3 Prognostic factors including baseline uptake in the patients using univariate (log-rank test) and multivariate (Cox proportional hazards) analysis

	Univariate	Multivariate		
Variable	*P*	HR[a]	95% CI	*P*
Age (>50 *vs.* ≤50 yrs)	0.74	0.94	0.31–2.87	0.92
Gender (female *vs.* male)	0.086	0.57	0.16–1.97	0.37
Size (>10 *vs.* ≤10 cm)	0.15	2.42	0.69–8.51	0.17
Tumor type (bone *vs.* soft tissue)	0.83	2.86	0.74–11.03	0.13
Stage (>IIB *vs.* ≤IIB)	0.98	0.38	0.07–2.18	0.17
Presentation (primary *vs.* others)	0.95	3.65	0.19–69.27	0.39
CMT preCIRT (with *vs.* without)	0.41	0.81	0.25–2.61	0.73
META postCIRT (with *vs.* without)	0.0014	6.11	1.61–23.09	0.0077
Recurrence postCIRT (with *vs.* without)	0.014	4.31	1.28–14.46	0.018
Baseline uptake (>6.0 *vs.* ≤6.0)	0.012	0.18	0.06–0.56	0.0031

[a] HR, hazard ratio; CI, confidence interval; CMT preCIRT, chemotherapy before carbon ion radiotherapy; META postCIRT, metastases post carbon ion radiotherapy; Recurrence postCIRT, recurrence post carbon ion radiotherapy; Baseline uptake, tracer uptake before carbon ion radiotherapy.

≤4.4 had a better survival (2-year survival rate of 63.7%) than that with a post-CIRT ratio >4.4 (2-year survival rate of 41.3%; $P = 0.012$; Fig. 4*B*). On the other hand, patients with a percentage of change of >30% had a much better survival (2-year survival rate of 74.6%) than those with a percentage of change ≤30% (2-year survival rate of 41.6%; $P = 0.049$; Fig. 5*B*).

Multivariate Survival Analysis. To evaluate the impact of the metabolic response to carbon ion radiotherapy on overall survival rates, Cox proportional hazards model analysis was performed. Factors significantly influencing survival in univariate analysis were included in the Cox models. In addition, the other factors (patient age, gender, tumor size, stage, tumor

Table 4 Prognostic factors including postCIRT uptake in the patients using univariate (log-rank test) and multivariate (Cox proportional hazards) analysis

	Univariate	Multivariate		
Variable	*P*	HR[a]	95% CI	*P*
Age (>50 *vs.* ≤50 yrs)	0.74	1.01	0.34–3.54	0.88
Gender (female *vs.* male)	0.086	0.43	0.14–1.33	0.14
Size (>10 *vs.* ≤10 cm)	0.15	2.61	0.77–8.81	0.12
Tumor type (bone *vs.* soft tissue)	0.83	1.88	0.50–7.10	0.35
Stage (>IIB *vs.* ≤IIB)	0.98	0.90	0.16–5.12	0.35
Presentation (primary *vs.* others)	0.95	10.03	0.52–192.72	0.13
CMT preCIRT (with *vs.* without)	0.41	0.57	0.19–1.76	0.33
META postCIRT (with *vs.* without)	0.0014	6.12	1.81–23.67	0.004
Recurrence postCIRT (with *vs.* without)	0.014	2.17	0.66–7.22	0.20
PostCIRT uptake (>4.4 *vs.* ≤4.4)	0.012	0.16	0.049–0.55	0.0032

[a] HR, hazard ratio; CI, confidence interval; CMT preCIRT, chemotherapy before carbon ion radiotherapy; META postCIRT, metastases post carbon ion radiotherapy; Recurrence postCIRT, recurrence post carbon ion radiotherapy; PostCIRT uptake, tracer uptake post carbon ion radiotherapy.

Table 5 Prognostic factors including percentage of change uptake in the patients using univariate (log-rank test) and multivariate (Cox proportional hazards) analysis

	Univariate	Multivariate		
Variable	*P*	HR[a]	95% CI	*P*
Age (>50 *vs.* ≤50 yrs)	0.74	0.88	0.28–2.80	0.84
Gender (female *vs.* male)	0.086	0.60	0.20–1.82	0.37
Size (>10 *vs.* ≤10 cm)	0.15	2.01	0.62–6.96	0.23
Tumor type (bone *vs.* soft tissue)	0.83	1.84	0.50–6.72	0.36
Stage (>IIB *vs.* ≤IIB)	0.98	0.44	0.074–2.56	0.34
Presentation (primary *vs.* others)	0.95	2.59	0.15–44.22	0.51
CMT preCIRT (with *vs.* without)	0.41	0.63	0.21–1.89	0.41
META postCIRT (with *vs.* without)	0.0014	3.58	0.99–12.88	0.051
Recurrence postCIRT (with *vs.* without)	0.014	3.23	0.96–10.83	0.058
%change uptake (>30% *vs.* ≤30%)	0.049	0.42	0.11–1.64	0.21

[a] HR, hazard ratio; CI, confidence interval; CMT preCIRT, chemotherapy before carbon ion radiotherapy; META postCIRT, metastases post carbon ion radiotherapy; Recurrence postCIRT, recurrence post carbon ion radiotherapy; %change uptake, percent tracer uptake change after CIRT.

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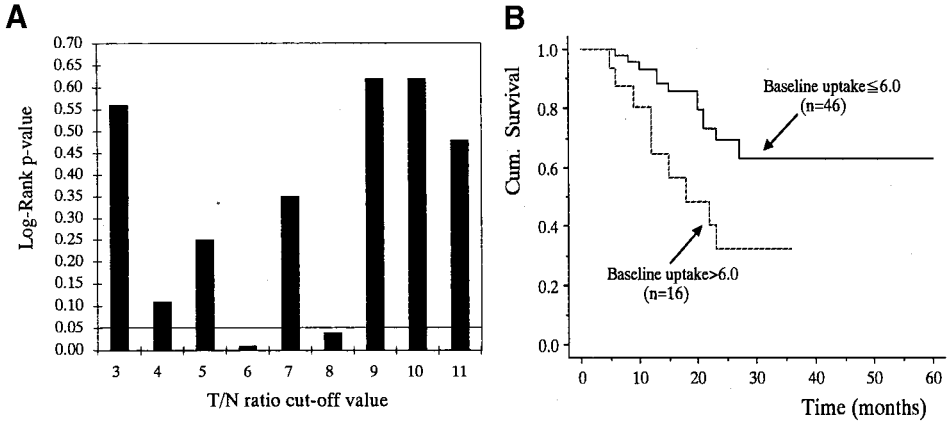

Fig. 3 *A*, relationship between various baseline tumor:nontumor ratio (*T/N ratio*) cutoff values and their discriminative value for survival, as assessed by the log-rank test. Tumor whether L-[methyl-^{11}C]-methionine uptake T/N ratios of 6 and 8 gave log-rank *P*s < 0.05. *B*, cumulative (*Cum.*) survival of the 62 patients according to the baseline whether L-[methyl-^{11}C]-methionine uptake T/N ratio cutoff of 6.

presentation, and previous treatment) were included because of their high potential effect on patient survival. Variables were dichotomized as categorical variables (age $>$ *versus* $<$ 50, gender female *versus* male, tumor size $>$ *versus* $<$ 10 cm, tumor type bone *versus* soft tissue, stage above *versus* below IIB, tumor presentation primary *versus* others, chemotherapy before CIRT with *versus* without, metastases after CIRT with *versus* without, recurrence after CIRT with *versus* without, baseline MET uptake $>$ *versus* $<$ 6.0, post-CIRT MET uptake $>$ *versus* $<$ 4.4, and percentage of change of MET uptake $>$ *versus* $<$ 30%). Tables 3–5 showed the results of this analysis of survival as a function of those variables. Table 3 showed that metastases ($P = 0.0077$) and recurrence ($P = 0.018$) after CIRT, and baseline MET uptake ($P = 0.0031$) were the significant predictors of overall survival in the multivariate predication model using our patient data. If post-CIRT MET uptake instead of baseline MET uptake is used in multivariate analysis, as shown in Table 4, only metastases ($P = 0.004$) and post-CIRT MET uptake ($P = 0.0032$) were the significant predictors of overall survival. However, none of the factors were significant predictors when the percentage of change included uptake into multivariate analysis (Table 5). The baseline MET uptake and post-CIRT MET uptake were the most highly significant. None of the other prognostic factors included in the analysis (age, gender, tumor size, tumor type, stage, tumor presentation, and previous chemotherapy) were found to be statistically significant. The hazard ratios and the 95% confidence interval associated with these factors were also listed in Table 3–5.

DISCUSSION

The MET PET method has been found recently to be feasible for the evaluation of treatment effects after medical therapy. In a large series of pituitary adenomas and in some meningiomas, a decrease in the uptake of MET has been shown to represent a positive treatment effect (24). This technique has also been helpful in assessing the effect of chemotherapy earlier than is feasible with other methods (25, 26). Furthermore, MET PET has been shown to improve the quality of follow-up of other treatment such as brachytherapy (27, 28), and to permit evaluation of the effect of synchronous radiochemotherapy treatment in patients in whom CT has revealed no notable changes (28, 29). Also, MET PET has proved effective in the differentiation of recurrence of tumor and radiation-induced tissue changes (30). In experimental studies, MET PET has been sensitive enough to detect and differentiate viable cells in a residual tumor mass after radiotherapy (31). All of these findings could explain the relationship between MET uptake on PET

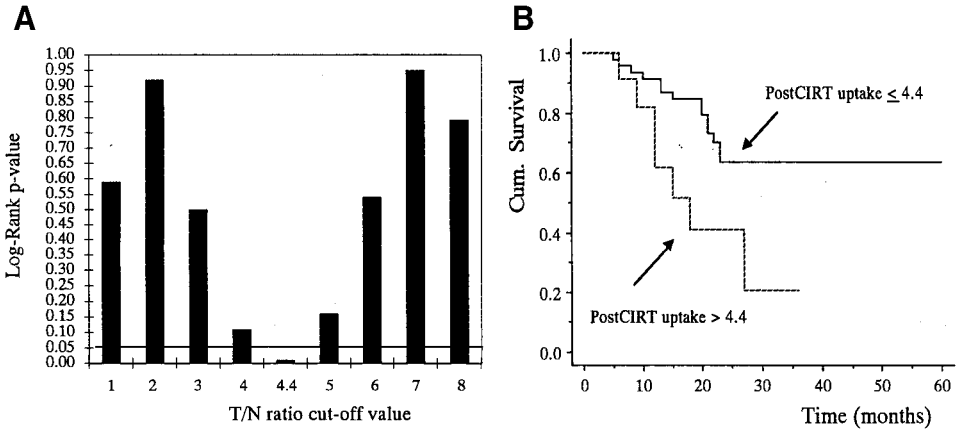

Fig. 4 *A*, relationship between various post-carbon ion radiotherapy tumor:nontumor ratio (*T/N ratio*) cutoff values and their discriminative value for survival, as assessed by the log-rank test. Tumor whether L-[methyl-^{11}C]-methionine uptake T/N ratios of 4.4 gave log-rank *P*s <0.05. *B*, cumulative (*Cum.*) survival of the 62 patients according to the post carbon ion radiotherapy whether L-[methyl-^{11}C]-methionine uptake T/N ratio cutoff of 4.4.

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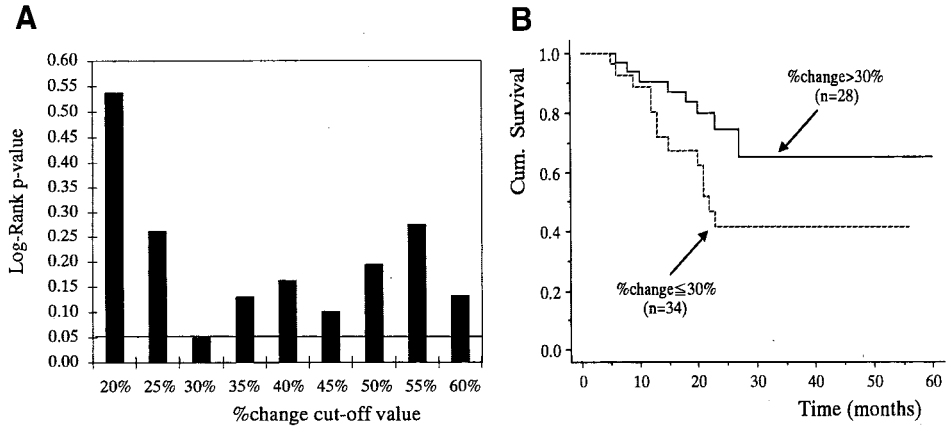

Fig. 5 *A*, relationship between various percentage of change cutoff values and the discriminative value for survival, as assessed by the log-rank test. Tumor whether L-[methyl-^{11}C]-methionine uptake change of 30% after carbon ion radiotherapy gave log-rank *P*s <0.05. *B*, cumulative (*Cum.*) survival of the 62 patients according to the whether L-[methyl-^{11}C]-methionine uptake change cutoff of 30%.

and biological aggressiveness, and, thus, prognosis. In our study, we chose to focus on MET PET to evaluate the outcome of the novel carbon ion radiotherapy in patients with bone and soft tissue sarcomas. In this univariate survival analysis of the 62 patients, we were able to demonstrate that baseline MET uptake T/N ratio of 6, post-CIRT MET uptake T/N ratio of 4.4, and its change of 30% after therapy had the best discriminative value for survival. Patients with a baseline MET uptake T/N ratio of $\leq$6 and post-CIRT MET uptake T/N ratio of $\leq$4.4 had a better survival than the patients with larger T/N ratios. The average reduction of tumor MET uptake was 20.4% after carbon ion radiotherapy. Patients with a reduction of MET uptake $>$30% showed a better survival than patients with a reduction $\leq$30%. This is our first experience to predict the prognosis using MET PET in the very early stage after CIRT. Although MET PET has been proposed to have less influence from inflammation as compared with FDG PET (16), in this study we still considered that inflammation due to the treatment might have slightly increased the post-CIRT MET uptake within 1 month and, thus, affected the percentage of change of uptake to some extent. Even so, this study initially verified that it was possible to determine the cutoff value of MET uptake (within 1 month) to estimate patient prognosis. Gudjonsson *et al.* (32) studied a small population of 19 patients with intracranial meningiomas who underwent high-energy proton irradiation treatment. In 15 of the 19 patients, MET uptake was reduced 36 months after irradiation compared with the pretreatment uptake of the tracer. In the total patient group the average reduction was 19.4%. It showed that proton beam irradiation of meningiomas had an inhibitory effect on the MET uptake, although tumor size remained unchanged. The combination of unchanged tumor morphology and a reduction in MET uptake after irradiation suggested that MET PET might enable earlier evaluation of the treatment effect than is possible with CT or magnetic resonance imaging. It has been also reported that tumor size measured by CT scan could not be considered an adequate prognostic factor in extraosseous solid tumors, soft tissue tumors, and Ewing's sarcoma (33). In our study, CT scan was preformed at 6 months after CIRT, and the changes of tumor size were not statistically significant because most of the sarcomas were osseous solid tumors. Another small population of 16 patients with breast cancer was investigated by Jansson *et al.* (25), 5 patients with FDG PET only, 7 patients with both MET and FDG PET, and 4 patients with only MET PET before polychemotherapy. PET showed a significant decrease in tracer uptake after the first course of therapy in 8 of 12 patients with a clinical response after completion of chemotherapy. They concluded that therapy response could be determined earlier with PET than with any other conventional therapy evaluation method.

Our patient population was large enough to allow multivariate survival analysis, and we have shown the most statistical significance of the baseline MET uptake and post-CIRT MET uptake when adjusting for possible confounding factors (Tables 3 and 4). The prognostic impact of the percentage of change of MET uptake after carbon ion radiotherapy was not significant in this Cox model. In addition, a strong tendency was observed that the baseline MET uptake in patients with metastases was higher than that of patients without metastases after carbon ion radiotherapy, although the difference was not significant ($P = 0.055$; data not presented). Similarly, there was no significant difference of that for post-CIRT MET uptake. The local recurrence was well controlled in this clinical trial. In all 62 of the patients, only 7 patients were found recurrence after CIRT. No correlation was observed in baseline MET uptake or post-CIRT MET uptake between patients with and without recurrence ($P = 0.47$; data not presented). The association between MET PET results and duration until relapse was not found, probably due to the very small recurrent population. It is known that the bone and soft tissue sarcomas population represents a more heterogeneous group of tumors, both between tumor subtypes and tumor grade, and between patients in each group. Tumor heterogeneity in an individual patient is thought to be a major contributor to the failure of conventional clinical diagnosis. On the other hand, bone and soft tissue sarcomas are composed of mesenchymal elements that contain various amounts of relatively metabolically inactive tissue such as myxoid ground substance, osteoid, fluid, and necrosis. They also contain population of different cellularity, tissue type, and levels of dedifferentiation. The risk of metastases and recurrence of bone and soft tissue sarcomas has been correlated to different factors. The most important are pathological type and grade, followed by individual patient characteristics. Because of these characteristics, it was difficult

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to predict the risk of post-therapeutic metastases or recurrence using MET uptake in this study.

Until now, several previous studies have shown that pretreatment FDG PET can predict tumor behavior and prognosis of a variety of tumor types (34–38). However, some of these studies are hindered by the heterogeneity of their treatment options. The different therapeutic regimens, chosen because there were tumors in different stages, might have caused a bias in those studies. In this study, all of the patients received the same treatment of CIRT and underwent both pre- and post-treatment PET studies. Our study initially showed a statistically significant association between either baseline MET uptake or post-CIRT MET uptake and survival. In particular, this study verified that the post-CIRT MET T/N ratio uptake could be a significant independent predicator of patient survival in this sarcoma population. It can provide important prognostic information in a very early stage after treatment, whereas FDG PET can only be performed a few months after treatment to avoid false-positive findings on FDG images. Thus, this study suggested that pretreatment MET uptake can be used to evaluate therapeutic options in treatment planning for patients who are at highest risk for poor survival and who might benefit from carbon ion radiotherapy regardless of other prognostic factors. Furthermore, post-therapeutic MET uptake can also be used to monitor the effect of a therapy protocol or to plan modification of treatment in poor responders.

The potential of carbon ion radiotherapy in combination with the potential power of MET PET encouraged us to examine whether MET PET can play a role in the early evaluation of patient outcome with bone and soft tissue sarcomas. The current study has established a model for the correlation between MET PET and the outcome of patients with bone and soft tissue sarcomas treated by carbon ion radiotherapy. This method may contribute to the other clinical trials on various tumors in our institute for the same purpose.

In conclusion, MET uptake as measured by the T/N ratio, either baseline or post-CIRT MET uptake was an independent predictor of survival in patients with bone and soft tissue sarcomas treated by carbon ion radiotherapy. MET PET provided a promising method for early evaluation of prognosis of patients with bone and soft tissue sarcomas treated by carbon ion radiotherapy.

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