Lung Cancer xxx (2011) xxx–xxx

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

ALK fusion gene positive lung cancer and 3 cases treated with an inhibitor for ALK kinase activity

Hideki Kimura[a,*], Takahiro Nakajima[a,d], Kengo Takeuchi[b], Manabu Soda[c], Hiroyuki Mano[c], Toshihiko Iizasa[a], Yukiko Matsui[a], Mitsuru Yoshino[a], Masato Shingyoji[a], Meiji Itakura[a], Makiko Itami[e], Dai Ikebe[e], Sana Yokoi[f], Hajime Kageyama[f], Miki Ohira[g], Akira Nakagawara[h]

[a] *Division of Thoracic Diseases, Chiba Cancer Center, Chiba, Japan*
[b] *Pathology Project for Molecular Targets, Cancer Institute, Japanese Foundation for Cancer Research (JFCR), Koto, Tokyo, Japan*
[c] *Division of Functional Genomics, Jichi Medical University, Tochigi, Japan*
[d] *Division of Thoracic Surgery, Toronto General Hospital, University Health Network, Toronto, Canada*
[e] *Division of Pathology, Chiba Cancer Center, Japan*
[f] *Cancer Genome Center, Chiba Cancer Center Research Institute, Japan*
[g] *Laboratory of Cancer Genomics, Chiba Cancer Center Research Institute, Japan*
[h] *Chiba Cancer Center, Japan*

ARTICLE INFO

Article history:
Received 16 October 2010
Received in revised form 24 May 2011
Accepted 30 May 2011

Key words:
ALK
EML4
KIF5B
Fusion gene
Lung cancer
EBUS
TBNA
Crizotinib
ALK inhibitor

ABSTRACT

Background: Anaplastic lymphoma kinase (ALK) fusion gene-positive lung cancer accounts for 4–5% of non-small cell lung carcinoma. A clinical trial of the specific inhibitor of ALK fusion-type tyrosine kinase is currently under way.

Methods: ALK fusion gene products were analyzed immunohistochemically with the materials obtained by surgery or by endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA). The echinoderm microtubule-associated protein-like 4(EML4)-ALK or kinesin family member 5B (KIF5B)-ALK translocation was confirmed by the reverse transcription polymerase chain reaction (RT-PCR) and fluorescence in situ hybridization (FISH). After eligibility criteria were met and informed consent was obtained, 3 patients were enrolled for the Pfizer Study of Crizotinib (PF02341066), Clinical Trial A8081001, conducted at Seoul National University.

Results: Out of 404 cases, there were 14 of EML4-ALK non-small cell carcinoma (NSCLC) and one KIF5B-ALK NSCLC case (8 men, 7 women; mean age, 61.9 years, range 48–82). Except for 2 light smokers, all patients were non-smokers. All cases were of adenocarcinoma with papillary or acinar subtypes. Three were of stage IA, 5 of stage IIIA, 1 of stage IIIB and 6 of stage IV. Ten patients underwent thoracotomy, 3 received chemotherapy and 2 only best supportive care (BSC). One BSC and 2 chemotherapy cases were enrolled for the clinical trial. Patients with advanced stages who received chemotherapy or best supportive care were younger (54.0 ± 6.3) than those who were surgically treated (65.8 ± 10.1) ($p < 0.05$).

The powerful effect of ALK inhibitor on EML4-ALK NSCLC was observed. Soon after its administration, almost all the multiple bone and lymph node metastases quickly disappeared. Nausea, diarrhea and the persistence of a light image were the main side effects, but they diminished within a few months.

Conclusion: ALK-fusion gene was found in 3.7% (15/404) NSCLC cases and advanced disease with this fusion gene was correlated with younger generation. The ALK inhibitor presented in this study is effective in EML4-ALK NSCLC cases. A further study will be necessary to evaluate the clinical effectiveness of this drug.

1. Introduction

As the mechanisms of carcinogenesis become clearer, the target of cancer treatment is shifting from non-specific cytotoxic agents to specific agents that block key molecular events in the carcinogenesis of malignancy such as EGFR-TKI and anti-HER2 antibody (trastuzumab) [1–3]. Recently, Mano et al. [4–6] reported that a small inversion within chromosome 2p results in the formation of a fusion gene comprising portions of the

* Corresponding author at: Division of Thoracic Diseases, Chiba Cancer Center, 666-2, Nitona-cho, Chuo-ku, 260-8717 Chiba, Japan. Tel.: +81 43 264 5431; fax: +81 43 262 8680.
E-mail address: hkimura@chiba-cc.jp (H. Kimura).

0169-5002/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.lungcan.2011.05.027

echinoderm microtubule-associated protein-like 4 (EML4) gene and the anaplastic lymphoma kinase (ALK) gene in non-small-cell lung cancer. Transgenic mice that express EML4-ALK specifically in lung epithelial cells develop multiple foci of adenocarcinoma in the lung soon after birth, and the oral administration of a specific inhibitor of ALK tyrosine kinase activity eradicated completely the foci of adenocarcinoma. Clinical trials of specific inhibitors of EML4-ALK tumors are currently underway [7–11]. Kwak et al. [11] reported the effect of crizotinib in Clinical Trial A8081001 on the 82 patients with advanced ALK-positive disease. Over a mean treatment duration of 6.4 months, the overall response rate was 57% and the estimated probability of 6-month progression-free survival was 72%. We report 15 cases of ALK fusion gene-positive NSCLC cases and 3 cases in our experience with ALK inhibitor in the Pfizer Study of crizotinib (PF02341066), Clinical Trial A8081001, which was conducted at Seoul National University.

2. Materials and methods

Out of 404 patients who had undergone surgical resection (295 cases) or bronchoscopy (109 cases) in Chiba Cancer Center, Japan, from 2007 to 2009, 15 ALK fusion gene-positive NSCLC patients were initially screened by immunohistochemical procedures. Diagnoses were confirmed by RT-PCR and/or FISH for their molecular translocation.

2.1. ALK fusion protein detection by immunohistochemical methods

The intercalated antibody-enhanced polymer method of Takeuchi et al. [12,13] was used to detect ALK proteins. Formalin-fixed paraffin-embedded tissue was sliced at a thickness of 4 μm and the sections were placed on silane-coated slides. For antigen retrieval, the slides were heated for 40 min at 97 °C in target Retrieval Solution (pH 9.0; Dako). They were then incubated at room temperature, first with Protein Block Serum-free Ready-to-Use solution (Dako) for 10 min, and then with an anti-ALK antibody (5A4, Abcam) for 30 min. To increase the sensitivity of detection, we included an incubation step of 15 min at room temperature with rabbit polyclonal antibodies to mouse immunoglobulin (Dako). The immune complexes were then detected with the dextran polymer reagent and an AutoStainer instrument (Dako).

2.2. Confirmation of EML4-ALK fusion gene by RT-PCR and FISH

We confirmed the existence of ALK fusion gene expression by fluorescence in situ hybridization (FISH) and/or by the reverse transcription-polymerase chain reaction (RT-PCR).

2.3. Fluorescence in situ hybridization (FISH)

An EML4-ALK fusion assay was performed [10–12]. Unstained sections were processed with a Histology FISH Accessory Kit (Dako), subjected to hybridization with fluorescence-labeled bacterial artificial chromosome clone probes for EML4 and ALK (self-produced probes; EML4: RP11-996L7, ALK: RP11-984I21 and RP11-62B19), stained with 4,6-diamidino-2-phenylindole, and examined with a fluorescence microscope (BX51; Olympus). The FISH positivity criteria specified "over 50% cancer cells" for EBUS-TBNA samples.

2.4. Reverse transcription-polymerase chain reaction (RT-PCR)

The multiplex PCR method proposed by the Japanese ALK lung cancer study group (ALCAS) was used to confirm the expression of ALK fusion gene [4–6].

Table 1
Characteristics of ALK fusion gene positive lung cancer patients.

Patient no	Sex	Age	SI	Histology	Variant	p Stage	Therapy	Recurrence	Distant meta	Survival (M)	Prognosis	ALK inhibitor case no
1	f	64	0	Ad: papillary	3	IIIA	Surgery	Positive	Bone, brain	21	Dead	
2	m	82	0	Ad: solid	2	IIIA	Surgery	Positive	Ascites	36	Alive	
3	f	68	0	Ad: papillary	3	IIIB	Surgery	Positive	Brain	34	Alive	
4	f	60	0	Ad: solid	3	IIIA	Surgery	Negative	None	29	Alive	
5	m	73	0	Ad: acinar	3	IA	Surgery	Negative	None	21	Alive	
6	m	66	0	Ad: papillary	KIF5B	IA	Surgery	Negative	None	15	Alive	
7	m	56	300	Ad: papillary	1	IA	Surgery	Negative	None	13	Alive	
8	m	46	0	Ad: acinar	5	IIIA	Surgery	Negative	None	22	Alive	
9	m	71	0	Ad: papillary	1	IIIA	Surgery	Negative		17	Alive	
10	f	73	0	Ad: acinar	1	IV	Surgery	Negative		14	Alive	
11	m	55	100	Ad: muc+	3	IV	BSC		Bone, brain	5	Dead	
12	m	48	0	Ad: muc+	1	IV	Chemo		Bone, brain	29	Dead	Case 1
13	f	49	0	Ad: muc+	3	IV	BSC		Bone, brain	15	Alive	Case 2
14	f	54	0	Ad: muc+	1	IV	Chemo		Bone, brain, pul	22	Alive	Case 3
15	f	64	0	Ad: acinar	3	IV	Chemo		Pul	2	Alive	

SI, smoking index; f, female; m, male; Ad, adenocarcinoma; muc+, mucin production; Distant meta, at the recurrence (surgery group) at the diagnosis (non-surgery group); pul, pulmonary metastasis; Case 1 was already reported by Nakajima et al. [16].

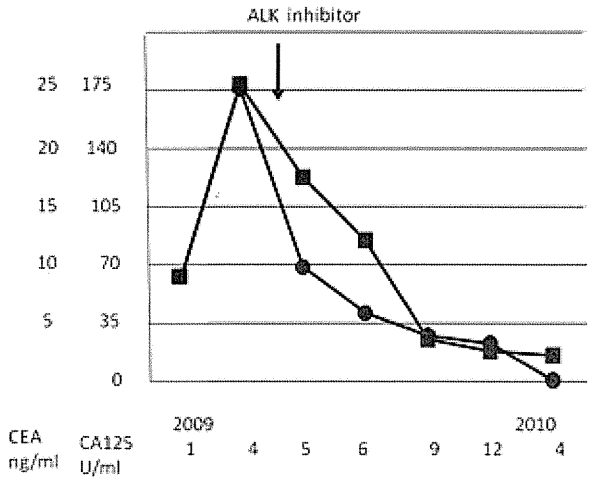

Fig. 1. Changes of tumor markers before and during the treatment with ALK inhibitor (Case 1) CEA (■), CA125 (●). Marked reduction of tumor markers was observed.

Total RNA was isolated from EBUS-TBNA or surgical samples using AllPrep DNA/RNA Mini Kit (Qiagen) and was reverse-transcribed into single strand cDNA using a High Capacity RNA-to-cDNA Kit (Applied Biosystems). To detect a fusion cDNA derived from EML4 or KIF5B and ALK, PCR analysis was performed with the AmpliTaq Gold PCR Master Mix (Applied Biosystems), the forward primers derived from EML4, EA-F-cDNA-S (5′-GTGCAGTGTTTAGCATTCTTGGGG-3′), EA-F-2-g-S (5′-AGCTACATCACACACCTTGACTGG-3′), EA-F-cDNA-v3-S-2 (5′-TACCAGTGCTGTCTCAATTGCAGG-3″) and EA-W-cDNA-in-S (5′-GCTTTCCCCGCAAGATGGACGG-3′) and the forward primers derived from KIF5B, KA-F-cDNA-S-e24 (5′-CAGCTGAGAGAGTGAAAGCTTTGG-3′), KA-F-cDNA-S-e17 (5′-GACAGTTGGAGGAATCTGTCGATG-3′), KA-F-cDNA-S-e11 (5′-ATCCTGCGGAACACTATTCAGTGG-3′), and KA-cDNA-S-e2 (5′-TCAAGCACATCTCAAGAGCAAGTG-3′) and the reverse primer derived from ALK, EA-F-cDNA-A (5′-TCTTGCCAGCAAAG-CAGTAGTTGG-3′). PCR products were purified from gel bands using QIAquick Gel Extraction Kit (Qiagen) and confirmed by direct sequencing analysis.

2.5. Enrolment of patients for the Clinical Trial A8081001

Informed consent was obtained from each patient to be enrolled for the study [10]. Eligibility criteria for the enrolment of ALK translocation positive patients into the ALK TKI PI Trial were as required by the Committee of Clinical Trials A8081001.

3. Results

There were 15 ALK fusion gene-positive cases which were screened immunohistochemically and confirmed by RT-PCR and FISH [14,15]. Eight patients were men and 7 women, of mean age

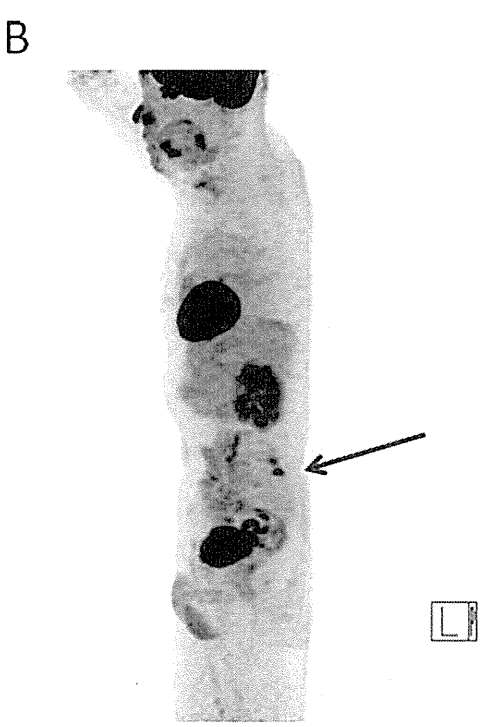

Fig. 2. FDG-PET scan of Case 1 performed at the same time (09/28/2009) as the previously reported Fig. 1D (Nakajima et al. [16]) shows bone metastasis of the left vertebral arch of L5 (arrow) in a sagittal view.

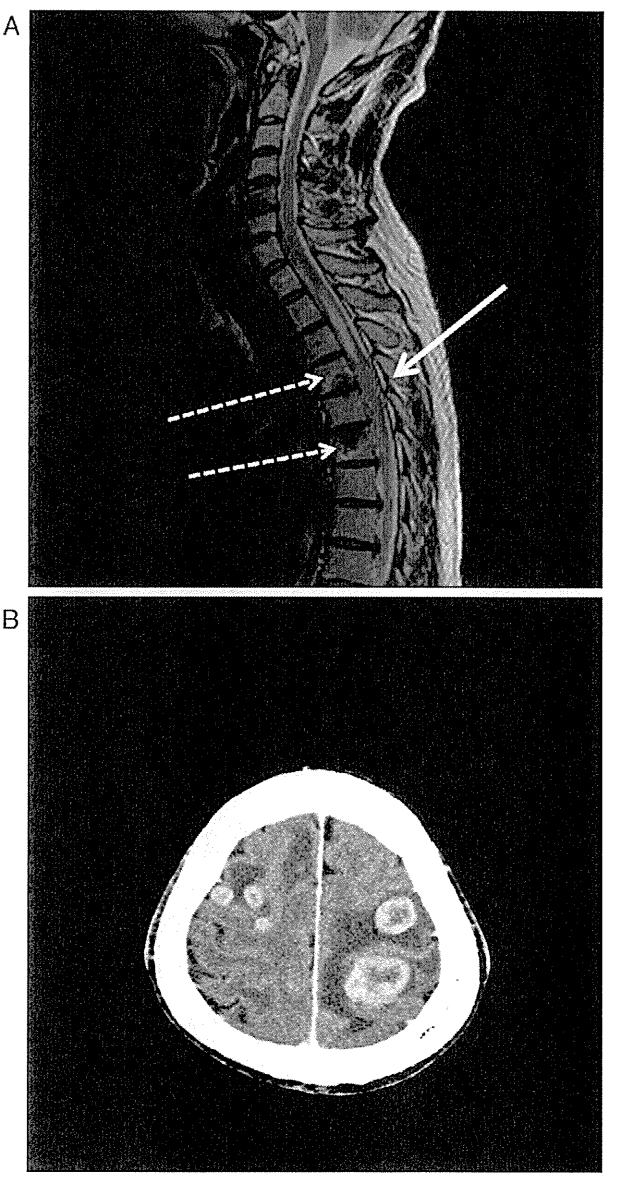

Fig. 3. MRI (Case 1) of the spinal cord on 04/05/2010 shows the metastases to the spinal cord (straight allow) and the spinal column (Th 4,6 dotted allow). B. CT scan (Case 1) of the brain on 04/05/2010 shows multiple brain metastases.

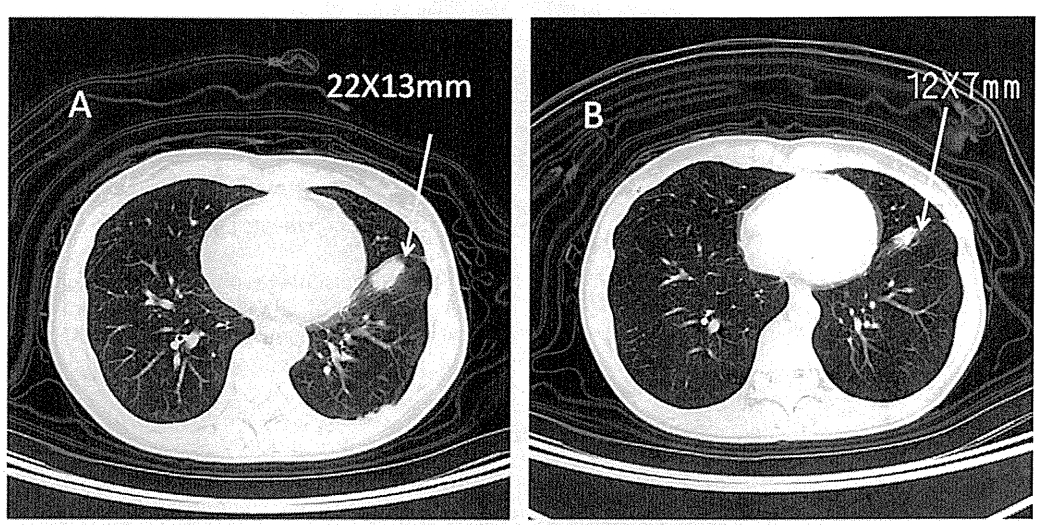

Fig. 4. CT scan (Case 2): A, 07/22/2009 (before ALK inhibitor) and B, 09/02/2009 (5 weeks after the initiation of the therapy). Left S8 tumor (arrow) decreased in size from 22X13 mm to 12X7 mm (PR).

61.9 years (range 48–82). Most were non-smokers, but 2 smoked lightly (Table 1). All tumors were adenocarcinomas with a papillary pattern predominant (5 cases), an acinar pattern predominant (3 cases), with mucin production (4 cases), etc. There were fourteen cases of fusion with EML4 and one KIF5B gene. There were 7 variant 3, 5 variant 1, and 1 each of variants 2 and 5. There were 3 stage IA, 5 stage IIIA, 1 stage IIIB and 6 stage IV cases. Ten cases were diagnosed after surgical resection, and 5, by tissue samples obtained with EBUS-TBNA. Ten cases underwent thoracotomy, 3 cases, chemotherapy, and 2 cases, only best supportive care. Of 5 cases diagnosed by EBUS-TBNA, 2 cases receiving chemotherapy and one receiving best supportive care were enrolled for the clinical trial. The mean age of the surgically treated group was 65.8 ± 10.1, and that of chemotherapy and BSC group was 54.0 ± 6.3. The difference was found by Student's t test to be statistically significant ($p < 0.05$), indicating that younger patients tend to have advanced cancer.

Out of 10 surgically treated cases, seven survived without a sign of recurrence, 3 had recurrence in both bone and brain tissue, and one died of bone and lymph node metastasis.

3.1. Case 1

Case 1 has already been reported in a case report (Nakajima et al.) [16] but without precise descriptions of the response to crizotinib, the adverse effects, the pattern of recurrence or the metastatic

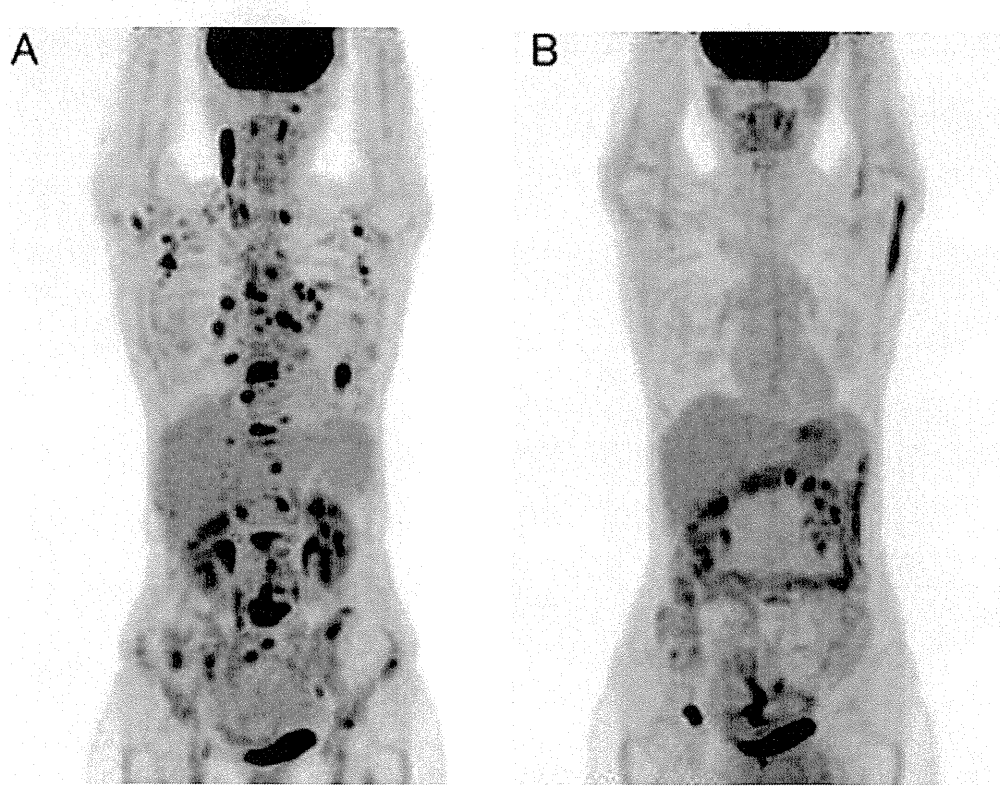

Fig. 5. FDG-PET scan (Case 2): A, 07/22/2009 (before ALK inhibitor) and B, 03/10/2010 FDG-PET scan shows marked reduction of accumulation in multiple bone and lymph node metastases 7 months after the initiation of the treatment.

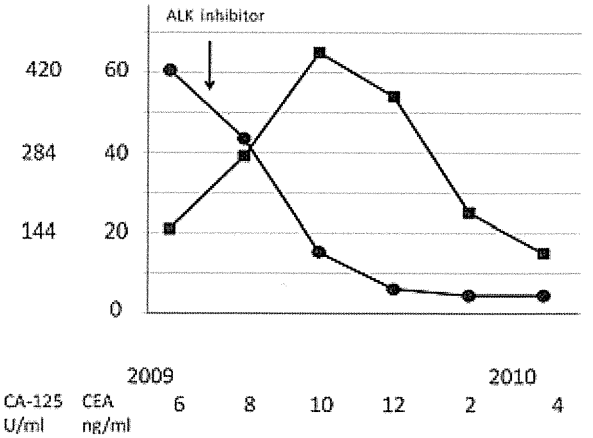

Fig. 6. Changes of tumor markers before and during the treatment with ALK inhibitor in case 2. CA125 (●) gradually decreased along with the treatment, but CEA (■) increased soon after the initiation of the therapy. The value of CEA then gradually decreased to 15.2 ng/ml in April 2010 (after 10 months).

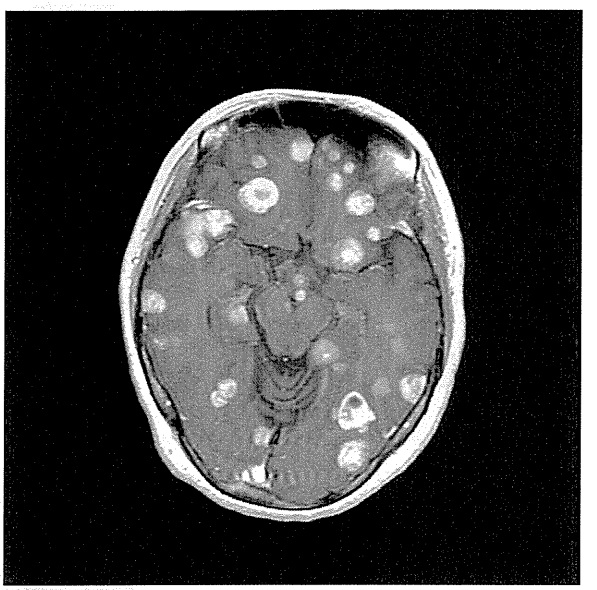

Fig. 7. Brain MRI of case 2 on 7/30/2010 showing multiple metastases.

tumor lesions. Such descriptions may contribute to a better understanding of the other cases, and so case 1 is described briefly below.

A 48-year-old non-smoking male patient had lung adenocarcinoma in the right lower lobe and multiple bone and lymph node metastases (T3N2M1 stage IV) at his first medical examination in November 2007. After several courses of chemotherapy, the patient was enrolled in a trial of crizotinib (PF02341066) from May 5th 2009 at Seoul National University, in which the drug was orally administered at 500 mg/day.

The effect of ALK inhibitor appeared rapidly. The patient's dyspnea improved within one week after drug administration. PS improved from 2 to 0 and a marked reduction in the tumor markers was observed (Fig. 1). Within 3 months after the start of therapy, almost all metastases disappeared except for those at the left vertebral arch of L5 (Fig. 2, arrow). The patient had severe adverse effects: diarrhea, nausea and persistence of light images started soon after the administration of the drug, but these gradually diminished over a 3-week period.

The control of the primary and metastatic tumors continued for 11 months until the patient visited Seoul University in April 2010, when he was hospitalized for paralysis of the lower extremities. MRI revealed spinal column (Th4-6) and spinal cord metastases (Fig. 3A). Soon after his hospitalization in our Cancer Center in April 2010, multiple brain metastases (Fig. 3B) were found, so the drug administration was stopped and he was transferred to a palliative care unit.

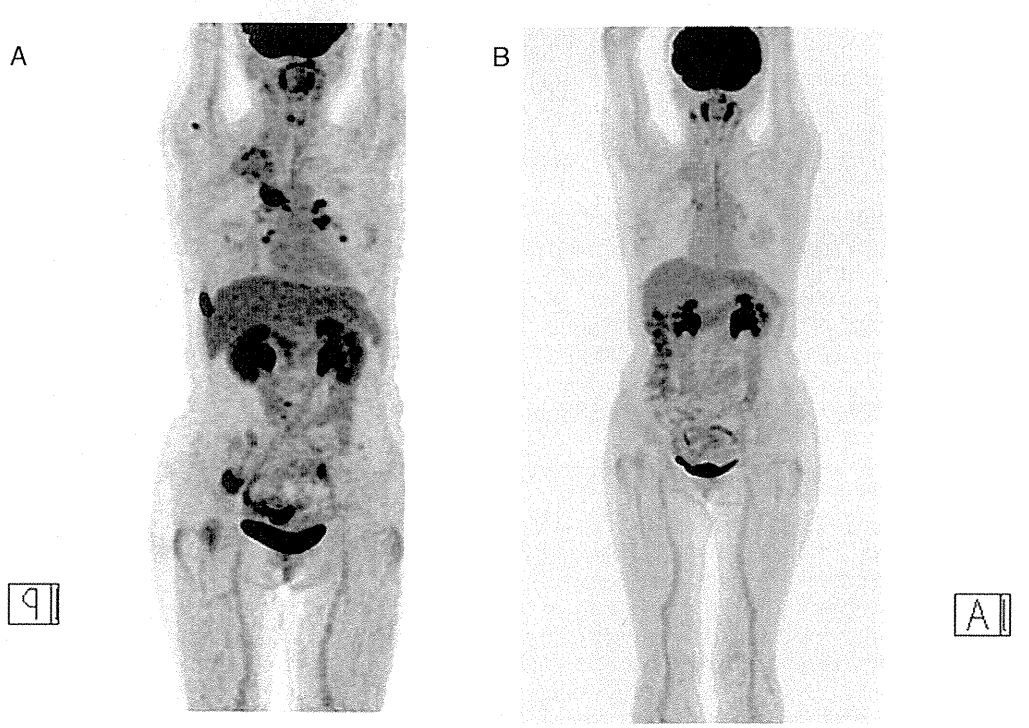

Fig. 8. FDG-PET scan: A, 09/08/2009 (before ALK inhibitor) and B, 07/05/2010 FDG-PET scan follow-up for 10 months indicated complete control of primary and distant metastases in case 3.

3.2. Case 2

A 49-year-old woman, a non-smoker with no history of illness, PS0, was introduced to the Orthopedics Department of our Center in April 2009 for back pain and multiple osteoplastic changes in the bones. Systematic examination revealed an abnormal shadow 22X13 mm in size in the left lower lobe (Fig. 4A). Bronchoscopy and a PET scan indicated left S8 adenocarcinoma with cervical, axial, mediastinal, hilar, pancreatic and retroperitoneal lymph node metastases, as well as cranial, thoracic (Th1-12), lumbar (L1-5), rib (1–12) pelvis, humerus, and femur metastases (Fig. 5A).

She refused any therapy except for best supportive care. One month after the examination, an additional immunohistochemical examination for EML4-ALK fusion protein was performed, and found to be positive. The presence of mRNA for EML4-ALK gene was also confirmed by RT-PCR and FISH from the mediastinal #4R lymph nodes obtained with EBUS-TBNA, which was performed 2 months later. EGFR mutation was negative, but the direct sequence of the EML4-ALK mRNA indicated that the translocation was variant 3 [9]. She decided to be enrolled to the crizotinib study (PF02341066) at a dosage of 500 mg/day at Seoul National University from July 2009.

She had nausea, diarrhea and light image persistence as in case 1, but her gastrointestinal symptoms were severer than those in case 1. Two weeks after the administration of ALK inhibitor, her back pain disappeared. A PET scan performed 5 weeks after the initiation of the therapy showed marked reduction of bone and lymph node metastases, and the primary tumor had decreased in size from 22X13 mm to 12X7 mm (Fig. 4A and B). Also, the SUV max dropped from 10.7 to 2.42. Changes of tumor markers were not parallel with the clinical course since the measured value of CA-125 dropped from 424 to 107 U/ml, but that of CEA increased from 21.5 to 65.4 ng/ml 4 months later. The value of CEA then gradually decreased to 15.2 ng/ml in April 2010 (10 months after that: Fig. 6). The PET scan conducted after 7 months indicated a partial response to multiple bone and lymph node metastases (Fig. 5B). The patient continued to take the drug until the end of July 2010, when brain metastases (Fig. 7) were found.

3.3. Case 3

A fifty-four-year-old woman, also a non-smoker, PS0, visited a doctor because of back pain in August 2008. Chest X-ray and CT scan showed an S3 59X22 mm tumor in the right upper lobe, combined with #4R, #2R mediastinal lymph nodes and intrapulmonary metastases. The tumor had invaded the SVC and the azygos vein. She had undergone bronchoscopy and EBUS-TBNA in October 2008. A diagnosis of lung adenocarcinoma was obtained with TBNA samples from #7 lymph nodes. Bone scans indicated cranial, costal, vertebral, scapular, pelvic and femoral metastases (T4N2M1 stage IV). She received 2 courses of CBDCA + GEM (1000 mg/m^2) and 7 courses of docetaxel (TXTL: 60 mg/m^2) from November 2008 to June 2009, but the effect was minimal.

EML4-ALK fusion gene was suggested immunohistochemically in August 2009 and confirmed by RT-PCR obtained by EBUS-TBNA samples from the primary tumor in September 2009. She was enrolled for the clinical trial from November 2009 with an oral administration of crizotinib 500 mg/day. Dyspnea and cough were alleviated within 2 weeks, and she complained of severe diarrhea, nausea, vomiting, light image persistence and perceived changes of taste. A PET scan one month after the start of the treatment demonstrated complete disappearance of the primary tumor as well as all the metastases except for a bone metastasis to the right 8th rib. A PET scan follow-up 8 months later indicated complete control of primary and metastatic tumors (Fig. 8A and B). CEA declined slowly from 1764 ng/ml to 79 ng/ml 6 months after the start of administration (Fig. 9). The patient had 12 brain metastases from 5 mm^3 to 309 mm^3 in volume and underwent gamma knife irradiation in August 2009, 2 months before the start of ALK inhibitor treatment. The irradiated field still showed little change for 5 months, but small new lesions appeared in the left occipital area 6 months after the start of the trial. Brain metastases grew very slowly, so we have maintained our observation until October 2010.

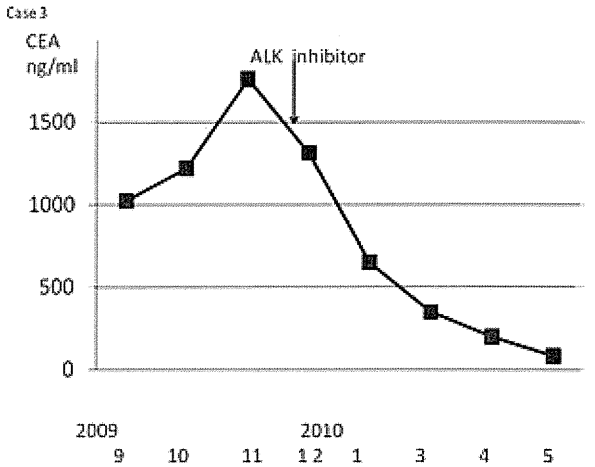

Fig. 9. CEA (■) declined slowly from 1764 ng/ml to 79 ng/ml 6 months after the start of the therapy in case 3.

4. Discussion

Above, we have reported the far-reaching effects of an ALK inhibitor on *EML4-ALK*-positive lung cancer patients. Soon after the administration of crizotinib, almost all metastases to bone and lymph nodes rapidly disappeared, followed by a marked reduction in the level of tumor markers in the sera. These observations clearly support the pivotal role of EML4-ALK oncokinase for the growth/survival of not only primary tumors but of the metastases. Such profound effects were rare among the patients when treated with conventional cytotoxic anticancer drugs.

The three cases which were enrolled for the study had surprisingly similar biological characteristics. They had multiple bone and lymph node metastases at the first medical examination, and were non-smokers at younger ages (48–54) who were resistant to chemotherapy. Adverse effects with crizotinib were also similar among them, including transient diarrhea, nausea, light image persistence, and subjective changes of taste. In addition, their response to ALK inhibitor was similar. Bone and lymph node metastases had disappeared within one month after the initiation of the therapy. The response of the primary tumor in case 2 was relatively slow compared with those of the metastases. The difference between the response of primary tumor and metastases to the ALK inhibitor in this case seems to indicate that the similar subclones of tumor cells in the primary tumors that were highly responsive to ALK inhibitor metastasized to distant organs and may give some explanation for the discrepancy in the time-course between CEA and CA125.

Molecular and immunohistochemical analyses in this cohort were conducted on the basis of the specimens obtained through EBUS-TBNA. Originally, EBUS-TBNA had been proposed useful for the pathological diagnosis of mediastinal involvement (N2 disease) of lung cancer [17–20]. However, we have already reported that EBUS-TBNA is also a versatile way of obtaining histological samples for the molecular analyses of cancer-related genes, such as *EGFR*, p53 et al. [21,22]. For those who have advanced NSCLC, it is often difficult to conduct surgery to obtain specimens from patients. Among such cases, however, EBUS-TBNA can usually be safely carried out to obtain specimens from enlarged mediastinal

lymph nodes or parathracheal tumors. We carried out EBUS-TBNA procedure for the reasons of its advantage in obtaining high quality core samples adequate for this purpose as well as its safety. We do not disregard the importance of TBB for the diagnosis of lung cancer; however, we needed histological samples to examine the immunohistochemistry and FISH for enrolment in a trial of crizotinib. Our experience with the three cases clearly demonstrates the importance and clinical relevance of obtaining such specimens for molecular analyses.

Although the initial effects of crizotinib are substantial in our cases, as well as in those reported by Bang et al. [10,11], such efficacy may not always last long. There was, for instance, development (case 1 and 2) and recurrence (case 3) of brain metastases while favorable control was maintained outside the brain. Given that the primary tumors and lymph node metastases were under control of crizotinib even at the appearance of brain metastases, the tumor cells outside the brain did not lose sensitivity to crizotinib. Relapses in the brain only may indicate either (i) subclones of the tumor acquired both the homing ability to the brain and resistance to crizotinib, or (ii) crizotinib may not penetrate the blood-brain barrier, leading to insufficient concentrations of crizotinib in the brain. It is thus highly important to examine in detail the molecular basis that would account for such acquired resistance to crizotinib, which may be secondary mutations within EML4-ALK itself or mutations/gene amplification of other genes, as demonstrated in the cases of acquired resistance of NSCLC to gefitinib/erlotinib [23–26].

Conflict of interest

None declared.

Acknowledgements

We are grateful to Dr. Yung-Jue Bang and the medical staff of Seoul National University Hospital for their support in the treatment of these patients. We also thank Mr. C.W.P. Reynolds of the Department of International Medical Communications, Tokyo Medical University, for his careful revision of the English of this manuscript.

References

[1] Janku F, Stewart DJ, Kurzrock R. Targeted therapy in non-small-cell lung cancer—Is it becoming a reality? Nat Rev Clin Oncol 2010 Jun 15;7(July (7)):401–14.
[2] Heinrich MC, Owzar K, Corless CL, et al. Correlation of kinase genotype and clinical outcome in the North American intergroup phase III trial of imatinib mesylate for treatment of advanced gastrointestinal stromal tumor: CALGB 150105 study by cancer and leukemia group B and southwest oncology group. J Clin Oncol 2008;26:5360–7.
[3] Mok TS, Wu YL, Yu CJ, et al. Randomized, placebo-controlled, phase II study of sequential erlotinib and chemotherapy as first-line treatment for advanced non-small-cell lung cancer. J Clin Oncol 2009;27:5080–7.
[4] Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. Nature 2007;448:561–6.
[5] Mano H. Non-solid oncogenes in solid tumors: EML4-ALK fusion genes in lung cancer. Cancer Sci 2008;99:2349–55.
[6] Soda M, Takada S, Takeuchi K, et al. A mouse model for EML4-ALK-positive lung cancer. Proc Natl Acad Sci USA 2008;105:19893–7.
[7] Christensen JG, Zou HY, Arango ME, et al. Cytoreductive antitumor activity of PF-2341066, a novel inhibitor of anaplastic lymphoma kinase and c-Met, in experimental models of anaplastic large-cell lymphoma. Mol Cancer Ther 2007;6:3314–22.
[8] Koivunen JP, Mermel C, Zejnullahu K, et al. EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. Clin Cancer Res 2008;14: 4275–83.
[9] Kwak EL, Camidge DR, Clark J, et al. Clinical activity observed in a phase I dose escalation trial of an oral c-met and ALK inhibitor, PF-02341066. J Clin Oncol 2009;27:15s.
[10] Bang Y, Kwak EL, Shaw AT, et al. Clinical activity of the oral ALK inhibitor PF-02341066 in ALK-positive patients with non-small cell lung cancer (NSCLC). J Clin Oncol 2010;28:18s [suppl; abstr 3].
[11] Kwak EL, Bang YJ, Camidge DR, et al. Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. N Engl J Med 2010;363:1693–703.
[12] Takeuchi K, Choi YL, Togashi Y, et al. KIF5B-ALK, a novel fusion oncokinase identified by an immunohistochemistry-based diagnostic system for ALK-positive lung cancer. Clin Cancer Res 2009;15:3143–9.
[13] Inamura K, Takeuchi K, Togashi Y, et al. EML4-ALK lung cancers are characterized by rare other mutations, a TTF-1 cell lineage, an acinar histology, and young onset. Mod Pathol 2009;22:508–15.
[14] Shaw AT, Yeap BY, Mino-Kenudson M, et al. Clinical features and outcome of patients with non-small-cell lung cancer who harbor EML4-ALK. J Clin Oncol 2009;27(September (26)):4247–53.
[15] Takahashi T, Snooze M, Kobayashi M, et al. Clinicopathologic features of non-small-cell lung cancer with EML4-ALK fusion gene. Ann Surg Oncol 2010;17(March (3)):889–97.
[16] Nakajima T, Kimura H, Takeuchi K, et al. Treatment of lung cancer with an ALK inhibitor after EML4-ALK fusion gene detection using endobronchial ultrasound-guided transbronchial needle aspiration. J Thorac Oncol 2010;5:2041–3.
[17] Yasufuku K, Chiyo M, Koh E, et al. Endobronchial ultrasound guided transbronchial needle aspiration for staging of lung cancer. Lung Cancer 2005;50:347–54.
[18] Yasufuku K, Chiyo M, Sekine Y, et al. Real-time endobronchial ultrasound-guided transbronchial needle aspiration of mediastinal and hilar lymph nodes. Chest 2004;126:122–8.
[19] Herth FJ, Eberhardt R, Vilmann P, Krasnik M, Ernst A. Real-time endobronchial ultrasound guided transbronchial needle aspiration for sampling mediastinal lymph nodes. Thorax 2006;61:795–8.
[20] Herth FJ, Ernst A, Eberhardt R, Vilmann P, Dienemann H, Krasnik M. Endobronchial ultrasound-guided transbronchial needle aspiration of lymph nodes in the radiologically normal mediastinum. Eur Respir J 2006;28: 910–4.
[21] Nakajima T, Yasufuku K, Suzuki M, et al. Assessment of epidermal growth factor receptor mutation by endobronchial ultrasound-guided transbronchial needle aspiration. Chest 2007;132:597–602.
[22] Mohamed S, Yasufuku K, Nakajima T, et al. Analysis of cell cycle-related proteins in mediastinal lymph nodes of patients with N2-NSCLC obtained by EBUS-TBNA: relevance to chemotherapy response. Thorax 2008;63:642–7.
[23] Kobayashi S, Boggon TJ, Dayaram T, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. N Engl J Med 2005;352:786–92.
[24] Lu L, Ghose AK, Quail MR, et al. ALK mutants in the kinase domain exhibit altered kinase activity and differential sensitivity to small molecule ALK inhibitors. Biochemistry 2009;48:3600–9.
[25] Gazdar AF. Activating and resistance mutations of *EGFR* in non-small-cell lung cancer: role in clinical response to EGFR tyrosine kinase inhibitors. Oncogene 2009;28:S24–31.
[26] Choi YL, Soda M, Yamashita Y, et al. EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. N Engl J Med 2010;363:1734–9.

UICC
global cancer control

IJC
International Journal of Cancer

Antitumor effect of sunitinib against skeletal metastatic renal cell carcinoma through inhibition of osteoclast function

Shinya Maita[1], Takeshi Yuasa[1,2,3], Norihiko Tsuchiya[1], Yoko Mitobe[1], Shintaro Narita[1], Yohei Horikawa[1], Kiyohiko Hatake[2], Iwao Fukui[3], Shinya Kimura[4], Taira Maekawa[5] and Tomonori Habuchi[1]

[1] Department of Urology, Akita University School of Medicine, Akita, Japan
[2] Department of Medical Oncology, Cancer Institute Hospital, Japanese Foundation for Cancer Research, Ariake, Tokyo, Japan
[3] Department of Urology, Cancer Institute Hospital, Japanese Foundation for Cancer Research, Ariake, Tokyo, Japan
[4] Department of Hematology, Saga University School of Medicine, Saga, Japan
[5] Department of Transfusion Medicine and Cell Therapy, Kyoto University Hospital, Kyoto, Japan

We investigated the inhibitory effect of sunitinib, a newly approved multitargeted tyrosine kinase inhibitor, against the progression of renal cell cancer (RCC) bone metastases *in vivo*. *In vitro* cell proliferation was determined using the MTS assay. To investigate the inhibitory effects of sunitinib *in vivo*, we established luciferase-labeled ACHN^{Luc} cells derived from papillary RCC. Mice in which ACHN^{Luc} cells had been transplanted into the left ventricle to establish bone metastases were treated orally with 40 mg/kg/day sunitinib or vehicle control for 3 weeks. Growth of the cancer cells was monitored using an *in vivo* imaging system. In addition, 16 patients with metastatic RCC were treated with sunitinib, and serum and urine levels of amino-terminal telopeptide (NTx) were measured as markers of bone resorption. Sunitinib did not inhibit the growth of RCC cells *in vitro* at clinically or experimentally achievable serum levels (100 nM–1 μM). To investigate the inhibitory effect of sunitinib *in vivo*, we established luciferase-labeled human RCC cells (ACHN^{Luc}). Sunitinib prevented the growth of ACHN^{Luc} RCC cells in the bone metastatic mouse model. The number of osteoclasts in sunitinib-treated mice was significantly less than that in control mice. Serum and urine levels of NTx in patients with metastatic RCC declined significantly during the first 4 weeks of sunitinib treatment ($p = 0.027$). Sunitinib is a potent anticancer agent for RCC bone metastases, at least for papillary RCC.

Bone is a common site of metastasis, with the frequency of solitary or multiple metastases to bone ranging from 24 to 51% in patients with metastatic renal cell cancer (RCC).[1–3] Although bone metastasis is not an independent prognostic factor associated with poor survival, the prognosis of patients with bone metastasis is not favorable when they are treated with cytokines, with an average life expectancy of 8–16 months.[2–4] Moreover, bone metastases are associated with poor performance status due to intractable pain and pathological fractures.[5] Because treatment options for RCC patients with bone metastasis are limited, appropriate treatment strategies are desired.

Sunitinib is a newly approved, multitarget, small-molecule tyrosine kinase inhibitor for the treatment of metastatic RCC. It inhibits various receptor tyrosine kinases, including vascular endothelial growth factor (VEGF) receptors 1, 2 and 3; stem cell factor receptor (KIT) and PDGF receptors α and β.[6–8] Moreover, sunitinib has been known to inhibit the phosphorylation of colony-stimulating factor (CSF)-1R, resulting in the prevention of osteoclast function and CSF-1R-dependent osteolysis in an experimental breast cancer bone metastasis model.[9,10] These findings led us to propose the hypothesis that sunitinib may inhibit tumor growth and osteolysis in bone metastatic lesions in RCC patients.

Although establishing a treatment strategy for bone metastases from RCC is important for urologists, the assessment of inhibitory effects on the growth of bone metastases is often difficult in clinical practice. In this study, we show that sunitinib has anticancer as well as inhibitory activities against osteolysis in an experimental mouse model of bone metastasis of RCC cells.

Key words: renal cell carcinoma, bone metastases, sunitinib, *in vivo* imaging system
Grant sponsors: Takeda Science Foundation, The Kobayashi Institute for Innovative Cancer Chemotherapy, The Shimadzu Science Foundation, The Sagawa Foundation for Promotion of Cancer Research, Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan and the GCOE program of the Ministry of Education, Culture, Sports, Science and Technology, Japan
DOI: 10.1002/ijc.26034
History: Received 15 Jun 2010; Revised 4 Jan 2011; Accepted 26 Jan 2011; Online 8 Mar 2011
Correspondence to: Takeshi Yuasa, Department of Medical Oncology and Genitourinary Oncology, Cancer Institute Hospital, Japanese Foundation for Cancer Research, Ariake, Tokyo 135-8550, Japan, E-mail: takeshi.yuasa@jfcr.or.jp

Material and Methods

Animals, cell lines and reagents

Approval for these studies was obtained from the institutional review board at Akita University School of Medicine. Specific pathogen-free BALB/c *nu/nu* mice (CLEA, Kyoto, Japan) aged 7 weeks were used. The human RCC lines ACHN, CCFRC-1, CCFRC-2 and NC65 were obtained from the American Type Culture Collection (ATCC, Rockville, MD) and sunitinib was obtained from Pfizer (New York, NY).

Patients

A total of 16 native Japanese patients with metastatic RCC, who were treated at the Department of Urology at Akita University School of Medicine between 2008 and 2009, were enrolled, and the serum and urine levels of amino-terminal telopeptide (Serum NTx, normal range: 9.5–17.7 nmol/l) were measured as markers of bone resorption. The patients' characteristics are shown in Table 1. The median dose was 37.5 (25–50) mg/day and the median number of treatment cycles was 4.6 (1–21). Written informed consent was provided according to the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of Akita University Graduate School of Medicine. The response was assessed by computed tomography (CT) after at least every two cycles of treatment, according to the Response Evaluation Criteria in Solid Tumors (RECIST ver. 1.0).[11]

Growth inhibitory effects of sunitinib in vitro

Cell proliferation was determined by the MTS assay using CellTiter96 (Promega Corporation, Madison) as described previously.[12]

Generation of a stable luciferase-expressing cancer cell line

Among the RCC cell lines we tested (ACHN, CCFRC-1, CCFRC-2 and NC65), ACHN was the only line that was transplanted into the left ventricle and formed bone metastases successfully. Therefore, we used $ACHN^{Luc}$ in the *in vivo* experiment. ACHN cells were stably transfected with the pGL3 control vector (Promega Corporation, Madison) and with pSV2Neo (ATCC), as described previously.[12] In brief, the cells were treated with 10 μg pGL3 control vector and 1 μg pSV2Neo vector using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) in Opti-MEM (Invitrogen) and selected using geneticin (400 μg/ml). Stable clones expressing luciferase were isolated and the clone with the highest level of luciferase expression (as determined by bioluminescence) was selected using luciferin (Xenogen, Alameda, CA) and an *in vivo* imaging system (IVIS; Xenogen).

In vivo effects of sunitinib

To produce bone metastasis models, RCC cell suspensions (3×10^6/100 μl phosphate-buffered saline) were injected into the left ventricle of mice under inhalation anesthesia with isoflurane (Abbott Japan, Tokyo, Japan). From 21 days after implantation, 14 mice with bone metastases were selected and divided into two matched groups on the basis of bioluminescence quantified by IVIS. On the same day, we started daily oral administration of 40 mg/kg (body weight) sunitinib or the solution used to dissolve sunitinib as vehicle control. According to the human 4 weeks on/2 weeks off schedule, mice were treated with sunitinib for 4 weeks before being sacrificed. Mice were observed by IVIS once per week.

Table 1. Patients characteristics

Factors			
Age (years old)	Median	60.5	Range: 37–80
Sex (*n*)	Male	13	(81%)
	Female	3	(19%)
Metastatic sites	Lung	12	(75%)
	Liver	4	(25%)
	Bone	5	(31%)
	Brain	3	(19%)
	Lymph node	3	(19%)
Follow-up period (month)	Median	4.5	Range: 1–37
Performance status	0 or 1	15	(94%)
	>1	1	(6%)
Diagnosis to initial treatment	>1 year	5	(31%)
	<1 year	11	(69%)
Blood hemoglobin	Normal range	7	(44%)
	ULN>	9	(56%)
Serum calcium	<10 mg/dl	15	(94%)
	≥10 mg/dl	1	(6%)
Serum LDH	<1.5 × ULN	16	(100%)
	≥1.5 × ULN	0	(0%)
MSKCC risk classification	Favorable	6	(38%)
	Intermediate	9	(56%)
	Poor	1	(6%)

ULN: upper limit of normal range.

Measurement of bone metastatic lesions by in vivo imaging

An aqueous solution of luciferin (150 mg/kg) was injected intraperitoneally 10 min before imaging. The animals were anesthetized with isoflurane and placed in the light-tight chamber of a CCD camera system (Xenogen) and photons emitted from the luciferase-expressing cells within the animal were quantified for 5 min using the software program Living Image (Xenogen) as an overlay on Igor (Wavemetrics, Seattle, WA). Using this *in vivo* imaging system, we evaluated the efficacy of sunitinib by measuring the photon counts of the metastatic lesions in the mandible and both hip joints in a blinded manner as described previously.[13]

Measurement of serum VEGF and M-CSF in the mouse bone metastasis model in vivo

The serum concentrations of VEGF and M-CSF in mice were determined using Quantikine ELISA (R&D Systems, Minneapolis, MN) according to the manufacturer's protocol. To investigate the serum concentrations of VEGF and M-CSF, sera from each of seven treated and seven untreated mice were collected and analyzed 4 weeks after $ACHN^{Luc}$ inoculation.

Histological analysis

After imaging studies, the femora of the mice were removed, frozen immediately and stored at −80°C. To detect osteoclasts, 4-μm-thick sections were stained with tartrate-resistant acid phosphatase (TRAP) using the TRAP and ALP double-stain kit (Takara Bio, Otsu, Japan), as described previously.[14] Three sections were examined in each femur. The number of TRAP-positive osteoclasts was counted per ten high-power microscope fields by two blinded examiners, as described previously.[14]

Statistical analysis

The influence of sunitinib on the growth of bone metastases was analyzed by Student's t test. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS version 13.0; SPSS, Chicago, IL), and two-sided p values <0.05 were considered statistically significant.

Results

Effect of sunitinib on RCC growth in a mouse bone metastasis model in vivo

Injection of cancer cells via the left ventricle is an established method of inducing bone metastases, as reported previously.[12,13] In the present study, all mice that were successfully implanted with $ACHN^{Luc}$ cancer cells developed bone metastases 3 weeks after injection. Of these mice, we excluded those that showed brilliant bioluminescence in the lungs. The remaining 14 mice were then divided into two matched groups according to bioluminescence quantified by IVIS, and we administered either sunitinib or vehicle control for 4 weeks and monitored the growth of bone metastases in the lesions in the maxilla and bilateral hip joints, as described previously[12,13] (Fig. 1*b*). Metastatic bone lesions in the control group progressed during the 3 weeks. On the other hand, photon emission was significantly suppressed in the sunitinib treatment group ($p < 0.001$) (Figs. 1*a* and 1*c*). The mean body weights of the mice did not differ significantly between the two groups.

Serum VEGF and M-CSF in a mouse bone metastasis model in vivo

To examine the indirect antitumor effect of sunitinib, we measured the concentrations of VEGF and M-CSF. However, no significant difference was present in the serum concentrations of these growth factors between the two groups (Fig. 1*d*).

Effect of sunitinib on osteoclasts in a mouse bone metastasis model

Next, we investigated the efficacy of sunitinib against osteoclasts in the tumor-bearing mice. Femoral bone sections were stained with TRAP to enable counting of the number of osteoclasts, as described previously.[13] The mean number of TRAP-positive osteoclasts in mice treated with sunitinib was significantly lower than that in mice treated with vehicle control (23.1 ± 4.7 *vs.* 33.2 ± 7.9 osteoclasts/100 high-power fields, respectively; $p = 0.013$).

Sunitinib did not inhibit cell proliferation in vitro at a clinically achievable serum concentration

To assess the direct antitumor effect of sunitinib, four RCC cell lines (ACHN, CCFRC-1, CCFRC-2 and NC65) were cultured in the presence of various concentrations of sunitinib (0.1 nM–10 μM). Sunitinib inhibited the proliferation of these cell lines in a concentration-dependent manner (Fig. 2). However, sunitinib was not effective *in vitro* at the clinically achievable serum concentration (~80 nM), as demonstrated previously.[8] On the other hand, the serum concentration of sunitinib was reported to be ~100 nM on administration to mice at 40 mg/kg/day.[14] The IC50s of sunitinib for these cell lines were estimated to be >1 μM. These results suggest the involvement of an indirect growth inhibitory mechanism of sunitinib, at least partially, for bone metastatic lesions in mice.

Effect of sunitinib on serum and urine levels of NTx in patients with metastatic RCC

The characteristics and demographic data of the patients are shown in Table 1. As shown in Figure 3, both serum and urine levels of NTx significantly declined during the first 4 weeks of treatment with sunitinib ($p = 0.027$). During the holiday period when the administration was discontinued following 4 weeks of administration of sunitinib, the serum and urine levels of NTx showed gradual recovery (Fig. 3). Of these 16 patients, five had bone metastatic lesions, but we could not evaluate the efficacy of sunitinib quantitatively. Regarding the extraosseous sites, nine of 14 patients demonstrated a partial response (PR) or stable disease (SD) whereas the remaining five demonstrated progressive disease (PD). The reduction rate of the serum NTx level from the baseline in patients with favorable efficacy (PR/SD; 30.8%) was higher than that in patients with poor efficacy (PD; 22%), although the difference was not significant ($p = 0.6404$).

Discussion

In patients with metastatic RCC, bone is the major metastatic organ, second only to the lung.[1–3] Bone metastases were shown to be associated with severe bone pain, pathological fractures, spinal cord compression and a short survival

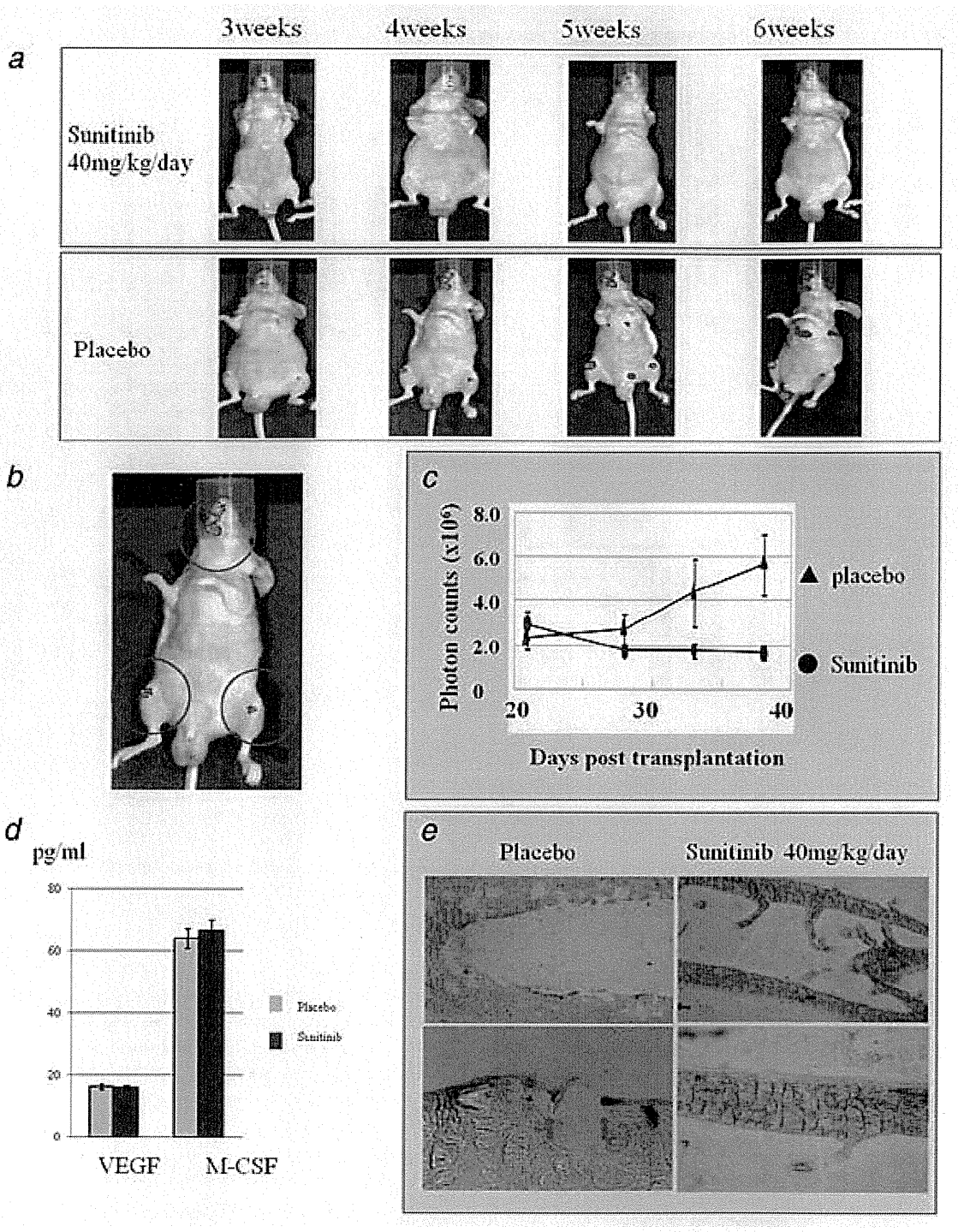

Figure 1. Growth inhibitory effect of orally administered sunitinib in an RCC bone metastatic mouse model. We established an RCC bone metastatic mouse model using the cell line ACHN^{Luc}. Images were obtained using an *in vivo* imaging system 3–6 weeks after cell transplantation by intracardiac injection (*a*). To evaluate the growth inhibitory effect of orally administered sunitinib, we selected metastatic lesions from the maxilla and bilateral hip joints as examples of bone metastasis (*b*). Average real-time growth curves of ACHN^{Luc} cells of bone metastatic lesions in sunitinib- and control vehicle-treated groups demonstrated that sunitinib significantly prevented the growth of metastatic bone lesions ($p < 0.001$; *c*). Serum levels of VEGF and M-CSF did not differ significantly between sunitinib-treated and control mice (*d*). The mean number of TRAP-positive osteoclasts in mice treated with sunitinib was significantly lower than that in mice treated with vehicle control ($p = 0.013$; *e*).

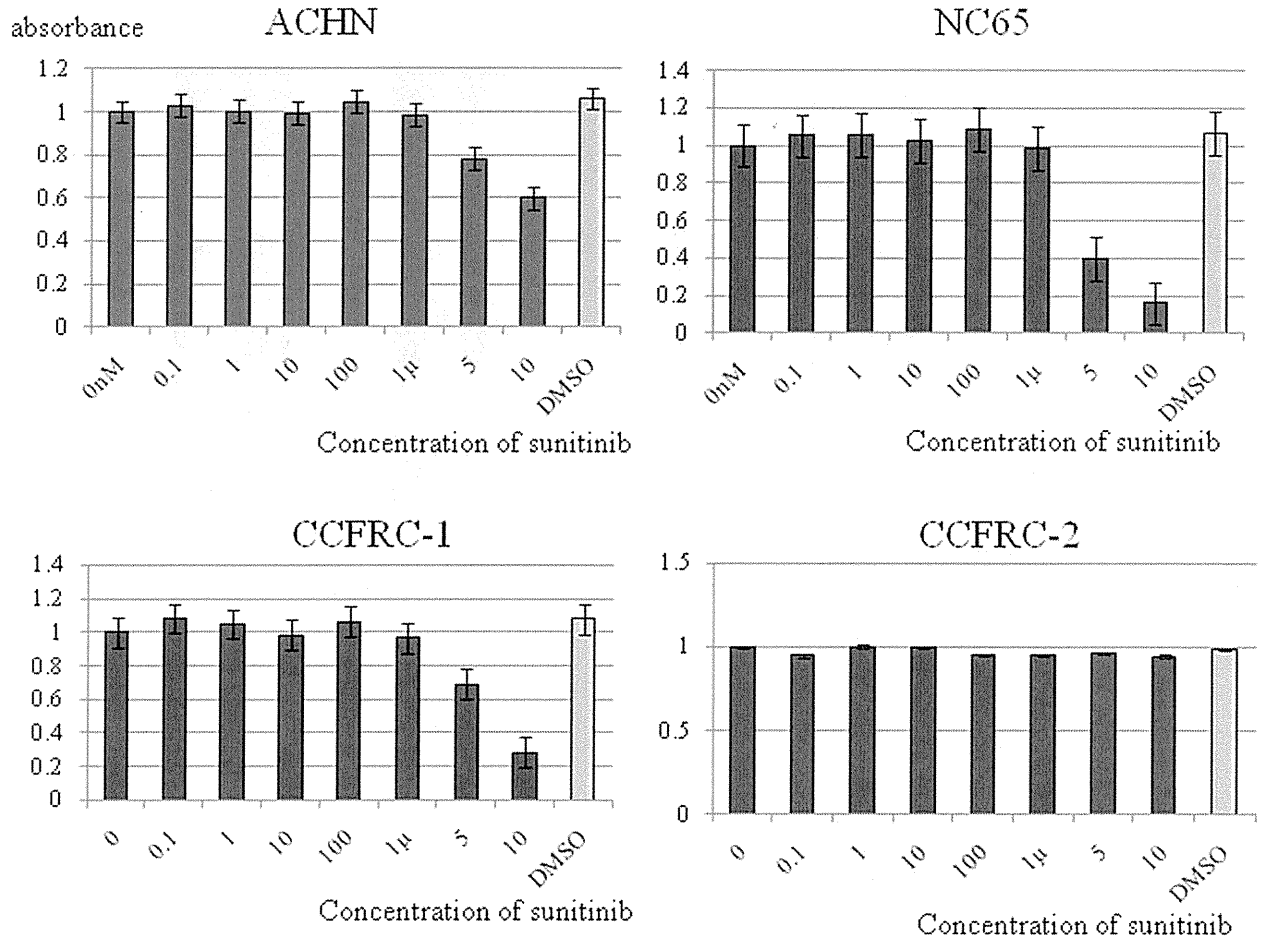

Figure 2. Sunitinib does not inhibit the growth of RCC at a clinically achievable concentration *in vitro*. Cells of the RCC lines ACHN, NC65, CCFRC-1 and CCFRC-2 were plated at 3,000 cells/well in 96-well plates, incubated for 24 hr, and then treated with various concentrations (0–100 mM) of sunitinib. After 72 hr of incubation, relative cell growth was measured in an MTS assay. Data are Mean ± SD. Sunitinib did not inhibit the growth of any of the four RCC cell lines at the clinically achievable concentration (~80 nM) *in vitro*.

period.[4,11] Several studies have demonstrated that bone metastasis is one of the risk factors for poor prognosis in the cytokine era, although it was not identified as an independent prognostic factor.[1–4] Négrier *et al.* investigated the prognostic factors of 782 metastatic RCC patients treated with cytokines and found that 32% (248/776) had bone metastases, and that these patients had a significantly worse prognosis than those without bone metastases ($p = 0.008$).[2] Recently, Naito *et al.* retrospectively analyzed the prognosis of 1,463 Japanese metastatic RCC patients in the cytokine era and demonstrated that 24.6% (320/1,302) had bone metastases, and that these patients also had a significantly worse prognosis than those without bone metastases ($p = 0.003$).[3] Accumulated evidence suggests that systemic immunotherapy is not effective in the management of bone metastasis of RCC.

The efficacy of sunitinib against RCC bone metastasis, however, remains to be established and is difficult to evaluate in clinical practice. Thus, we sought to investigate the efficacy of sunitinib against bone metastatic RCC in the preclinical setting. The dose of sunitinib used in this study (40 mg/kg/day) was intended to provide a serum level of sunitinib similar to that attained in the clinical setting.[8,15] Pharmacokinetic and pharmacodynamic analyses showed that the clinical dose of 50 mg/day led to plasma concentrations ranging from 50 to 100 ng/ml in humans.[8] This dose is equivalent to the plasma concentration in mice administered sunitinib at 40 mg/kg/day.[15] Data from VEGF-induced vascular permeability assays also support 50–100 ng/ml as the range, including the minimum plasma concentrations required to inhibit VEGFR and PDGFR *in vivo*.[8] Therefore, our results obtained in the RCC bone metastatic model used in this study might be reflective of those obtained in the clinical setting.

Similar to several other *in vitro* analyses, our results showed that sunitinib at concentrations of 50–100 ng/ml did not inhibit the proliferation of RCC cells *in vitro*.[10,16] Therefore, we sought an indirect mechanism for this *in vivo* growth inhibition of RCC bone metastases. Bone is an abundant repository for immobilized growth factors, including

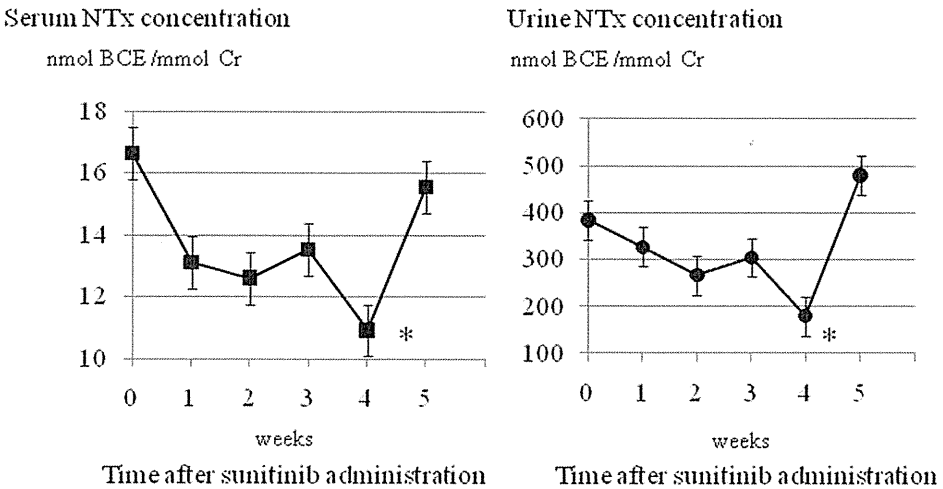

Figure 3. Alteration of bone resorption markers in sunitinib-treated patients with metastatic RCC. Serum and urine levels of NTx 28 days after oral administration of sunitinib were significantly lower than initial levels (*$p < 0.01$). Characteristics of the 16 sunitinib-treated patients are shown in Table 1.

transforming growth factor beta, fibroblast growth factor, insulin-like growth factors I and II, PDGF and bone morphogenetic proteins.[17] When osteoclasts absorb bone by secreting protons and proteases, these growth factors are released and they provide fertile ground for the growth of cancer cells. Therefore, osteoclasts are a suitable therapeutic target in the treatment of bone metastases. In this study, there were significantly fewer TRAP-positive osteoclasts in the mice treated with sunitinib than in those treated with vehicle control (Fig. 1*e*). This observation is consistent with previous reports.[10,18] Zwolak *et al.* reported that treatment with sunitinib decreased the percentage of active osteoclasts to 45.6% ± 5.8% compared with the percentage in untreated tumor-bearing mice (79.4% ± 8.6%), suggesting that sunitinib treatment (40 mg/kg/day) may inhibit osteoclast maturation.[18] Murray *et al.* reported that sunitinib inhibited osteoclast development and function mediated by M-CSF, which is one of the differentiating factors for osteoclasts and is a target tyrosine kinase of sunitinib, both *in vitro* and *in vivo*.[10] Our clinical observation of decreases in serum and urine NTx is also in line with these reports (Fig. 3).

NTx is a degradation product of Type I collagen and is often used as a marker of bone resorption both in serum and urine. Some clinical studies have suggested that levels of NTx correlate with the presence and extent of bone metastases, prognosis and response to treatment.[19,20] Although our data did not show an association between the reduction rate of NTx and the efficacy of sunitinib, further investigation is necessary to clarify this association, especially in bone metastatic lesions.

During the completion of this manuscript, we found that ACHN originated from papillary renal cancer in a 22-year-old patient (Reference [21] and by personal communication from Dr. Ernest Borden). Recent studies have suggested the possible clinical efficacy of sunitinib for patients with clear and non-clear cell cancer.[22,23] However, there are no prospective Phase 2 or Phase 3 studies clarifying this question. We therefore have to wait for the results of a large prospective study on the use of sunitinib for non-clear cell cancer. Since bone is the second most common site of metastases for RCC, we reported an indirect mechanism that may partly help to elucidate the reasons for the clinical efficacy of sunitinib.

Mesenchymal-epithelial transition factor (MET) and fumarate hydratase (FH) are considered to be the genes responsible for Type 1 and Type 2 papillary RCC, respectively.[24,25] MET, which is a proto-oncogene, encodes a tyrosine kinase membrane receptor, and activation of MET can indirectly promote angiogenesis and tumor growth through overexpression of VEGF.[26,27] FH is an enzyme in the mitochondrial tricarboxylic acid (TCA) cycle. Loss of FH leads to a state of pseudohypoxia through overexpression of hypoxia-inducible factor (HIF), resulting in an increase in downstream targets, including VEGF.[26,28] Therefore, activation of MET and loss of FH, which are considered to be responsible for Type 1 and Type 2 papillary RCC, lead to angiogenesis. Clinically, Ljungberg *et al.* demonstrated that the mRNA levels of VEGF, VEGF-receptor Type 1 and VEGF-receptor Type 2 above the median were related to adverse survival in papillary RCC.[29] Therefore, it is relevant to measure VEGF in a clear or non-clear cell RCC model.

To elucidate whether sunitinib has any other indirect effects, we measured the concentrations of VEGF and M-CSF. However, we found no significant difference between the two groups in the serum concentrations of these growth factors. This observation is consistent with previous findings. Ebos *et al.* reported a significant increase in the serum VEGF level on administration of 60–120 mg/kg sunitinib.[30] While it has been shown that sunitinib is a multikinase inhibitor that

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.

Acknowledgements

The authors thank Ms. Yuka Izumida and Ms. Tomomi Kawamura for their technical assistance.

References

1. Motzer RJ, Michaelson MD, Redman BG, Hudes GR, Wilding G, Figlin RA, Ginsberg MS, Kim ST, Baum CM, DePrimo SE, Li JZ, Bello CL, et al. Activity of SU11248, a multitargeted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor, in patients with metastatic renal cell carcinoma. *J Clin Oncol* 2006;24:16–24.
2. Négrier S, Escudier B, Gomez F, Douillard JY, Ravaud A, Chevreau C, Buclon M, Pérol D, Lasset C. Prognostic factors of survival and rapid progression in 782 patients with metastatic renal carcinomas treated by cytokines: a report from the Groupe Français d'Immunothérapie. *Ann Oncol* 2002;13:1460–68.
3. Naito S, Yamamoto N, Takayama T, Muramoto M, Shinohara N, Nishiyama K, Takahashi A, Maruyama R, Saika T, Hoshi S, Nagao K, Yamamoto S, et al. Prognosis of Japanese metastatic renal cell carcinoma patients in the cytokine era: a cooperative group report of 1463 patients. *Eur Urol* 2009; 3.
4. Toyoda Y, Shinohara N, Harabayashi T, Abe T, Akino T, Sazawa A, Nonomura K. Survival and prognostic classification of patients with metastatic renal cell carcinoma of bone. *Eur Urol* 2007;52: 163–8.
5. Kuczyk MA, Anastasiadis AG, Zimmermann R, Merseburger AS, Corvin S, Stenzl A. Current aspects of the surgical management of organ-confined, metastatic, and recurrent renal cell cancer. *BJU Int* 2005;96:721–7; quiz i–ii.
6. Oudard S, George D, Medioni J, Motzer R. Treatment options in renal cell carcinoma: past, present and future. *Ann Oncol* 2007; 18 (Suppl 10):x25–x31.
7. Hutson TE, Figlin RA, Kuhn JG, Motzer RJ. Targeted therapies for metastatic renal cell carcinoma: an overview of toxicity and dosing strategies. *Oncologist* 2008;13: 1084–96.
8. Faivre S, Delbaldo C, Vera K, Robert C, Lozahic S, Lassau N, Bello C, Deprimo S, Brega N, Massimini G, Armand JP, Scigalla P, et al. Safety, pharmacokinetic, and antitumor activity of SU11248, a novel oral multitarget tyrosine kinase inhibitor, in patients with cancer. *J Clin Oncol* 2006;24: 25–35.
9. Motzer RJ, Hoosen S, Bello CL, Christensen JG. Sunitinib malate for the treatment of solid tumours: a review of current clinical data. *Exp Opin Invest Drugs* 2006;15:553–61.
10. Murray LJ, Abrams TJ, Long KR, Ngai TJ, Olson LM, Hong W, Keast PK, Brassard JA, O'Farrell AM, Cherrington JM, Pryer NK. SU11248 inhibits tumor growth and CSF-1R-dependent osteolysis in an experimental breast cancer bone metastasis model. *Clin Exp Metastasis* 2003;20:757–66.
11. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000;92:205–16.
12. Yuasa T, Nogawa M, Kimura S, Yokota A, Sato K, Segawa H, Kuroda J, Maekawa T. A third-generation bisphosphonate, minodronic acid (YM529), augments the interferon alpha/beta-mediated inhibition of renal cell cancer cell growth both in vitro and in vivo. *Clin Cancer Res* 2005;11: 853–9.
13. Nogawa M, Yuasa T, Kimura S, Kuroda J, Sato K, Segawa H, Yokota A, Maekawa T. Monitoring luciferase-labeled cancer cell growth and metastasis in different in vivo models. *Cancer Lett* 2005;217:243–53.
14. Henriksen K, Tanko LB, Qvist P, Delmas PD, Christiansen C, Karsdal MA. Assessment of osteoclast number and function: application in the development of new and improved treatment modalities for bone diseases. *Osteoporos Int* 2007;18: 681–5.
15. Mendel DB, Laird AD, Xin X, Louie SG, Christensen JG, Li G, Schreck RE, Abrams TJ, Ngai TJ, Lee LB, Murray LJ, Carver J, et al. In vivo antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. *Clin Cancer Res* 2003;9:327–37.
16. Sonpavde G, Jian W, Liu H, Wu MF, Shen SS, Lerner SP. Sunitinib malate is active against human urothelial carcinoma and enhances the activity of cisplatin in a preclinical model. *Urol Oncol* 2009;27: 391–9.
17. Yuasa T, Kimura S, Ashihara E, Habuchi T, Maekawa T. Zoledronic acid—a multiplicity of anti-cancer action. *Curr Med Chem* 2007;14:2126–35.
18. Zwolak P, Jasinski P, Terai K, Gallus NJ, Ericson ME, Clohisy DR, Dudek AZ. Addition of receptor tyrosine kinase inhibitor to radiation increases tumour control in an orthotopic murine model of breast cancer metastasis in bone. *Eur J Cancer* 2008;44:2506–17.
19. Brown JE, Cook RJ, Major P, Lipton A, Saad F, Smith M, Lee KA, Zheng M, Hei YJ, Coleman RE. Bone turnover markers as predictors of skeletal complications in prostate cancer, lung cancer, and other solid tumors. *J Natl Cancer Inst* 2005;97: 59–69.
20. Coleman RE, Major P, Lipton A, Brown JE, Lee KA, Smith M, Saad F, Zheng M, Hei YJ, Seaman J, Cook R. Predictive value of bone resorption and formation markers in cancer patients with bone metastases receiving the bisphosphonate zoledronic acid. *J Clin Oncol* 2005;23:4925–35.
21. Ruzicka FJ, Schmid SM, Groveman DS, Cummings KB, Borden EC. Variation in the binding of 125I-labeled interferon-beta ser to cellular receptors during growth of human renal and bladder carcinoma cells in vitro. *Cancer Res* 1987;47: 4582–9.
22. Choueiri TK, Plantade A, Elson P, Negrier S, Ravaud A, Oudard S, Zhou M, Rini BI, Bukowski RM, Escudier B. Efficacy of sunitinib and sorafenib in metastatic papillary and chromophobe renal cell carcinoma. *J Clin Oncol* 2008;26:127–31.

23. Gore ME, Szczylik C, Porta C, Bracarda S, Bjarnason GA, Oudard S, Hariharan S, Lee SH, Haanen J, Castellano D, Vrdoljak E, Schöffski P, et al. Safety and efficacy of sunitinib for metastatic renal-cell carcinoma: an expanded-access trial. *Lancet Oncol* 2009;10:757–63.
24. Schmidt L, Duh FM, Chen F, Kishida T, Glenn G, Choyke P, Scherer SW, Zhuang Z, Lubensky I, Dean M, Allikmets R, Chidambaram A, et al. Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. *Nat Genet* 1997;16:68–73.
25. Tomlinson IP, Alam NA, Rowan AJ, Barclay E, Jaeger EE, Kelsell D, Leigh I, Gorman P, Lamlum H, Rahman S, Roylance RR, Olpin S, et al. Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. *Nat Genet* 2002;30:406–10.
26. Linehan WM, Pinto PA, Srinivasan R, Merino M, Choyke P, Choyke L, Coleman J, Toro J, Glenn G, Vocke C, Zbar B, Schmidt LS, et al. Identification of the genes for kidney cancer: opportunity for disease-specific targeted therapeutics. *Clin Cancer Res* 2007;13 (2 Part 2): 671–9.
27. Pennacchietti S, Michieli P, Galluzzo M, Mazzone M, Giordano S, Comoglio PM. Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. *Cancer Cell* 2003;3:347–61.
28. Isaacs JS, Jung YJ, Mole DR, Lee S, Torres-Cabala C, Chung YL, Merino M, Trepel J, Zbar B, Toro J, Ratcliffe PJ, Linehan WM, et al. HIF overexpression correlates with biallelic loss of fumarate hydratase in renal cancer: novel role of fumarate in regulation of HIF stability. *Cancer Cell* 2005;8:143–53.
29. Ljungberg BJ, Jacobsen J, Rudolfsson SH, Lindh G, Grankvist K, Rasmuson T. Different vascular endothelial growth factor (VEGF), VEGF-receptor 1 and −2 mRNA expression profiles between clear cell and papillary renal cell carcinoma. *BJU Int* 2006;98:661–7.
30. Ebos JM, Lee CR, Christensen JG, Mutsaers J, Kerbel RS. Multiple circulating proangiogenic factors induced by sunitinib malate are tumor-independent and correlate with antitumor efficacy. *Proc Natl Acad Sci USA* 2007;104:17069–74.

BJUI
BJU INTERNATIONAL

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

Takeshi Yuasa*†‡, Norihiko Tsuchiya‡, Shinji Urakami*, Yohei Horikawa‡, Shintaro Narita‡, Takamitsu Inoue‡, Mitsuru Saito‡, Shinya Yamamoto*, Junji Yonese*, Iwao Fukui*, Kenji Nakano†, Shunji Takahashi†, Kiyohiko Hatake† and Tomonori Habuchi‡

*Departments of *Urology and †Medical Oncology, Cancer Institute Hospital, Japanese Foundation for Cancer Research, Ariake, Tokyo, ‡Department of Urology, Akita University School of Medicine, Akita, Japan*

Accepted for publication 10 June 2011

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

 | doi:10.1111/j.1464-410X.2011.10534.x

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 (≤4.5×10^5/μL vs >4.5×10^5/μL); corrected calcium (≤10 mg/dL vs >10 mg/dL) and lactate dehydrogenase (LDH; ≤1.5 × 230 IU/dL vs >1.5 × 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)

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

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.

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.

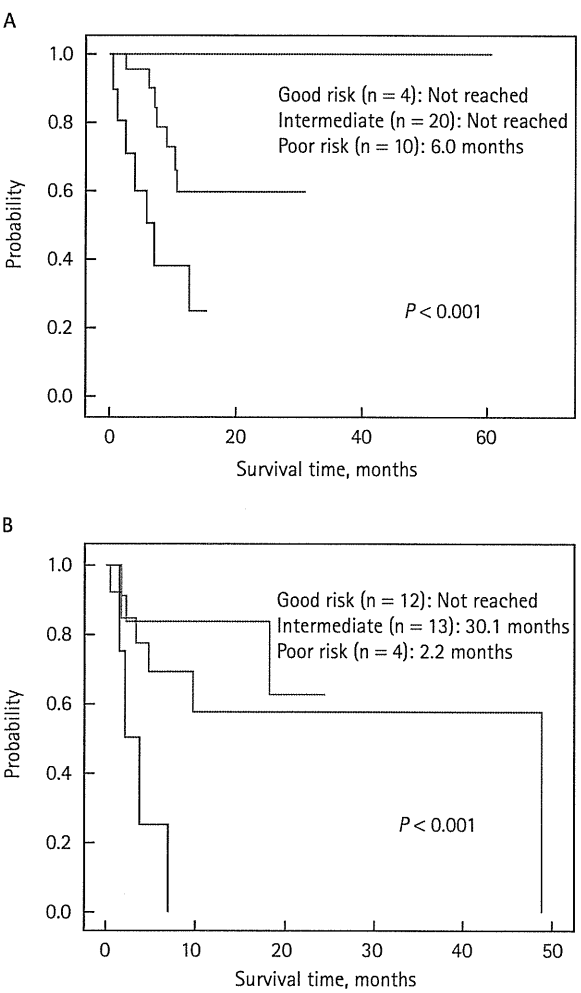

FIG. 1.
*Stratification of the overall survival for the patients with metastatic renal cell cancer by the respective Memorial Sloan-Kettering Cancer Center (MSKCC) risk scores. Stratification of the treatment naïve (**A**) and the treatment refractory (**B**) patients by MSKCC risk scores.*

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.

ACKNOWLEDGEMENTS

This work was partly supported by the Takeda Science Foundation, the Kobayashi Institute for Innovative Cancer Chemotherapy, and Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

CONFLICT OF INTEREST

None declared.

REFERENCES

1 **Hutson TE, Figlin RA.** Novel therapeutics for metastatic renal cell carcinoma. *Cancer* 2009; **115**: 2361–7
2 **Christensen JG.** A preclinical review of sunitinib, a multitargeted receptor tyrosine kinase inhibitor with anti-angiogenic and antitumour activities. *Ann Oncol* 2007; **18**: x3–10
3 **Motzer RJ, Hutson TE, Tomczak P *et al.*** Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. *N Engl J Med* 2007; **356**: 115–24
4 **Motzer RJ, Hutson TE, Tomczak P *et al.*** Overall survival and updated results for sunitinib compared with interferon alfa in patients with metastatic renal cell carcinoma. *J Clin Oncol* 2009; **27**: 3584–90