inhibits Class III and Class V RTKs, including PDGF receptors, VEGF receptors, KIT and FLT3, with low nanomolar potency,[30] other growth factor-mediated signals might be inhibited by sunitinib. Further investigation is necessary to clarify the precise mechanism of action of sunitinib and its clinical efficacy against bone metastases.

Conclusion

In conclusion, we demonstrated that oral administration of a clinically achievable dose of sunitinib prevented the growth of RCC bone metastases *in vivo*. Because RCC cell lines are resistant to clinically and preclinically achievable plasma concentrations *in vitro*, prevention of osteoclast activity and/or maturation is one of the mechanisms of growth inhibition in metastatic bone lesions. Our study supports the use of sunitinib as an initial treatment for RCC patients with bone metastasis.

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BJU INTERNATIONAL

Clinical efficacy and prognostic factors for overall survival in Japanese patients with metastatic renal cell cancer treated with sunitinib

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Study Type – Therapy (case series)
Level of Evidence 4

What's known on the subject? and What does the study add?
A randomized prospective phase III clinical trial for systemic treatment-naïve metastatic renal cell cancer (RCC) patients demonstrated the superiority of sunitinib over interferon with an acceptable safety profile. However, a commonly asked question is whether patients with RCC in clinical trials are representative of those with this disease being seen in ordinary clinical practice.

To our knowledge, this is the first report of sunitinib for the Japanese patients with metastatic RCC in ordinary clinical practice. The estimated median PFS and OS in this study were 9.3 and 32.2 months, respectively. The application of the MSKCC model distinctly separated OS curves ($P<0.001$), suggesting that MSKCC prognostic factors might be still valid to predict survival in metastatic RCC in the era of molecular targeted therapy.

OBJECTIVES

- To report the treatment efficacy and safety profile of sunitinib for patients with metastatic renal cell carcinoma (RCC) in ordinary clinical practice.
- In addition, to investigate the prognostic clinicopathological factors in these patients.

PATIENTS AND METHODS

- The present study consisted of native Japanese patients with metastatic RCC, comprising 29 pretreated and 34 systemic treatment-naïve patients.
- Univariate and multivariate analyses were performed by the log-rank test and the Cox proportional hazards model, respectively.

RESULTS

- Estimated median progression-free survival and overall survival (OS) were 9.3 months (95% confidence interval, CI, 5.0–13.7) and 32.2 months (95% CI, 24.4–40.0), respectively.
- Among the patients pretreated before sunitinib, two patients were treated with initialized systemic therapy with sorafenib and the remaining 27 were initialized with interferon-α.
- The OS from the initial systemic therapy of the patients in pretreated groups was 79.6 months (95% CI, 14.6–144.5).
- The application of the Memorial Sloan-Kettering Cancer Center model distinctly separated the OS curves ($P<0.001$).
- The most common grade 3 adverse events were fatigue (53%), thrombocytopaenia (48%), hand-foot syndrome (16%), anaemia (20%), hypertension (10%) and leucopaenia (9%), although these events were manageable and reversible.

CONCLUSIONS

- Sunitinib has a favourable efficacy/safety profile for Japanese metastatic RCC patients in clinical practice.
- The estimated median OS was >2 years with acceptable tolerability.
- The median OS from the initial systemic therapy of the pretreated patients was >6 years.
- Memorial Sloan-Kettering Cancer Center prognostic factors still appear to be valid for predicting survival in metastatic RCC in the era of molecular targeted therapy.

KEYWORDS

MSKCC score, outcome, prognostic factor, renal cell cancer, sunitinib

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INTRODUCTION

Sunitinib is an orally administered, multitargeted tyrosine kinase inhibitor of vascular endothelial growth factor receptors 1–3 and platelet-derived growth factor receptors α and β [1,2]. A randomized, prospective phase III clinical trial for systemic treatment-naïve metastatic RCC patients showed the superiority of sunitinib over interferon with respect to objective response rate (ORR) (31% vs 6%), progression-free survival (PFS) (11 vs 5 month) and overall survival (OS) (26.4 vs 21.8 months), with an acceptable safety profile [3,4]. These results indicate an improved prognosis in patients with RCC in the era of targeted therapy.

The first Japanese phase II study of single-agent sunitinib, which was conducted in 51 patients with metastatic RCC, also showed efficacy and tolerability comparable to that observed in Western patients [5]. In that study, the ORR was 52.0% in treatment-naïve patients, 53.8% among cytokine-pretreated patients and 52.9% in the overall population [5]. As a result of these findings, the multinational approvals of sunitinib for treatment of first- and second-line advanced RCC now include Japan.

However, a commonly asked question is whether patients with RCC in clinical trials are representative of those with this disease who are seen in ordinary clinical practice. Many patients with RCC do not meet the inclusion criteria, particularly those with a poorer prognosis. Patients with a poor performance status (PS), brain metastasis or other clinical parameters predicting shorter survival are often excluded from clinical trials. In the present study, we report the treatment efficacy and safety profile of sunitinib for patients with metastatic RCC in ordinary clinical practice. In addition, we also investigated the prognostic clinicopathological factors associated with OS in this population.

PATIENTS AND METHODS

PATIENT POPULATION

The present study consisted of native Japanese patients with metastatic RCC. The study group comprised 29 patients pretreated before sunitinib and 34 systemic treatment-naïve patients, who were all undergoing treatment at the Akita University Medical Center or the Cancer Institute Hospital, Japanese Foundation for Cancer Research, from March 2006 until January 2011. The patients included in the retrospective study were not consecutive. In this period, patients with metastatic renal cell cancer were treated by sunitinib or interferon-α as an initial treatment and sunitinib or sorafenib as a secondary treatment, fundamentally. All RCC patients were diagnosed on the basis of histological analysis of specimens obtained by radical nephrectomy or ultrasonographically-guided needle biopsy.

TREATMENT AND ASSESSMENT

Each patient signed a protocol-specific informed consent, approved by an institutional review board, in accordance with national and institutional guidelines. Sunitinib was administered orally at a dose of 50 mg daily, for 4 weeks followed by a 2-week rest period. Sunitinib was discontinued in the case of grade 3 or 4 toxicity and was re-administrated when toxicity was ≤grade 1. In the case of grade 3 non-haematological toxicity or grade 4 haematological toxicity, a dose reduction of sunitinib (to 37.5 mg and then to 25 mg) was allowed. The response was assessed by CT scans performed at least every two cycles of treatment, in accordance with the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.0 [6]. Safety and tolerability were assessed at regular intervals with adverse event monitoring using National Cancer Center Common Toxicity Criteria for Adverse Events, version 3.0, to document adverse events and classify severity; haematology and biochemistry; body weight; vital signs; and Eastern Cooperative Oncology Group (ECOG) PS.

STATISTICAL ANALYSIS

OS and PFS were measured from the initial administration of sunitinib until death from any cause, and from the date of initial administration of sunitinib until objective tumour progression or death, respectively. Time-to-event distributions were estimated using Kaplan–Meier curves. Univariate and multivariate analyses were performed by the log-rank test and the Cox proportional hazards model, respectively, aiming to assess the relationship between OS and the laboratory, as well as clinical variables. The laboratory variables included haemoglobin (Hb; male: <13 g/dL vs ≥13 g/dL; female: <11.5 g/dL vs ≥11.5 g/dL); neutrophil count (≤6600/μL vs >6600/μL); platelet count ($\leq 4.5 \times 10^5$/μL vs $>4.5 \times 10^5$/μL); corrected calcium (≤10 mg/dL vs >10 mg/dL) and lactate dehydrogenase (LDH; $\leq 1.5 \times 230$ IU/dL vs $>1.5 \times 230$ IU/dL). The clinical variables included ECOG PS (≤1 vs >1); time from diagnosis of RCC to systemic therapy initiation (<12 months vs ≥12 months); time from diagnosis to sunitinib initiation (<12 months vs ≥12 months); history of nephrectomy (no vs yes); number of metastatic sites (1 vs ≥1); presence or absence of lung, bone, lymph node and brain metastasis (yes vs no); tumour grade (I, II vs III); clear cell histology (clear cell or no clear cell histology); and the presence or absence of sarcomatoid component (without sarcomatoid component vs with sarcomatoid component). The corrected serum calcium level was calculated using Payne's formula [7]. SPSS software was used for statistical analysis (SPSS for Windows, version 17.0, SPSS Inc., Chicago, IL, USA).

RESULTS

PATIENT CHARACTERISTICS

The characteristics of the patients treated with sunitinib are shown in Table 1. Among the patients pretreated before sunitinib, two patients were initialized with systemic therapy with sorafenib because the approval of sorafenib had been given 3 months before that of sunitinib in Japan. The remaining 27 patients were initialized with interferon-α. The median (interquartile range) follow-up from the initial systemic therapy and sunitinib start was 17.3 (8.0–40.1) months and 7.7 (3.3–16.4) months, respectively. Overall, 19 (30%) patients showed a partial response and 32 (51%) patients showed stable disease longer than 3 months by RECIST criteria, indicating that 81% of the patients experienced a clinical benefit from sunitinib. Progression within 3 months was observed in 12 (19%) patients, and none experienced an early treatment failure before the initial assessment.

TABLE 1 Patient characteristics

Variable	Value
Age (years), median (range)	62 (27–81)
Sex, *n* (%)	
Male	50 (79)
Female	13 (21)
Diagnosis to systemic therapy, *n* (%)	
1 year	16 (25)
>1 year	47 (75)
Diagnosis to sunitinib, *n* (%)	
≤1 year	30 (48)
>1 year	33 (52)
Previous systemic therapy, *n* (%)	
Yes	29 (46)
No	34 (54)
Nephrectomy, *n* (%)	
Done	49 (78)
None	14 (22)
Number of metastatic sites, *n* (%)	
1	31 (49)
<1	32 (51)
Metastatic sites, *n* (%)	
Lung	43 (68)
Liver	16 (25)
Bone	19 (30)
Lymph node	24 (38)
Brain	5 (8)
Histological subtype, *n* (%)	
Clear cell type	51 (81)
Papillary type	6 (10)
Chromophobe type	2 (3)
Others	4 (6)

TABLE 2 Univariate analysis of factors associated with overall survival

Category	*N*	Median OS	95% CI	*P*
ECOG PS				
0–1	52	35.8	12.5–44.2	
≤2	11	6.03	2.8–9.2	0.001
Haemoglobin				
Normal	26	Not reached		
Anaemia	37	9.8	5.1–14.6	0.002
Calcium				
>10 mg/dL	56	35.0	26.7–43.3	
≤10 mg/dL	7	7.0	0.0–15.2	0.008
Lactate dehydrogenase				
≥1.5 × ULN	51	36.0	27.6–44.4	
<1.5 × ULN	12	2.3	0–8.2	<0.001
Dx to systemic Tx				
≤1 year	16	Not reached		
>1 year	47	12.5	3.2–21.8	0.037
Neutrophil count				
≥ULN	55	34.8	26.4–43.2	
<ULN	8	4.9	0–10.6	0.054
Platelet count				
≥ULN	59	33.9	25.8–42.1	
<ULN	4	3.4	1.9–4.8	0.051
Dx to sunitinib				
≤1 year	30	38.2	12.3–21.3	
>1 year	33	10.6	6.5–14.7	0.106
Previous systemic therapy				
Yes	29	Not reached		
No	34	28.8	19.8–37.8	0.719
Nephrectomy				
Done	49	36.1	27.4–44.7	
None	14	10.3	5.8–15.1	0.039
Number of metastatic sites				
1	31	38.5	31.4–45.6	
<1	32	9.8	4.6–15.0	0.002
Lung metastasis				
Yes	43	37.0	27.3–46.8	
No	20	31.2	22.5–40.0	0.188
Liver metastasis				
Yes	16	7.0	4.0–9.9	
No	47	Not reached		0.082
Bone metastasis				
Yes	19	11.6	7.5–15.9	
No	44	33.4	24.5–42.3	0.328
Lymph node metastasis				
Yes	24	10.6	7.7–13.5	
No	39	35.5	26.2–44.7	0.171
Brain metastasis				
Yes	5	6.4	0.0–13.1	
No	58	35.3	27.2–43.4	0.001
Grade				
1, 2	32	Not reached		
3	19	10.3	8.2–12.3	0.158
Histology				
Clear cell	51	33.3	24.9–41.8	
Non-clear cell	12	16.9	2.9–17.6	0.961
Sarcomatoid				
Without sarcomatoid	53	35.3	26.9–43.8	
With sarcomatoid	10	7.0	7.0–7.2	0.014

ECOG PS, Eastern Cooperative Oncology Group performance status; OS, overall survival; Dx, diagnosis; Tx, treatment; ULN, upper limit of normal range.

PFS AND OS

Estimated median PFS and OS were 9.3 months (95% CI, 5.0–13.7) and 32.2 months (95% CI, 24.4–40.0), respectively. Estimated 12-month PFS and 18-month OS rates were 47.8% and 53.7%, respectively. During follow-up, 28 (44%) patients died from RCC. In addition, we investigated the OS from the initial systemic therapy given to the patients in pretreated groups, and their median OS was 79.6 months (95% CI, 14.6–144.5).

PROGNOSTIC FACTORS ASSOCIATED WITH OS PERIOD

Finally, we investigated the prognostic factors associated with overall survival time. Univariate analysis showed that the various pretreatment factors were associated with worse OS (Table 2). All the factors in a

Memorial Sloan-Kettering Cancer Center (MSKCC) score [8], which include ECOG PS >1 ($P = 0.001$), low Hb levels ($P = 0.002$), high corrected calcium levels ($P = 0.008$) and high LDH levels ($P < 0.001$), and ≤12 months between diagnosis and initial systemic therapy ($P = 0.037$), were associated with worse OS (Table 2). Multivariate analysis by a Cox proportional hazard model showed that low Hb and high LDH were independently associated with poorer OS among MSKCC scores (Table 3). In addition, brain metastasis and no history of nephrectomy were also associated with poorer OS.

Because the patients included in the present study represent a mixture of treatment naïve and refractory RCC patients, we analyzed these two groups separately. The application of the MSKCC model (using PS, Hb, calcium, LDH and time from diagnosis to initiation of systemic therapy) to stratify patients into three risk groups for the treatment naïve patients (favourable: no risk factors, 12%, $n = 4$; intermediate: one or two risk factors, 59%, $n = 20$; poor: three to five risk factors, 29%, $n = 10$) distinctly separated the OS curves (Fig. 1A). On the other hand, the application of the MSKCC model for the treatment refractory patients [9] (using Hb, calcium and PS) to stratify patients into three risk groups (favourable: no risk factors, 13%, $n = 12$; intermediate: one risk factor, 62%, $n = 13$; poor: two or three risk factors, 25%, $n = 4$) also distinctly separated the OS curves (Fig. 1B).

TABLE 3 Multivariate analyses associated with poor survival

Parameter	Hazard ratio	95% CI	*P*
Laboratory data			
Haemoglobin <LLN	2.658	1.064–7.547	0.044
Lactate dehydrogenase >1.5 × ULN	2.678	1.105–6.490	0.029
Clinical data			
Brain metastasis	6.499	2.277–18.555	0.001
No history of nephrectomy	3.086	1.287–7.407	0.012

LLN, lower limit of normal range; ULN, upper limit of normal range.

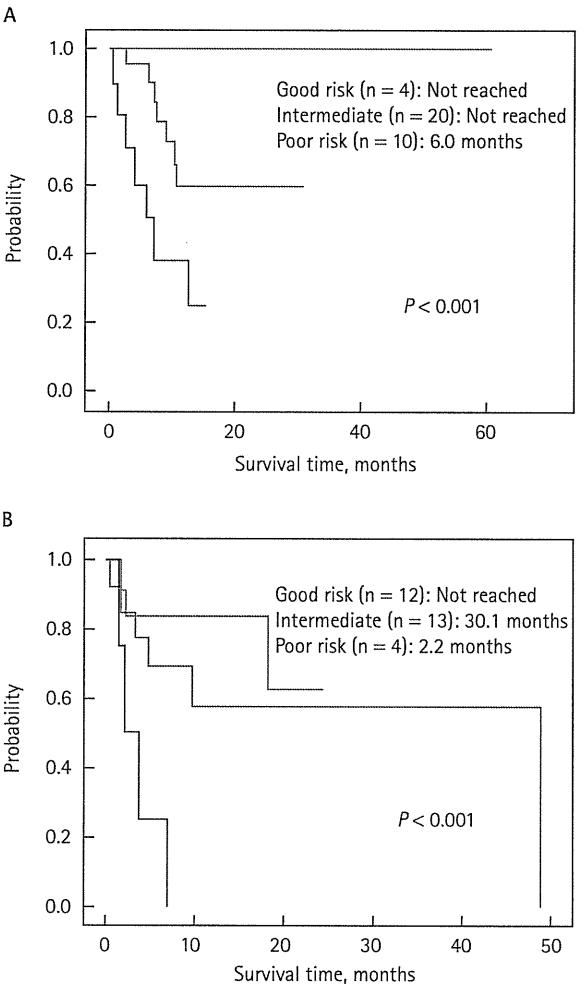

FIG. 1.
Stratification of the overall survival for the patients with metastatic renal cell cancer by the respective Memorial Sloan-Kettering Cancer Center (MSKCC) risk scores. Stratification of the treatment naïve (A) and the treatment refractory (B) patients by MSKCC risk scores.

ADVERSE EFFECTS

All 63 patients experienced treatment-related adverse events, most of which were grade 1 or 2 in severity. The most common grade 3 or 4 adverse events were fatigue (53%), thrombocytopaenia (48%), hand-foot syndrome (16%), anaemia (20%), hypertension (10%) and leucopaenia (9%). Most of these adverse events were manageable and reversible. Although most patients were able to resume therapy after treatment modification, only two (3%) patients and one (1%) patient discontinued because of adverse fatigue and hand-foot syndrome events, respectively. In particular, the incidence of hypothyroidism (76%) was remarkable. Among these patients, levothyroxine had to be administered to maintain thyroid function in 37 (59%) patients.

DISCUSSION

In the present study, we retrospectively analyzed the ORR, PFS, OS and prognostic factors in 63 native Japanese patients, suggesting that the results of the present study could reflect current clinical practice in metastatic RCC in our country. In this cohort, the ORR and clinical benefit (partial response plus stable disease >12 weeks) from sunitinib were 30% and 81%, respectively. These are somewhat lower than the results of a Japanese phase II study (52% and 78%) [5] but slightly better than the results from an expanded-access programme in Western countries (16% and 76%) [10]. The estimated median PFS and OS in the present study were 9.3 and 32.2

months, respectively. These are also somewhat lower than the results of the Japanese phase II study (12.2 and 33.1 months for treatment-naïve patients and 10.6 and 32.5 months for cytokine-refractory patients) but slightly better than the results from the expanded-access programme in Western countries (10.9 and 18.4 months) [5,10,11]. In addition, it should be noted that 42% of the patients in the present study were still on treatment, which may have resulted in an underestimation of the ORR, as suggested by a recent analysis showing a higher ORR after longer follow-up [3,12].

It is remarkable that the median OS from the initial systemic therapy of the pretreated patients was 79.6 months. In a phase III randomized clinical trial, sunitinib showed longer PFS and OS compared to interferon-α as a first-line therapy for patients with metastatic RCC [3,4]. Interestingly, the OS was not significantly different between the treatment naïve patients and pretreated patients in the present study (not reached and 24.2 months), in the expanded-access programme (18.4 and 18.1 months) [10] and in the Japanese phase II study (33.1 and 32.5 months, respectively [11]. These results suggest that sunitinib can give a favourable impact as a second-line therapy for patients refractory to cytokine therapy rather than for treatment-naïve patients.

All of the risk factors (ECOG PS >1, low Hb level, high corrected calcium level, high LDH level and ≤12 months between diagnosis and initial systemic therapy) were associated with worse OS in the MSKCC scores, which have been previously identified in patients treated with cytokines (Fig. 1A and Table 2) [8,9]. The application of the respect MSKCC models for the treatment naïve and refractory patients also distinctly separated the OS curves (Fig. 1). This is consistent with recent studies identifying patients who probably will benefit from tyrosine kinase inhibitors [12–15]. These results, as well as those obtained in the present study, indicate that MSKCC scores are associated with the behaviour of the disease rather than with specific forms of therapy. Therefore, MSKCC prognostic factors are still valid for predicting survival in metastatic RCC in the molecular targeted therapy era. However, the distribution of patients according to the MSKCC model is uneven: in the series from the present study, 13%, 62% and 25% of patients belonged to favourable, intermediate and poor risk groups, respectively. The disproportionately large number of patients in the intermediate group suggests that the outcome of this group may be somewhat heterogeneous. The prognostic significance of these factors remains to be verified in a larger study because the present study is only preliminary and has a small number of patients.

Apart from the MSKCC score, brain metastasis and no history of nephrectomy were also independently associated with poorer OS. The cumulative incidence of brain metastases is ≈10%, and these patients are considered to have a poor prognosis (median overall survival, 3–6 months) [16,17]. Although some studies have reported that sunitinib has activity against brain metastasis [18,19], and even against multiple brain lesions [19], there is insufficient information available about the activity of sunitinib for brain metastasis because most clinical trials have excluded patients with brain metastasis. Intracerebral haemorrhage in RCC patients with brain metastases should be considered as a cautious adverse effect to be treated with tyrosine kinase inhibitors, although the incidence of this remains to be reported. We consider radiotherapy as a primary treatment for patients with brain metastasis; thereafter, targeted therapies could be considered. However, in the present study, no patients suffered intracerebral haemorrhage.

Among the 49 patients who underwent nephrectomy, 22 patients did so as a cytoreductive strategy. Upfront cytoreductive nephrectomy, followed by systemic therapy, has been established as the standard care for metastatic RCC in the cytokine era [20,21]. Targeted agents, including sunitinib, have shown improved outcomes compared to cytokine therapy, transforming the treatment strategy of metastatic RCC. Although many studies focus on the role of cytoreductive nephrectomy in combination with targeted agents for patients with metastatic RCC, cytoreductive nephrectomy is still recommended at least for those patients with currently good PS.

All patients experienced treatment-related adverse events, most of which were grade 1 or 2 in severity, and most patients were able to resume therapy after treatment modification. In particular, the incidence of grade 3/4 haematological toxicities, including anaemia (20%), leucopaenia (9%) and thrombocytopaenia (48%), appears to be higher in the present study compared to the previous worldwide phase III clinical trial data [3,4], and also is consistent with the report from the Japanese phase II clinical trial [5,11].

In conclusion, sunitinib has a favourable efficacy/safety profile for Japanese metastatic RCC patients in clinical practice. The estimated median OS was >2 years with acceptable tolerability. In addition, it should be noted that the median OS from the initial systemic therapy of pretreated patients was >6 years. MSKCC prognostic factors appear to be still valid for predicting survival in metastatic RCC in the era of molecular targeted therapy.

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CONFLICT OF INTEREST

None declared.

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Abbreviations: **ECOG**, Eastern Cooperative Oncology Group; **Hb**, haemoglobin; **LDH**, lactate dehydrogenase; **MSKCC**, Memorial Sloan-Kettering Cancer Center; **ORR**, objective response rate; **OS**, overall survival; **PFS**, progression-free survival; **PS**, performance status.

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RESEARCH PAPER

Treatment outcome and prognostic factors in renal cell cancer patients with bone metastasis

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Abstract We retrospectively analyzed treatment outcomes and factors for poor prognosis for patients with renal cell cancer (RCC) bone metastases. Patients with bone metastases at initial diagnosis of metastasis secondary from RCC, treated at our hospital between 1984 and 2009, were retrospectively reviewed and statistically analyzed. Among 214 RCC patients with metastasis, 71 patients (33%) were found to have bone metastases at initial diagnosis of metastasis. The median follow-up was 21.1 months (intra-quartile range: IQR, 9.1–47.4 months). The estimated median overall survival time from the diagnosis of bone metastasis was 27.7 months. The probability of patients surviving at 1, 2, and 5 years was 63.7, 52.2, and 19.3%, respectively. When they were stratified by MSKCC scores, the probability of the median overall survival of the populations classified as favorable, intermediate, and poor was not reached, 32.9, and 10.5 months, respectively ($P = 0.002$). In addition, poor performance status (PS) (hazard ratio [HR]: 1.938, $P = 0.035$) and no prior nephrectomy (HR: 3.008, $P = 0.004$) were extracted as independent poor prognostic factors by multivariate analysis. All treatment modalities—including radical en bloc surgery, radiation therapy, cytokine therapy, molecular targeted therapy, and administration of zoledronic acid—seemed to contribute to favorable survival. More than half of the patients with bone metastases secondary from RCC were predicted to survive more than 24 months. In this population, MSKCC scores were valid predictors of survival. With increased treatment options, RCC patients with bone metastasis may benefit further from subsequent modalities and/or agents.

Keywords Renal cell cancer · Sunitinib · Outcome · Bone metastasis · Prognostic factor

Abbreviations

RCC	Renal cell cancer
PS	Performance status
Ca	Calcium
Lower Hb	Hemoglobin
LDH	Lactate dehydrogenase
MSKCC	Memorial Sloan Kettering Cancer Center
CT	Computed tomography
MRI	Magnetic resonance imaging
ECOG	Eastern Cooperative Oncology Group
HRs	Hazard ratios
CIs	Confidence intervals

Introduction

In patients with metastatic renal cell cancer (RCC), bone is a common metastatic site, second only to the lung, with estimates of frequency ranging from 24 to 51% [1–3]. Bone metastases create serious problems for these patients as they often bring poor performance status (PS) due to pathologic fractures, spinal cord compression, and intractable pain [4]. Although bone metastasis secondary from RCC would appear to predispose toward poor prognosis, until now it has been controversial [4–6]. Neither of two large retrospective studies has identified bone metastasis as an independent

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poor prognostic factor [3, 7]. Instead, the time from the RCC diagnosis to the initial systemic treatment being less than one year, poor PS, elevated serum adjusted calcium (Ca), lower hemoglobin (Hb), and elevated serum lactate dehydrogenase (LDH) are well-known independent prognostic factors in the cytokine era, as measured by MSKCC scores (named for Memorial Sloan Kettering Cancer Center investigators). These factors are often used as stratification factors in various clinical trials for patients with metastatic RCC [7].

Because RCC is commonly sensitive neither to chemotherapy nor radiation therapy, treatment options for these RCC patients with bone metastasis have long been limited. Recently, several new agents have been introduced in the clinical treatment of metastatic RCC. The third-generation bisphosphonate, zoledronic acid, demonstrated significant reduction in skeletal-related complications in these patients [8]. In addition, various molecularly targeted agents for metastatic RCC may possibly result in improved survival [9, 10] although their efficacies remain to be established. In this study, we retrospectively analyzed treatment outcomes and the factors for poor prognosis in patients with RCC bone metastases.

Materials and methods

Patients and treatment

The medical records of patients with bone metastases secondary from RCC, who were treated in our hospital between 1980 and 2009, were retrospectively reviewed. In all patients, bone metastasis was confirmed by bone scans and computed tomography (CT) and/or magnetic resonance imaging (MRI). We considered clinical and geometric factors including age, gender, Eastern Cooperative Oncology Group (ECOG) PS, presence or absence of extra-osseous metastases, solitary or multiple bone metastases, pain due to osseous metastasis, the interval from the diagnosis of RCC to the initial systemic therapy, surgical treatment, and radiation therapy of bone metastasis, as well as systemic medical treatment including cytokine therapy, zoledronic acid, and targeted agents (sorafenib and sunitinib). In addition, common laboratory blood and serum data, including Hb, LDH, and adjusted Ca levels, were determined by using a multichannel autoanalyzer (LX-20, Beckman-Coulter, Los Angeles, CA). The corrected serum Ca level was calculated using Payne's formula [11]. Fundamentally, administered radiation dose was 30 Gy given over two weeks with single fractions of 3 Gy. Regarding the medical therapy, three million international unit (IU) of interferon-alpha (Sumiferon, Dainippon Sumitomo Pharma, Osaka, Japan) were administered subcutaneously three times a week to the most patients who were treated by cytokine therapy. Sunitinib (Sutent; Pfizer Inc, New York, NY) was administered orally at a dose of 50 mg daily, consisting of four weeks of treatment followed by a two-week rest period. Sorafenib (Nexaval, Bayer Pharmaceuticals Corporation, West Haven, CT) was administered orally at a dose of 800 mg daily, continuously. In addition, 4 mg of zoledronic acid (Zometa, Novartis Pharma AG, Basel, Switzerland) was administered once a 3/4 weeks. Dose reduction of each agent was performed depending on the type and severity of adverse events.

Statistical analysis

Survival time was defined as the time from the diagnosis of bone metastasis to death or the last follow-up date. The duration of follow-up was calculated from the date at the initial diagnosis of bone metastasis to death or the last follow-up. In this study, we consider the initial cytokine or molecular targeted therapy as the start of the systemic treatments. Overall survival was estimated by using the Kaplan–Meier method. The relationship between survival and each of the variables was analyzed by using the log-rank test for categorical variables. The associations of the pre-treatment and treatment features with death from RCC were assessed by using the Cox proportional hazards regression model and summarized with hazard ratios (HRs) and 95% confidence intervals (CIs). Statistical analyses were performed using the Statistical Package for Social Sciences, version 17.0 for Windows (SPSS Inc., Chicago, Ill). Two-tailed $P < 0.05$ was considered significant.

Results

Characteristics of the patients and their bone metastases

Among 214 RCC patients with metastasis, 71 (33%) patients were diagnosed with bone metastasis. The median follow-up period was 21.1 months (intra-quartile range: IQR, 9.0–47.3 months). Patient characteristics and demographic data are shown in Table 1. The estimated median overall survival time from the diagnosis of bone metastasis was 27.7 months. The probability of patients surviving at 1, 2, and 5 years was 63.7, 52.2, and 19.3%, respectively (Fig. 1). While bone metastases were diagnosed at the same time as the initial diagnosis of RCC in 53 patients, metastatic bone lesions were detected after nephrectomy in the remaining 18 patients, 13 of whom were found to have bone metastasis 12 months or more postoperatively. Among these patients, 41 (58%) of the 71 patients had extra-osseous metastasis at the time of diagnosis of bone metastasis. These extra-osseous metastatic sites included 35 lung (85%), 15 lymph nodes (37%), 5 liver (12%), 4 adrenal gland (10%), and 2 brain (5%) metastases.

Table 1 Patient characteristics

Gender	
Male *n* (%)	55 (77)
Female *n* (%)	16 (23)
Age at Dx of bone metastasis	
Median (IQR)	62 (55.8–69.9)
Interval from initial Dx of RCC to bone metastasis	
Bone metastasis at initial Dx n (%)	53 (75)
<12 month *n* (%)	3 (4)
≥12 month *n* (%)	15 (21)
ECOG PS	
PS0 *n* (%)	22 (31)
PS1 *n* (%)	28 (39)
PS ≥ 2 *n* (%)	21 (30)
Hb at Dx of bone metastasis	
Male Median (IQR) g/dl	13.4 (12.5–14.4)
Female Median (IQR) g/dl	11.5 (10.7–13.6)
Serum LDH at Dx of bone metastasis	
Median (IQR) IU/l	222 (167–315)
Serum Ca at Dx of bone metastasis	
Median (IQR) g/dl	9.5 (9–9.8)
Follow-up	
Median (IQR) month	20.4 (8.7–44.5)

Dx diagnosis, *IQR* interquartile range, *ECOG* Eastern Cooperative Oncology Group, *PS* performance status

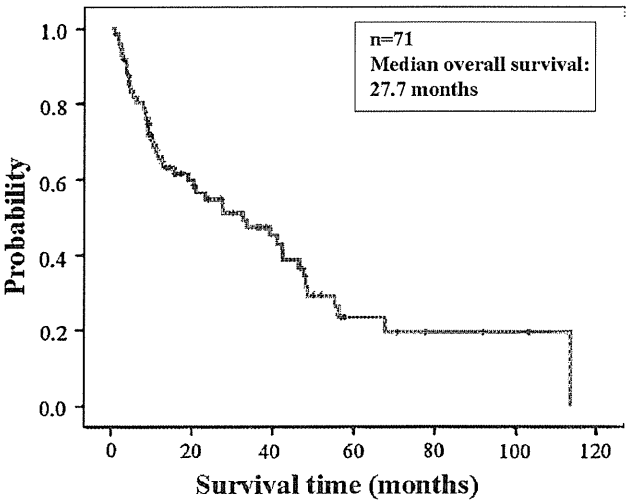

Fig. 1 Kaplan–Meier estimates of overall survival of 71 patients with bone metastasis secondary from renal cell cancer

Meanwhile, the primary bone metastatic sites were 34 vertebra (48%) including 16 lumbar (23%), 14 thoracic (20%), and 3 lumbar bones (4%), 26 lower extremities (37%), 24 thoracic cage (34%), 19 pelvis (27%), 13 upper extremities (18%), and 3 skull bone (4%). There was only a single metastasis in 39 patients (55%), whereas the remaining 32 patients (45%) had multiple bone metastases. Surgical management for bone metastasis was performed in 33 patients, including 13 (18%) and 20 (28%) for radical en bloc resection and palliative curettage procedure, respectively. In addition, radiation therapy was performed in 24 patients (34%). As systemic therapy, 48 patients (68%) and 18 (25%) patients received immune and molecular targeted therapies for their metastatic disease, respectively. Among these patients, 13 patients (18%) received both immune and molecular targeted therapies. Regarding the molecular targeted therapies, sorafenib and sunitinib, sorafenib alone, and sunitinib alone were administered to 3, 7, and 8 patients, respectively. In addition, 29 patients (41%) received zoledronic acid for their bone metastases.

Risk factors for poor outcome in univariate analysis

Univariate analysis of several clinical features associated with poor prognosis is summarized in Table 2. Among the factors in MSKCC scores, the time between RCC diagnosis and the initial systemic therapy of less than 1 year, poor PS (PS ≥ 2), elevated serum adjusted Ca were associated with short survival period, whereas lower Hb and elevated serum LDH were not statistically significant (Table 2). When we applied the MSKCC model, which stratifies patients into three risk groups: favorable: 0 risk factors ($n = 8$); intermediate: 1 or 2 risk factors ($n = 44$); and poor: 3, 4, or 5 risk factors ($n = 19$), the estimated median overall survival time from the diagnosis of bone metastasis of the populations classified as favorable, intermediate, and poor was not reached, 32.9, and 10.5 months, respectively, and resulted in distinctly separate overall survival curves ($P = 0.002$) (Fig. 2a).

In addition, elevated serum level of CRP and prior nephrectomy were associated with short survival period, whereas multiple bone metastasis, extra-osseous metastasis, and osseous pain due to metastatic lesion seemed not to be significant poor prognostic factors.

Regarding the treatment factors, all treatment modalities—including radiation therapy, cytokine therapy, molecular targeted therapy, and administration of zoledronic acid—seemed to contribute to longer survival (Table 2). As to surgical treatment, radical and palliative surgery were performed in 13 (18%) and 20 (28%) patients, respectively, which also seemed to contribute to longer survival ($P = 0.05$) (Table 2).

Multivariate analysis for predictors of poor prognosis

Among the pre-treatment factors, the time between RCC diagnosis and the initial systemic therapy of less than one year, poor PS, elevated serum adjusted Ca and CRP level,

Table 2 Univariate analysis of various prognostic factors

Factors	Yes			No			
	n	mOS	95%CI	*n*	mOS	95%CI	*P*
Gender	16	42.5	4.2–80.8	55	27.7	9.0–46.4	0.322
Age (> 64)	36	42.4	15.4–69.4	35	27.6	9.6–45.7	0.341
From Dx of RCC to initial Tx < 1 year or no treatment	60	20.4	3.9–37.0	11	NR		0.014
PS > 1	50	11.2	5.5–16.8	21	42.5	33.6–51.4	<0.001
Anemia	22	12.6	0.0–25.7	49	41.2	28.9–53.5	0.088
Serum LDH > 1.5xULN	32	20.4	0.0–42.1	36	42.4	26.7–58.1	0.091
Serum adjusted Ca > ULN	13	4.5	0.5–8.6	56	39.3	23.1–55.5	0.004
Serum CRP > ULN	33	10.0	6.3–13.8	33	44.2	33.9–54.5	0.016
Multiple bone metastases	32	27.6	4.9–50.4	39	42.4	16.1–68.7	0.164
Extra-osseus metastasis	41	11.2	0.0–26.6	30	42.5	20.7–64.3	0.087
Pain	48	32	10.0–55.8	23	13.7	3.1–24.2	0.415
Prior nephrectomy	45	48.3	44.9–51.7	26	6.4	1.4–11.5	<0.001
Radiation therapy	24	64	44.5–83.6	47	20.4	2.6–38.3	0.006
Cytokine therapy	48	41.2	30.0–52.3	23	11.2	8.1–14.2	0.018
Molecular targeted therapy	13	81.3	55.8–106.7	58	20.4	7.2–33.7	0.002
Zoledronic acid	29	77.9	56.9–99.0	42	11.7	7.2–16.2	<0.001
MSKCC score							
Good	8	NR					0.002
Intermediate	44	32.9	10.6–55.1				
Poor	19	10.7	6.6–14.9				
Surgical treatment							
Radical en bloc resection	13	48.7	21.4–75.9				0.05
Palliative curettage procedure	20	32.6	6.8–58.5				
No surgery	38	12.6	4.8–20.5				

mOS median overall survival, 95%CI: 95% confidence interval, *Dx* diagnosis, *Tx* systemic treatment, *NR* not reached, *ULN* upper limit of normal range, *MSKCC* Memorial Sloan Kettering Cancer Center

and prior nephrectomy were associated with short survival period by univariate analysis. Then a multivariate analysis was performed using Cox proportional hazard model and it showed that poor PS (HR: 1.938, 95% CI: 1.048–3.584, $P = 0.035$) and no prior nephrectomy (HR: 3.008, 95%CI: 1.416–6.390, $P = 0.004$) were extracted as independent poor prognostic factors. When we applied the model using these two risk factors, which stratifies patients into three risk groups: favorable: 0 risk factors ($n = 21$); intermediate: 1 risk factor ($n = 32$); and poor: 2 risk factors ($n = 18$), the estimated median overall survival time from the diagnosis of bone metastasis of the populations classified as favorable, intermediate, and poor was not reached, 21.1 and 10.1 months, respectively, and resulted in distinctly separate overall survival curves ($P = 0.001$) (Fig. 2b).

Discussion

Motzer et al. and recently Naito et al. [3, 7] analyzed the prognosis of 463 Western and 1463 Japanese metastatic RCC patients in the cytokine era. These large, multicenter, retrospective studies demonstrated similar poor prognostic factors, using MSKCC scores [3, 7]. In the Japanese study, the median overall survival times from diagnosis and the favorable, intermediate, and poor prognoses as classified by the MSKCC criteria were 21.5 months and 55.3, 29.6, and 9.8 months, respectively [3]. Interestingly, these median overall survival times are quite similar to those in our study (Figs. 1, 2a), suggesting that bone metastasis might not be a poor prognostic factor among Japanese RCC patients with metastasis.

In this study, although MSKCC scores had been previously identified as prognostic in patients treated with cytokines, these factors were significantly or marginally associated with poor OS (Table 2). This is similar to recent reports that have identified patients who are more likely to benefit from the tyrosine kinase inhibitors [12–14]. Their results suggest that MSKCC scores are associated with the behavior of the disease rather than with a specific form of therapy. Our study suggests that MSKCC prognostic factors are still valid to predict survival in patients with bone

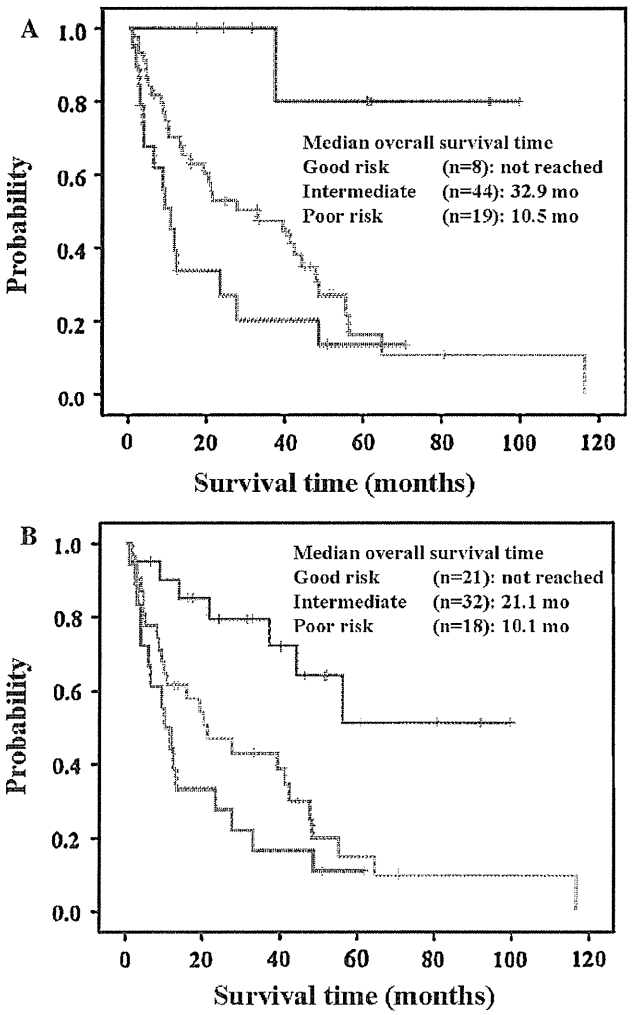

Fig. 2 A Kaplan–Meier estimates of overall survival of patients stratified by the risk factors into favorable, intermediate, and poor risk categories. Kaplan–Meier survival curves stratified by MSKCC score (**a**) and stratified by two risk factors, the performance status and prior nephrectomy, which were associated the poor prognosis in this study (**b**)

metastasis secondary from RCC (Fig. 2a). However, the distribution of patients according to the MSKCC model is uneven: in our series, 15, 58, and 27% patients belonged to favorable, intermediate, and poor risk groups, respectively. The disproportionately large number of patients in the intermediate group suggests that it may be somewhat heterogeneous and could be divided into subclasses. On the basis of our results, we classified the risk by combining the two prognostic factors, poor PS and no prior nephrectomy. In the present study, 21 (30%), 32 (45%), and 18 (25%) of 71 patients had a good, intermediate, and poor prognosis, respectively. This classification distinctly separated overall survival curves and seemed to classify proportionately ($P = 0.001$, Fig. 2b).

It is remarkable that all of the treatment modalities—including radical en bloc surgery, radiation therapy, prior nephrectomy, cytokine therapy, molecular targeted therapy, and administration of zoledronic acid—were identified as factors contributing to longer survival (Table 2) although this is quite a matter of course as various types of treatment tend to be administrated for the patients who survive longer. In other words, we can say that patients who underwent several types of treatment seemed to survive longer. Regarding surgical treatment, when radical resection surgery was performed, excellent local tumor control was reported [15, 16]. Lin et al. [15] reported that the local relapse-free survival rates at one and five years were 94 and 91% for 117 patients with en bloc resections. Jung et al. [16] also reported the superiority of surgical treatment on the basis of records from 99 patients. In this study, the eight patients who had radical en bloc surgical resection for a solitary metastasis in combination with a nephrectomy had a cancer-specific survival rate of 100% (mean follow-up, 69 months; range, 24–76 months). These studies, along with ours, suggest that when patients have good PS and are good candidates for surgical treatment with curative intent, we may need to consider aggressive surgical treatment including nephrectomy in order to achieve complete remission.

In prospective studies for patients with metastatic RCC, palliative radiotherapy can result in significant relief of local symptoms and improved quality of life [17, 18]. Therefore, external beam radiation therapy has been standard palliative treatment for symptomatic bone metastases. In addition, our recent retrospective study demonstrated that combining radiation therapy with administration of zoledronic acid achieved a higher objective response rate and prolonged skeletal related event-free survival than radiation therapy alone in patients with bone metastases from RCC [19]. Although further prospective investigation is necessary, a combination of radiation therapy and zoledronic acid, with or without targeted therapy, may prove effective.

Recently available agents, such as zoledronic acid and molecular targeted agents, might possibly be administered to long-time survivors. With increased treatment options, these RCC patients may benefit further from subsequent modality and/or agents. Zoledronic acid has been demonstrated to exhibit anti-tumor effects, not only inhibiting proliferation but also inducing apoptosis of various cancer cells in vitro and in vivo, and demonstrating survival benefits in recent clinical trials [20–22]. Regarding RCC, zoledronic acid was shown to significantly reduce the risk of skeletal complications in a large randomized, double-blind, placebo-controlled Phase III trial [8]. In this trial, zoledronic acid was also demonstrated to delay time to

progression of bone lesions and clinically benefited 48% of the patients, including 2 (7%) partial response and 30 (41%) stable disease [8]. Moreover, although the difference did not reach statistical significance ($P = 0.179$), the survival of the patients in the zoledronic acid arm was improved by at least three months [8]. In our study, in the most recent cases, 29 patients (41%) received zoledronic acid for their bone metastases.

Finally, the efficacy of sunitinib and sorafenib against RCC bone metastasis remains to be established. One retrospective study suggested that targeted agents appeared slightly more effective than cytokine therapy at extending mean time to progression of pre-existing bone lesions [23]. On the other hand, in prospective clinical trials, interestingly, the efficacy of targeted agents was apparently not affected by prior cytokine treatment. In fact, the overall survival was not significantly different between the treatment naïve patients and pretreated patients in a sunitinib expanded-access program (18.4 and 18.1 months [24]), and in a Japanese phase 2 study (33.1 and 32.5 months, [25]). These studies (including ours) suggested that some patients may derive survival benefit from cytokine therapy and then gain further benefit from subsequent sequential treatment with a targeted agent.

In conclusion, more than half of the patients with bone metastasis secondary from RCC were predicted to survive more than 24 months. The MSKCC score is still valid to predict survival in patients with bone metastasis secondary from RCC. Moreover, all of the treatment modalities seemed to contribute to longer survival in patients with RCC bone metastasis. With increased treatment options for these RCC patients with bone metastasis, they may gain further benefit from subsequent modality and/or agents. Therefore, in patients with favorable PS who are good candidates for surgical treatment with curative intent, we should consider aggressive multimodal treatment, including surgical and/or medical treatment.

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ORIGINAL ARTICLE

Detection of circulating tumor cells in peripheral blood of heavily treated metastatic breast cancer patients

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Abstract
Background Circulating tumor cells (CTCs) are detected in peripheral blood of breast cancer patients, and they may play an important role as a prognostic and predictive marker. We conducted this study to determine the presence of CTCs with the CellSearch System™ and the clinical significance in treatment of metastatic breast cancer (MBC).
Method Twenty-eight MBC patients were enrolled. These patients were followed by assessing CTCs, imaging studies, and serum tumor markers. Blood samples were collected before starting a new treatment and at the treatment evaluation period (2–3 months after starting chemotherapy). The cutoff for CTC level was 5.
Results At baseline, 9 of 28 patients (32%) had ≥5 CTCs per 7.5 mL of blood. At the evaluation period, 5 of 23 patients (22%) had ≥5 CTCs. The baseline CTC number did not contribute to determine their overall survival (OS); however, CTCs at the evaluation period were available to predict their OS ($p < 0.001$). In two cases, both CTCs and tumor markers were available as predictors of treatment efficacy. In two other cases, although alterations of tumor markers might not reflect disease condition, CTC alteration corresponded to their condition. One patient who had multiple skeletal metastasis only, experienced a decrease in her CTCs in spite of tumor marker alteration.
Conclusions We suggest that monitoring the number of CTCs may be helpful in predicting the efficacy of the treatment and the prognosis. CTCs might be especially useful with patients whose lesions are difficult to assess.

Keywords Circulating tumor cell · Metastatic breast cancer · Tumor marker

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Introduction

Breast cancer is the most frequent type of cancer in women. In Japan, approximately 50,000 women are newly diagnosed with breast cancer each year, and 10,000 patients die of breast cancer every year. Recently, many chemotherapeutic agents, endocrine therapy, and targeted therapy have been introduced to treat metastatic breast cancer patients, but the curability rate is very low. The aim of treatment is to improve the patient's quality of life, not to cure completely. Therefore, useful prognostic and predictive markers would help to select an appropriate treatment modality to obtain maximum treatment effects and minimize unnecessary side effects.

Circulating tumor cells (CTCs) are detected in peripheral blood of cancer patients, and their existence has been known for a long time [1]. Although the source of CTCs is unknown and the clinical significance is not yet established, it is reported that circulating epithelial cells in breast cancer patients are malignant, that the cells are derived from clones in the primary tumor site which suggests that they may reflect the tumor burden at all stages of tumor progression [2], and that CTCs could be scattered seeds to develop distant metastasis. The presence of CTCs

may predict the presence of micrometastasis or of aggressive primary tumors. Some reports have related the existence of CTCs to shorter survival time and progression-free survival [3, 4]. In short, CTCs might be important as a prognostic and predictive marker. Cristofanilli et al. [3] described a cutoff of 5 CTCs per 7.7 mL of blood to distinguish patients with a favorable prognosis.

We conducted a small study to determine the presence of CTCs and the clinical significance in treatment of metastatic breast cancer patients in our institution.

Materials and methods

Patients

A total of 28 metastatic breast cancer patients were enrolled. For all patients, the Eastern Cooperative Oncology Group score of performance status was 0–1. The institutional review board approved this study protocol, and all patients provided written informed consent. These patients were followed by assessing CTCs, imaging studies, and blood chemistry (especially tumor markers). Blood samples were collected before starting a new treatment and at the treatment evaluation period (2–3 months after starting chemotherapy). At the same points, imaging studies including computed tomography (CT), tumor markers, carcinoembryonic antigen (CEA), and carbohydrate antigen 15-3 (CA15-3) were also evaluated.

Sample preparation and sample analysis

Of the several approaches to detecting CTCs, we employed The CellSearch System™ (Immunicon Corp., Huntington Valley, PA, USA). First, blood samples were drawn into 10-mL EDTA Vacutainer tubes (Becton–Dickinson, Franklin Lakes, NJ, USA), to which a cell preservative was subsequently added. Samples were maintained at room temperature and processed within 72 h after collection. The CellSearch System™ includes the CellPrep system, the CellSearch Epithelial Cell Kit, and the CellSpotter Analyzer. The CellPrep system is a semiautomated sample preparation system, and the CellSearch Epithelial Cell Kit contains ferrofluids coated with epithelial cell specific EpCAM (epithelial cell adhesion molecule) antibodies to immunomagnetically enrich epithelial cells. Isolated cells were fluorescently labeled with nucleic acid dye 4′,6-diamidino-2-phenylindole (DAPI) and monoclonal antibodies specific for leukocytes (CD45-allophycocyanin), and epithelial cells (cytokeratin 8,18,19-phycoerythrin), and were then put through the CellSpotter Analyzer (Veridex LLC, Warren, NJ, USA). The CellSpotter Analyzer reveals images of candidate CTCs in a sample. To qualify as a CTC, a cell must be round or oval with a nucleus (as determined by positive DAPI staining) contained within the cytoplasm (negative CD45-allophycocyanin staining, positive cytokeratin 8,18,19-phycoerythrin staining). As a characteristic of this system, nonviable cells are removed in counting the CTCs, thereby reducing false positive cells. Finally, results are always expressed as the number of cells per 7.5 mL of whole blood. To ensure reproducibility, these processes were performed two times by two different technical experts.

Study analysis

The objective of this study was to evaluate the prediction of response to therapy with the CTCs and progression-free survival (PFS) and overall survival (OS) as patient's prognosis. PFS was defined as the period between the date when the treatment was started and tumor progression or stopping treatment because of severe adverse events and a patient's unfavorable condition, and OS was the period until death. Statistical analysis was performed using SPSS 17.0 (Chicago, IL, USA). Survival distributions were estimated with the Kaplan–Meier method, and the log-rank statistic was used to test for difference groups. All p values were two-sided, and $p < 0.05$ was considered significant. Tumor response was evaluated after 2 or 3 months for measurable or evaluable lesions according to Response Evaluation Criteria in Solid Tumors (RECIST).

Results

Patient characteristics

The average age of the 28 metastatic breast cancer patients enrolled was 54.5 years (range 37–73). Fifty-four percent of the patients were positive for estrogen receptors (ER) and/or progesterone receptors (PgR). Twenty-nine percent of the tumors were HER2/neu overexpressed or amplified (immunohistochemistry (IHC) 3+ or fluorescence in situ hybridization (FISH)+) (Table 1). In this study, 82% of patients were starting a regimen containing vinorelbine: either vinorelbine monotherapy for HER2/neu negative patients, or vinorelbine and trastuzumab combination therapy for HER2/neu positive patients. Almost all patients (82%) had been heavily pretreated as the 3rd line treatment or more (Table 1). Except for lymph nodes, lung was the major metastatic site, and 43% of patients had three or more metastatic sites (Table 1). Before evaluation, six patients were withdrawn. Two of them changed hospitals, three died, and one had unexpected severe cardiac symptoms and stopped her treatment. Therefore, their CTC samples could not be collected.

Table 1 Patient demographics

	All patients ($N = 28$)	
	No.	%
Age (years)		
Mean	54.5	
Range	37–73	
ER/PgR status		
ER and/or PgR positive	16	57
ER and PgR negative	12	43
HER2		
IHC 3+ or FISH+	10	36
Negative	16	57
Unknown	2	7
Type of chemotherapy		
Vinorelbine	15	54
Vinorelbine + trastuzumab	8	29
Docetaxel	3	11
Paclitaxel + trastuzumab	2	7
Treatment line		
1st line	3	11
2nd line	2	7
3rd line	7	25
4th line	6	21
5th line or more	10	36
Site of metastasis		
Lung	10	36
Pleura	6	21
Liver	8	29
Bone	8	29
Lymph node	13	46
Skin	3	11
Breast	5	18
Brain	1	4
Adrenal	1	4
Number of metastasis		
1	7	25
2	9	32
3	9	32
4	3	11

ER estrogen receptor, *PgR* progesterone receptor, *IHC* immunohistochemistry, *FISH* fluorescence in situ hybridization

Number of CTCs and clinical efficacy

The number of patients who had at least one CTC was 15 (53.6%) out of 28. The range of CTC count was 0–223. In this study, five or more CTCs were regarded as positive in accordance with a previous report [3]. At baseline, nine of 28 patients (32.1%) had five or more CTCs per 7.5 mL of blood. Five of 22 patients (22.7%) had five or more CTCs at the evaluation period (Table 2).

According to the clinical efficacy determined with the radiodiagnostic procedure at the evaluation period, only one patient (3.6%) had complete response (CR), five patients (17.9%) had partial response (PR), seven patients (25.0%) had stable disease (SD), 14 (50%) patients had progressive disease (PD), and one patient (3.6%) was not evaluated. Unfortunately, CTCs were not detected in the CR patient. Although with the PD case, the positive rate of CTCs increased between baseline and the treatment evaluation period, with the PR and SD cases, this rate decreased (Table 2). Figure 1 shows that, especially for patients whose baseline CTCs number was above the cutoff, with one PR patient the CTCs disappeared, two of three SD patients' CTCs dropped below the cutoff, and one PD patient's CTCs level increased. In contrast, one PD patient had CTCs below the cutoff at first, but the number of CTCs had increased at the evaluation period.

Among all positive CTC patients at baseline ($n = 9$), except for 2 patients whose CTCs were not collected at the evaluation period, CTCs of all ER and/or PgR positive patients ($n = 4$) did not decrease below the cutoff, and three of these four patients' disease progressed. By contrast, CTCs of all ER and PgR negative patients ($n = 3$) decreased below the cutoff at the evaluation period, and their disease did not become PD.

Overall survival

For all 28 patients, the median OS was approximately 23.8 months (1.3–44.7), and the median OS of the patients who had positive and negative CTCs at baseline was 12.7 (1.3–44.7) and 29.1 (2.6–44.2) months respectively. The median OS of the patients who had positive and negative CTCs at the evaluation period was 4.7 (3.4–21.4) and 37.9

Table 2 Frequency of positive CTCs (≥5 CTCs)

Treatment efficacy	Patients (%)	≥ 5 CTCs at baseline (%)	≥ 5 CTCs at evaluation (%)
Complete response	1 (3.6)	0/1 (0.0)	0/1 (0.0)
Partial response	5 (17.9)	1/5 (20.0)	0/5 (0.0)
Stable disease	7 (25.0)	3/7 (42.8)	1/7 (14.3)
Progressive disease	14 (50.0)	5/14 (35.7)	4/9[a] (44.4)
Not examined	1 (3.6)	0/1 (0.0)	0/1 (0.0)
Total	28	9/28 (32.1)	5/23[a] (21.7)

[a] Changing hospital 2, death 3

(16.8–44.7) months respectively. Figure 2 shows Kaplan–Meier curves of OS according to the number of CTCs at baseline and the evaluation period. According to these curves, baseline CTCs did not contribute significantly to determine the prognosis ($p = 0.477$). On the other hand, the patients with negative CTCs at evaluation had a better prognosis than positive CTCs patients ($p < 0.001$).

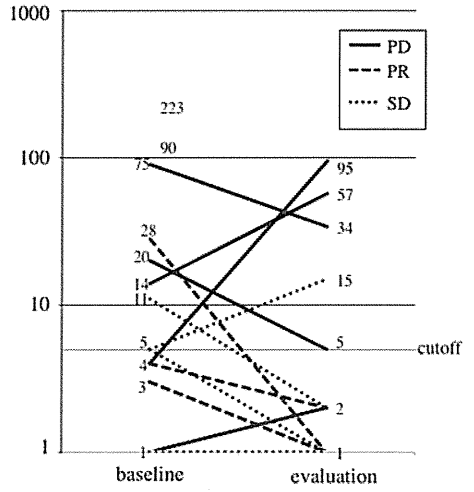

Fig. 1 Alterations of the number of CTCs between baseline and evaluation period. Of the patients whose baseline CTCs number was above the cutoff, one PR patient's CTCs disappeared, two of three SD patients' CTCs dropped below the cutoff, and one PD patient's CTCs level increased. In contrast, one PD patient had CTCs below the cutoff at first, but the number of CTCs had increased at the evaluation period

Progression-free survival

For all 28 patients, the median PFS was approximately 5.2 months (0.2–44.2), and the median PFS of the patients who had positive and negative CTCs at baseline was 1.9 (0.3–33.9) and 5.2 (0.2–44.2) months respectively. The median PFS of the patients who had positive and negative CTCs at the evaluation period was 1.9 (1.1–6.8) and 8.3 (0.2–44.2) months respectively. Figure 3 shows Kaplan–Meier curves of PFS according to the number of CTCs at baseline and the evaluation period. According to these curves, the baseline CTC level did not contribute to predict the treatment efficacy ($p = 0.542$). However, the patients with negative CTCs at evaluation had a better prognosis than positive CTCs patients ($p = 0.002$).

Alterations of CTCs level between pre- and post-treatment

In addition, although the correlations between the PFS and the difference of CTC numbers between baseline and the evaluation period were inconsistent, some patients whose pre-treatment CTC number was greater than their post-treatment CTCs number had longer a PFS (Fig. 4). In Fig. 5, the patients who had positive CTCs during treatment had a worse PFS and OS than patients whose CTCs level decreased below the cutoff between the pre- and post-treatment period.

Examples

Figures 6, 7, and 8 reveal the alterations of CTCs and tumor markers (CEA, CA15-3) of typical patients between

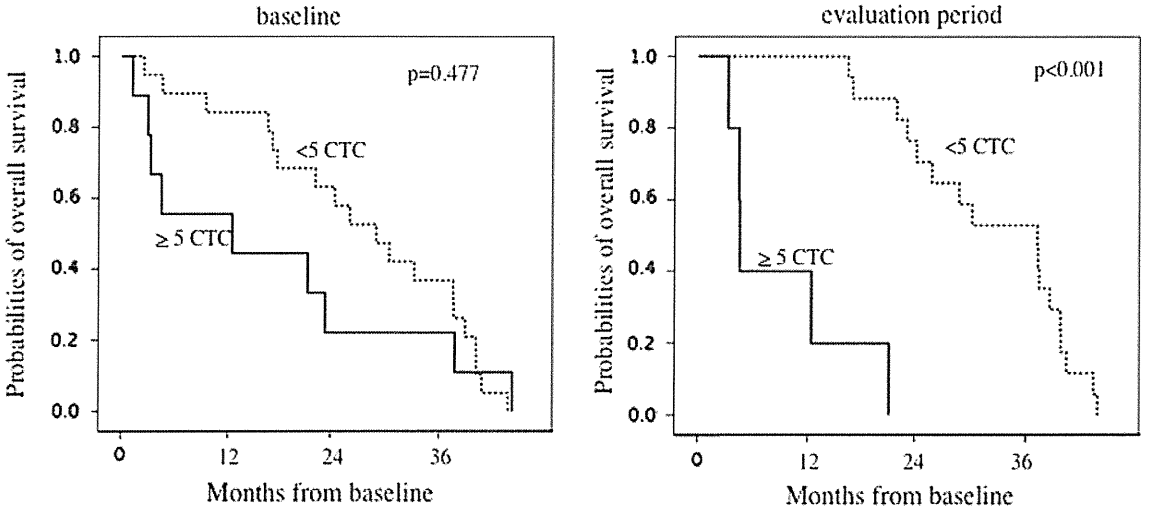

Fig. 2 Kaplan–Meier estimates of probabilities of overall survival for those positive and negative CTCs at baseline and evaluation period. Baseline CTCs did not contribute significantly to determine the prognosis; patients with negative CTCs at evaluation had a better prognosis than positive CTCs patients

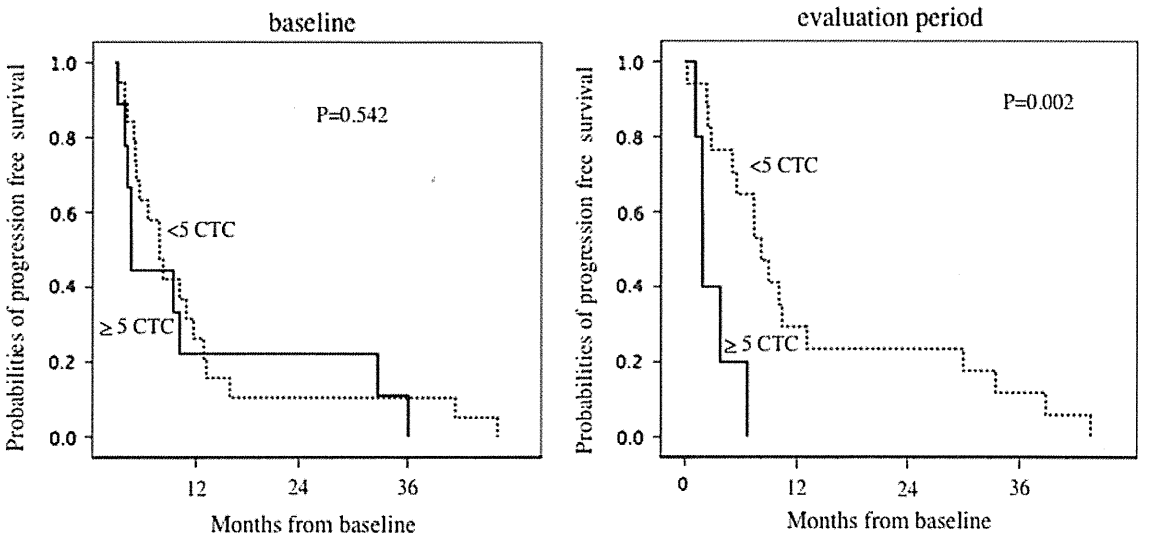

Fig. 3 Kaplan–Meier estimates of probabilities of progression-free survival for those positive and negative CTCs at baseline and evaluation period. Baseline CTC level did not contribute to predict treatment efficacy. Patients with negative CTCs at evaluation had a better prognosis than positive CTCs patients

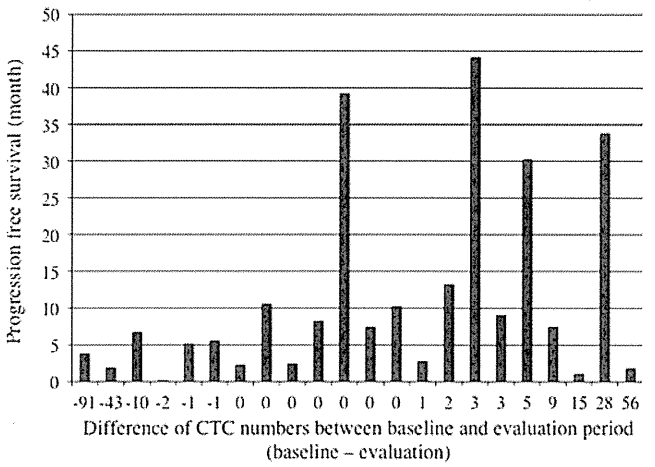

Fig. 4 Correlation of progression-free survival and the difference of CTC numbers between baseline and evaluation period. Although correlations between PFS and the difference of CTC numbers between baseline and evaluation period were inconsistent, some patients whose pre-treatment CTC number was greater than post-treatment CTCs had longer PFS

baseline and the treatment evaluation period. Figure 6 shows that in case A (PD patient) and case B (PR patient), both CTCs and tumor markers altered according to the patients' condition. In Fig. 7, although alterations of tumor markers did not reflect the patient's condition, CTC alteration corresponded to each patient's condition. With case C, though her metastatic sites shrank, tumor markers did not change. On the other hand, her CTC level decreased; therefore CTC alteration was suitable to evaluate efficacy. In case D, in spite of decreased tumor markers, the CTC level increased. Four months later her metastatic sites progressed.

In case E who had multiple skeletal metastasis only, both CTCs and tumor markers decreased and the patient had a long SD (SD more than 6 months) with treatment (Fig. 8).

Discussion

CTCs have been detected in the peripheral blood of all major carcinomas, such as prostate, breast, colorectal, and lung. Fehm et al. [2] reported that CTC chromosomal abnormalities resembled those in primary epithelial cancer lesions, indicating that the CTCs were derived from the tumor sites. Furthermore, the number of CTCs in the blood of healthy control and nonmalignant disease is extremely low [5], as confirmed more recently by many studies on the various methods used to detect CTCs. As for biological techniques, immunomagnetic isolation, flow cytometry, immunofluorescent microscopy, reverse transcriptase-polymerase chain reaction (RT-PCR), polymerase chain reaction (PCR), and fluorescence in situ hybridization (FISH) are used to achieve high specificity and accuracy. For example, elevated CTCs were found in 50–75% of metastatic breast cancer patients by using either immunofluorescent approach or RT-PCR methods [6].

The CellSearch System™ contains immunomagnetic and immunofluorescent microscopic procedures to identify CTCs with high sensitivity and specificity and has been approved for clinical use the by Food and Drug Administration (FDA). This system is semiautomated to be highly