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.

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

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Breast Cancer (2011) 18:195–202
DOI 10.1007/s12282-011-0259-4

ORIGINAL ARTICLE

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

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Received: 25 October 2010 / Accepted: 17 February 2011 / Published online: 5 April 2011

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

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

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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 ($p < 0.001$).

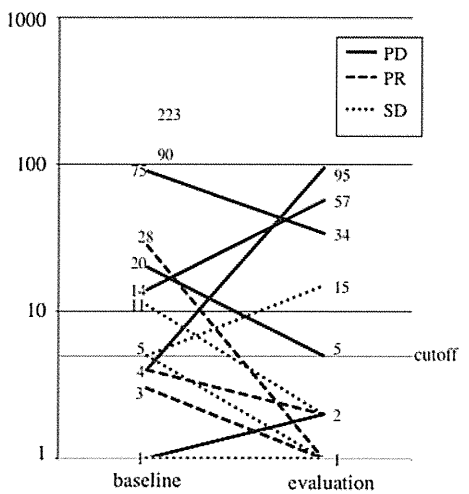

Fig. 1 Alterations of the number of CTCs between baseline and evaluation period. Of the patients whose baseline CTCs number was above the cutoff, one PR patient's CTCs disappeared, two of three SD patients' CTCs dropped below the cutoff, and one PD patient's CTCs level increased. In contrast, one PD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

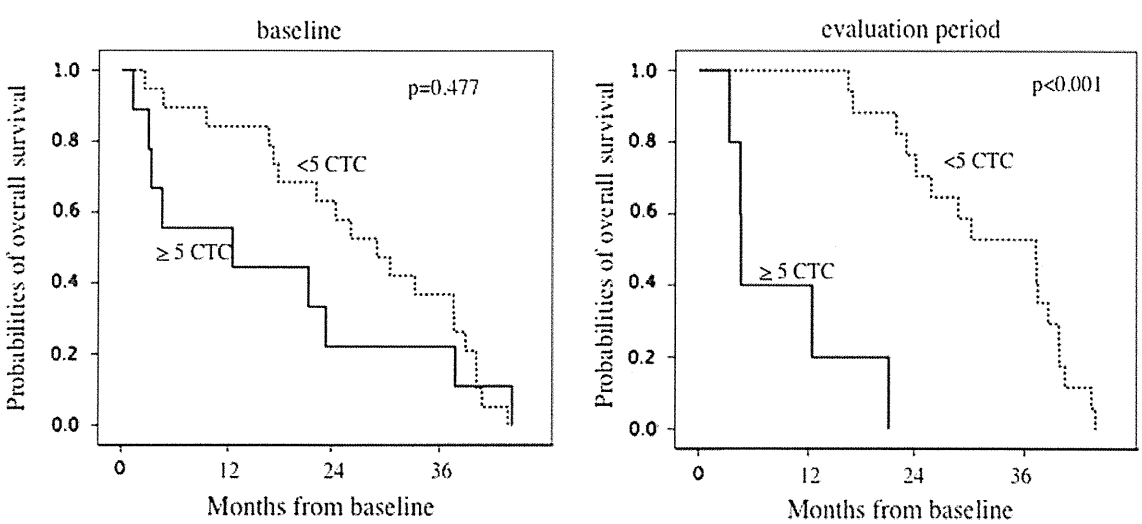

Fig. 2 Kaplan–Meier estimates of probabilities of overall survival for those positive and negative CTCs at baseline and evaluation period. Baseline CTCs did not contribute significantly to determine the prognosis; patients with negative CTCs at evaluation had a better prognosis than positive CTCs patients

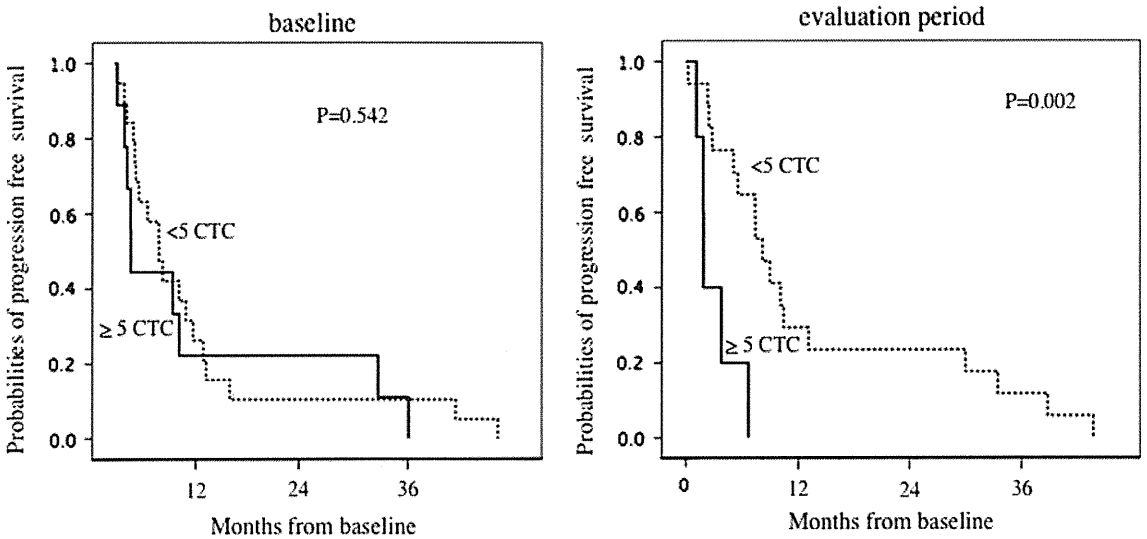

Fig. 3 Kaplan–Meier estimates of probabilities of progression-free survival for those positive and negative CTCs at baseline and evaluation period. Baseline CTC level did not contribute to predict treatment efficacy. Patients with negative CTCs at evaluation had a better prognosis than positive CTCs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

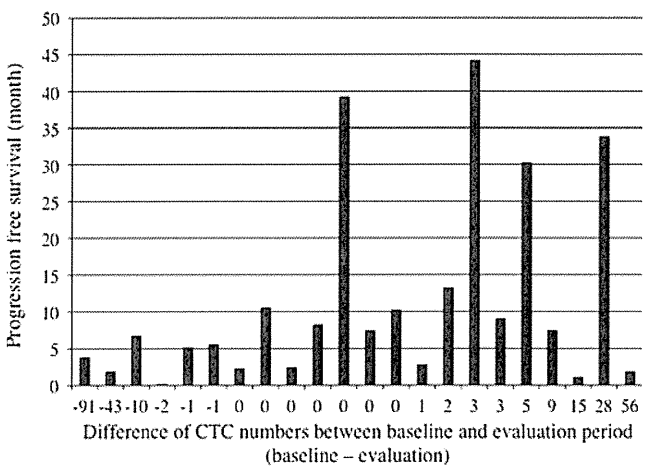

Fig. 4 Correlation of progression-free survival and the difference of CTC numbers between baseline and evaluation period. Although correlations between PFS and the difference of CTC numbers between baseline and evaluation period were inconsistent, some patients whose pre-treatment CTC number was greater 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^{TM} 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

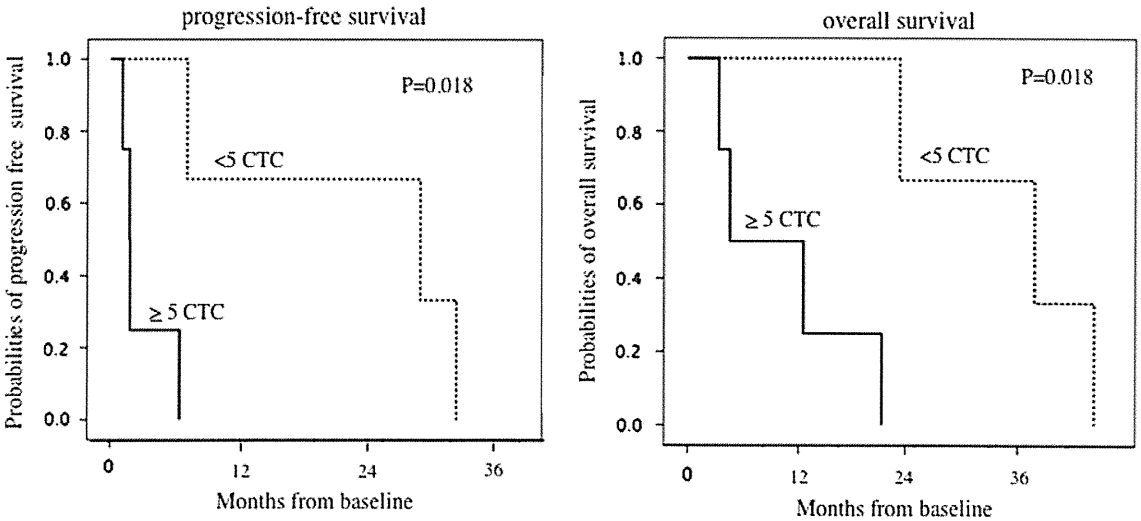

Fig. 5 Kaplan–Meier estimates of probabilities of progression-free survival and overall survival for those with positive CTCs at both pre- and post-treatment and those positive with CTCs changed to negative at evaluation period. Patients who had positive CTCs during treatment had worse PFS than patients whose CTCs level changed to negative during treatment. Patients who had positive CTCs during treatment had worse OS than patients whose CTCs level decreased below the cutoff during treatment

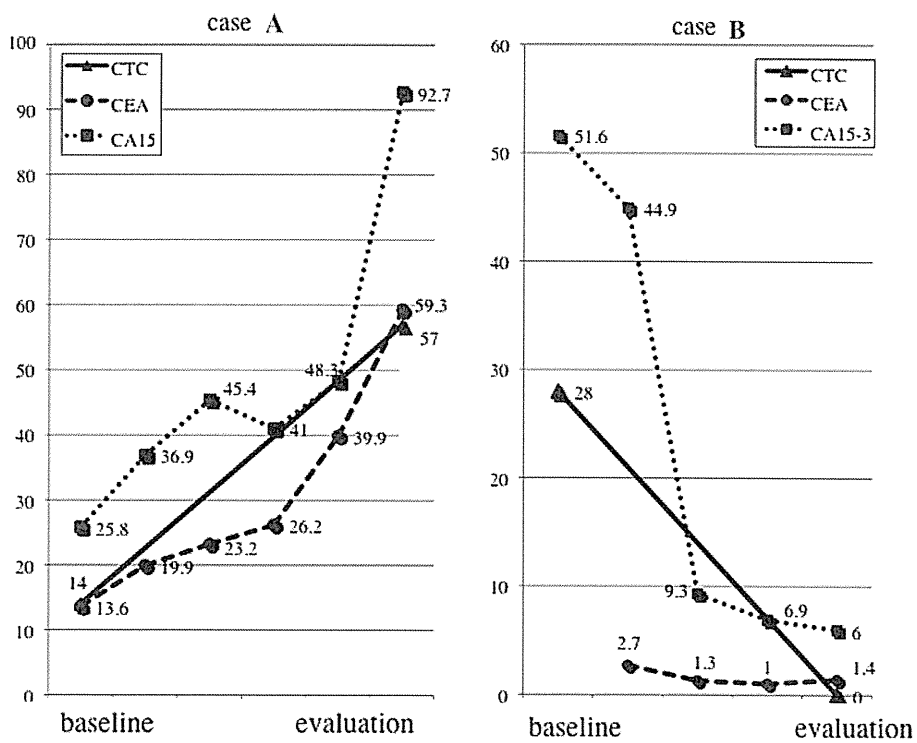

Fig. 6 *Case A* Both CTCs and tumor markers increased in this PD patient. *Case B* CTCs disappeared and tumor markers showed a tendency to decrease in this PR patient

reproducible, enabling the enumeration and characterization of CTCs [5]. It appears to provide reasonable prognostic and predictive utility in metastatic breast cancer. Commonly, clinical features including the time to first recurrence, metastatic sites, number of lesions, and tumor burden are used as prognostic factors, whereas hormone receptor status and HER2 status are usually used as predictive factors. In addition to these tools, Cristofanilli et al. described in their use of a cutoff level of CTC defined as 5 per 7.5 mL of blood [3]. The presence of more than 5 CTCs before initiation of treatment was associated with a short PFS and OS, and predicted a poorer outcome than for patients who have less than 5 CTCs detected. Similarly, the CTC level at first follow-up also predicted the treatment efficacy, PFS, and OS [3, 4]. In short, more than 5 CTCs after the initiation of treatments predicted no therapeutic

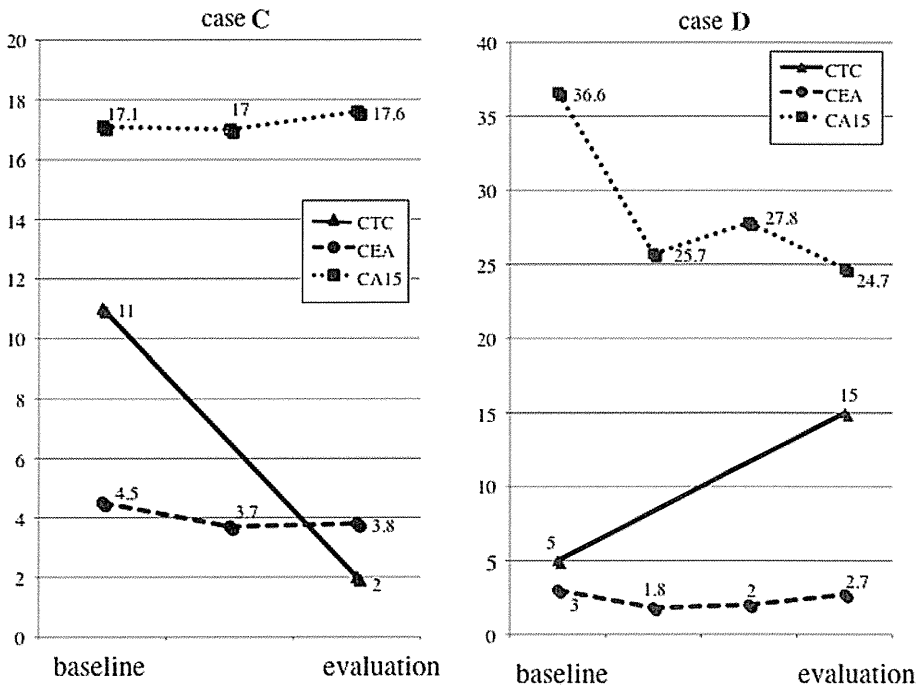

Fig. 7 *Case C* A patient whose metastatic sites showed a tendency to shrink, tumor markers did not change. On the other hand, her CTC level decreased; therefore CTC alteration was suitable to evaluate efficacy. *Case D* In spite of decreased tumor markers, the CTC level increased. Four months later, her metastatic sites progressed

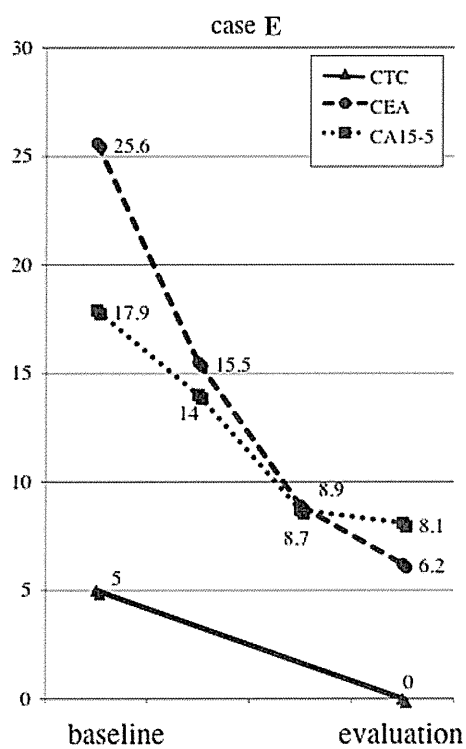

Fig. 8 *Case E* Both CTCs and tumor markers decreased in this patient with long SD who had skeletal metastasis only

benefit from the treatments. Furthermore, Hayes et al. [7]. reported that continuous detection of more than 5 CTCs at any time during therapy accurately indicated subsequent treatment failure and mortality. Although the present small study could not demonstrate that patients who initially had 5 or more 5 CTCs were associated with a worse prognosis, these data suggest that the CTC status after the treatment may be a prognostic marker. In addition, CTCs were useful to estimate treatment efficacy as a predictive marker. These results suggest that following up the number of CTCs may contribute to predict the efficacy of the treatment like tumor markers.

A subsequent report showed that CTC enumeration might provide an earlier, more reproducible indication of disease status than imaging examination. In particular, the prognosis of radiologically responding patients (stable disease and partial response) was divided into good and unfavorable prognosis groups according to the number of CTCs (5 or more, less than 5). Similarly, radiologically non-responding patients (progressive disease) were also divided into these two groups depending on the number of CTCs [8]. Furthermore, in this report, obvious radiological disease progression patients with more than 5 CTCs demonstrated a significantly worse prognosis than the patients whose CTCs level was fewer than 5.

Commonly, for monitoring patients with metastatic disease during therapy, some tumor markers can be used in conjunction with diagnostic imaging and physical examination. CA15-3 and CEA are often used in monitoring a patient's condition. However, during the first few weeks of new treatment, these serous tumor marker levels sometimes rise temporarily, and we experience difficulties in evaluating the therapeutic response [6]. As mentioned above, although alterations of tumor markers sometimes did not reflect disease condition, CTC alteration corresponded to each disease condition [9]. In our study and previous

reports, CTCs may be usable as a kind of tumor marker or surrogate marker to avoid unnecessary toxic therapy and help us develop better therapeutic strategies for our patients. After a few cycles or treatment, patients with elevated CTCs would be assigned to either the same treatment until clinical disease progression or switched to the next chemotherapeutic agent(s).

On the other hand, it is often difficult to judge treatment efficacy in the absence of measurable disease, especially with patients who have only skeletal metastasis. Usually, skeletal metastatic sites of breast cancer contain both osteolytic and osteoblastic changes. If treatment has been effective, the cancer nests sometimes reveal osteoplastic change. It is difficult to judge by radiography whether osteoblastic change is due to disease progression or to treatment efficacy. Therefore, in addition to other tumor markers, CTCs might be a useful predictor of treatment efficacy, especially with the patients whose lesions are difficult to assess.

According to the American Society of Clinical Oncology 2007 update of recommendations for the use of tumor markers in breast cancer, the measurement of CTCs should not be used to diagnose breast cancer or to influence any treatment decision in patients with breast cancer [10]. Similarly, the use of the recently FDA-cleared test for CTC (CellSearch Assay) in patients with metastatic breast cancer cannot be recommended until further validation confirms the clinical value of this test. Therefore, a prospective trial should be held with metastatic breast cancer patients.

Acknowledgment We greatly appreciate our laboratory staff, Ms. Shibata and Ms. Minowa, who supported our study by searching for and counting numerous CTC numbers.

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Cancer Science
The official journal of the Japanese Cancer Association JCA

Review Article

Biomarkers to predict response to sunitinib therapy and prognosis in metastatic renal cell cancer

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(Received May 26, 2011/Revised July 27, 2011/Accepted July 28, 2011/Accepted manuscript online August 4, 2011/Article first published online September 14, 2011)

Sunitinib is an orally-administered, multitargeted tyrosine kinase inhibitor. The main targets are vascular endothelial growth factor receptor (VEGFR)-1, VEGFR-2, VEGFR-3, platelet-derived growth factor receptor (PDGFR)-α, and PDGFR-β. Among therapeutic targeting agents, it is the best available in the USA for patients with metastatic renal cell cancer (RCC). Well-constructed clinical trials have led to the worldwide approval of various agents for RCC. However, in clinical practice, it remains difficult to determine the best treatment strategy with these agents. Therefore, the identification of biomarkers to predict response and side-effects and to select optimal dosages is urgently needed. Potential mechanisms of action and resistance need to be understood in order to make accurate predictions. This article briefly reviews candidate biomarkers of sunitinib therapy in terms of clinical variables, genetic factors, and circulating proteins and endothelial cells. Although further validation and implementation is necessary, if validated, biomarkers will help measure the therapeutic response in individual patients and establish treatment strategies for metastatic RCC. (*Cancer Sci* 2011; 102: 1949–1957)

Renal cell cancer (RCC) is the most lethal urologic malignancy, and its incidence is currently rising.[1] Radical nephrectomy remains the standard and only curative therapy for patients with localized RCC. However, at initial diagnosis, one-third of RCC patients exhibit visceral metastasis, and up to half of remaining patients eventually develop distant metastases.[2,3] For a long time, the only effective therapeutic and preventive agents for distant metastases and local recurrence have been interferon (IFN) and interleukin (IL)-2, although these agents only achieve a response rate of 15%.[2,3] Major recent breakthroughs have broadened our knowledge of the genetics and transduction pathways involved in various malignancies, including RCC.[4] This greater understanding of the molecular biology of RCC has led to the identification of the vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and mammalian target of rapamycin (mTOR) signaling pathways as rational targets for anticancer therapy for metastatic RCC.[4] Currently, two major subgroups of molecular-targeted agents are available in clinical practice: angiogenesis inhibitors, which include sorafenib (Nexavar; Bayer, West Haven, CT, USA), sunitinib (Sutent; Pfizer, New York, NY, USA), bevacizumab (Avastin; Genentech/Roche, Basle, Switzerland), pazopanib (Votrient; GlaxoSmithKline, Brentford, UK), and axitinib (AG-013736; Pfizer, Philadelphia, PA, USA);[5–9] and two specific inhibitors of the mTOR kinase, temsirolimus (Torisel; Pfizer) and everolimus (Afinitor; Novartis, Basel, Switzerland).[10,11] The RCC growth signals and the rationale behind these molecular-targeted agents are shown in Figure 1.

Among these targeted agents, sunitinib is an orally-administered, multitargeted tyrosine kinase inhibitor (TKI). The main targets of sunitinib are VEGF receptor (VEGFR)-1, VEGFR-2, VEGFR-3, PDGF receptor (PDGFR)-α, and PDGFR-β.[12] Sunitinib is the most readily-available therapeutic targeting agent in the USA (Research from the Synovate Healthcare US Tandem Oncology Monitor 2007–2010) for patients with RCC. A randomized, multicenter, phase-III trial enrolled 750 patients with previously-untreated metastatic RCC to receive either repeated 6-week cycles of sunitinib (at a dose of 50 mg, given orally once daily for 4 weeks, followed by 2 weeks without treatment) or IFN-α (at a dose of 9 million units, given subcutaneously three times/week). This trial demonstrated the superiority of sunitinib over IFN in the objective response rate (ORR; 31% *vs* 6%), progression-free survival (PFS; 11 *vs* 5 months), and overall survival (OS; 26.4 *vs* 21.8 months) and its acceptable safety profile.[6,13] These results and those of the clinical trials for other targeting agents indicate an improved prognosis for patients with RCC in the era of targeted therapy.

Currently, various agents, including molecular-targeted agents and immunotherapeutic agents, are treatments of choice in clinical practice. Risk algorithms, molecular diagnostics, pharmacogenomics, and pharmacodynamics are important tools to improve treatment outcomes. One of the treatment goals is personalized medicine, which offers the right treatment for the right patient at the right time.[14] Therefore, the identification of biomarkers to predict response and side-effects, and to select optimal dosages, is urgently needed. This article provides a brief overview of the genetic changes of RCC and introduces sunitinib biomarkers in terms of clinical variables, genetic factors, and circulating proteins and endothelial cells.

Renal Cell Cancer and the Hypoxia-inducible, Factor-mediated Pathway

Renal cell cancer is the most frequently-occurring solid lesion within the kidney.[1] Among RCC, clear-cell RCC (75%), alternatively called ''conventional RCC'', is the most common, followed by papillary RCC (10%), chromophobe RCC (5%), and renal oncocytoma (5%). In addition, the World Health Organization system includes rare, newly-recognized cancers defined by genetic factors, such as Xp11.2 translocation-associated renal cancer.[15–17]

The most important molecular disorder in RCC involves the von Hippel-Lindau (VHL) tumor suppressor gene, which is responsible for clear-cell RCC. The protein product of the *VHL* gene, which is located on chromosome 3p25, prevents angiogenesis and suppresses tumors.[15] Inactivating the phosphorylated VHL protein activates hypoxia-inducible factor (HIF) and the induction of VEGF in clear-cell RCC. In addition, mesenchymal–epithelial transition factor (MET) and fumarate hydratase (FH) are the genes responsible for types 1 and 2 papillary RCC,

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doi: 10.1111/j.1349-7006.2011.02054.x

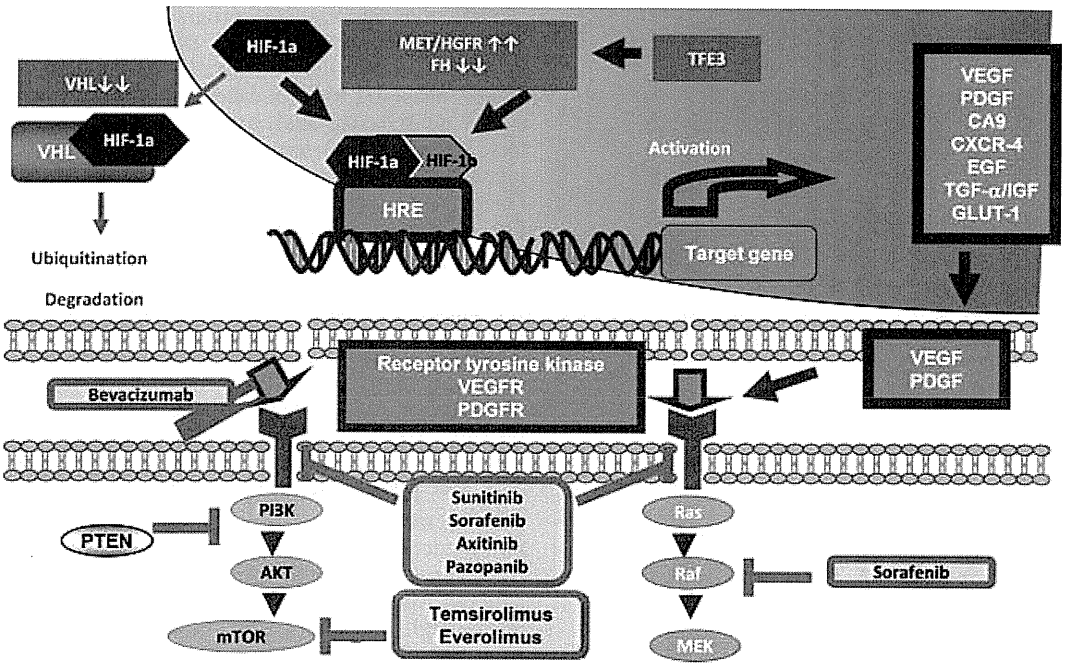

Fig. 1. Genetic alterations and growth signals in renal cell cancer and various molecular-targeted agents. CA9, carbonic anhydrase 9; CXCR-4, C-X-C chemokine receptor type 4; EGF, epidermal growth factor; FH, fumarate hydratase; GLUT1, glucose transporter 1; HGFR, hepatocyte growth factor receptor; HIF, hypoxia inducible factor; HRE, hypoxia response element; IGF-1, insulin-like growth factor 1; MET, mesenchymal–epithelial transition factor; mTOR, mammalian target of rapamycin; PDGF, platelet-derived growth factor; PI3K, phosphatidyl-inositol; TFE3, transcription factor E3; TGF-α, transforming growth factor alpha; VEGF, vascular endothelial growth factor; VHL, von Hippel-Lindau tumor suppressor gene product, 3-kinases.

respectively.[18,19] Mesenchymal–epithelial transition factor, which is a proto-oncogene, encodes a tyrosine kinase membrane receptor, and the activation of MET can indirectly promote angiogenesis and tumor growth through the overexpression of VEGF.[20,21] Fumarate hydratase is an enzyme in the mitochondrial tricarboxylic acid (TCA) cycle. The loss of FH leads to pseudohypoxia through the overexpression of HIF, resulting in an increase in downstream targets, including VEGF.[20,22] Therefore, the activation of MET and the loss of FH lead to angiogenesis.[4,15] Moreover, transcription factor E3 (TFE3) and transcription factor EB (TFEB), members of the microphthalmia transcription factor/TFE, are highly expressed in the nucleus as a result of chromosomal translocations, and are responsible for the development of juvenile renal cancer.[17] They also induce the HIF-mediated angiogenesis signaling cascade. The gene products responsible for RCC are indicated in Figure 1.

Biomarkers to Predict the Response to Sunitinib Therapy

Clinical factors. In the cytokine era, prognostic factors that could predict outcome in patients with metastatic RCC treated with IFN-α as initial systemic therapy were defined by Motzer at the Memorial Sloan Kettering Cancer Center (MSKCC).[23] The MSKCC group extracted five variable risk factors for short survival: low Karnofsky performance status (PS), high lactate dehydrogenase (LDH), low hemoglobin (Hb), high corrected serum calcium (Ca), and time from the initial RCC diagnosis to the start of IFN-α therapy of <1 year (Table 1).[23] Each patient was assigned to one of three risk groups: those with no risk factor (favorable risk), those with one or two risk factors (intermediate risk), and those with three or more risk factors (poor risk).[23] The median time to death was 30, 14, and 5 months in the favorable, intermediate, and poor-risk groups, respectively.[23] These five risk criteria are now widely used and are known as the Motzer score or the MSKCC score.

Later, the same group analyzed the prognostic factors of previously-treated RCC patients who had received new agents as salvage therapy in clinical trials at the MSKCC. The median survival time for the 251 patients was 10.2 months, and the pretreatment features associated with a poor prognosis extracted by multivariate analysis were low Karnofsky PS; low Hb level; and high corrected Ca level.[24]

In the molecular-targeted therapy era, several studies have investigated clinical prognostic factors, and the findings are summarized in Table 1. Heng *et al.*[25] first reported the results from a large, multicenter study of 645 patients with anti-VEGF, therapy-naïve metastatic RCC. The study included three groups of patients: 396, 200, and 49 patients, respectively, treated with sunitinib, sorafenib, and bevacizumab, respectively; 560 patients (94%) had clear-cell RCC, while the remaining 35 patients (6%) were diagnosed with non-clear-cell RCC.[25] Four of the five adverse prognostic factors according to the MSKCC score (low Hb, high corrected Ca level, low Karnofsky PS, and time from diagnosis to treatment of <1 year) emerged as independent predictors of poor OS.[25] In addition, neutrophils greater than the upper limit of normal (ULN) range, and platelets greater than the ULN, emerged as independent adverse prognostic factors.[25]

Choueiri *et al.*[26] retrospectively identified the clinical factors associated with outcome in patients with clear-cell RCC receiving anti-VEGF agents, the majority of whom (84%) received either sorafenib or sunitinib. In total, 120 patients with metastatic clear-cell RCC were studied, and a prognostic model was constructed using PFS as an end-point.[26] In this study, all patients had undergone prior nephrectomy, and 45 patients (37%) received anti-VEGF treatment as first-line therapy, while 75 patients (63%) had previously received non anti-VEGF therapies.[26] The interval between diagnosis and anti-VEGF therapy, high corrected Ca level, poor PS, and increased platelet and neutrophil counts were identified as independent prognostic factors for poor PFS (Table 1).

doi: 10.1111/j.1349-7006.2011.02054.x

Table 1. Clinical variables correlated with better/worse survival in patients with metastatic renal cell cancer

Investigators/ references	*n*	Agent	Setting	End-point	Hb <LLN	Corrected Ca >ULN	Poor PS	From Dx to Tx <1 year	LDH >ULN	Other prognostic factors
Motzer *et al.*[23]	670	Interferon	First line	OS	Significant	Significant	Significant	Significant	Significant	
Motzer *et al.*[24]	251	Various agents	Salvage	OS	Significant	Significant	Significant	NS	NS	
Heng *et al.*[25]	645	TKI	First line	OS	Significant	Significant	Significant	Significant	NS	Neutrophil count >ULN, Platelet count >ULN
Choueiri *et al.*[26]	120	TKI	Both	PFS	NS	Significant	Significant	Significant	NS	Neutrophil count >4500/uL, Platelet count >300 000/uL
Patil *et al.*[27]	375	Sunitinib	First line	OS	Significant	Significant	Significant	Significant	Significant	Bone metastasis
Patil *et al.*[27]	375	Interferon	First line	OS	Significant	Significant	NS	Significant	Significant	Neutrophil counts, bone metastasis, lymph node metastasis
Bamias *et al.*[28]	109	Sunitinib	Both	OS	NS	NS	Significant	Significant	NS	Multiple metastatic sites
Yuasa *et al.*[29]	63	Sunitinib	Both	OS	Significant	NS	NS	NS	Significant	No history of nephrectomy, brain metastasis

Both, both first line and second line therapies; Ca, calcium; Dx, diagnosis; Hb, hemoglobin; LLN, lower limit of normal range; NS, not significant; OS, overall survival; PFS, progression-free survival; PS, performance status; TKI, tyrosine kinase inhibitor; Tx, therapy; ULN, upper limit of normal range.

Patil *et al.* reported the prognostic factors for PFS and OS, with sunitinib or IFN-α as first-line systemic therapy for patients with clear-cell metastatic RCC in a randomized, multicenter, phase-III trial, as described earlier.[6,12,27] For sunitinib, a PFS multivariate analysis identified five independent predictors, including high-serum LDH level, the presence of multiple metastatic sites, no prior nephrectomy, Eastern Cooperative Oncology Group (ECOG) PS, and baseline platelet count. The OS correlated with high LDH level, corrected Ca level, the time from diagnosis to treatment, Hb level, ECOG PS, and the presence of bone metastasis (Table 1). The authors concluded that the MSKCC model was applicable to targeted therapy.[27]

A multi-institutional, retrospective analysis of patients with metastatic RCC treated with sunitinib in six Greek oncology units was reported.[28] In this study, 109 patients were included, of whom 100 (91%) had clear-cell RCC and 17 (15%) had been treated with IFN-α, while 86 (79%) had undergone nephrectomy.[28] The time from diagnosis to the start of sunitinib of <1 year, multiple metastatic sites ($P = 0.003$), and poor PS were independently correlated with OS (Table 1).[28]

Finally, in our retrospective study of 63 native Japanese patients, all five MSKCC risk factors (ECOG PS >1, low Hb levels, high corrected Ca levels, high LDH levels, and the time from diagnosis to initial systemic therapy of <1 year) were associated with poorer OS by univariate analysis.[29] A multivariate analysis using the Cox proportional hazard model demonstrated that low Hb and elevated LDH were independently associated with poorer OS among MSKCC scores. Brain metastasis and no history of nephrectomy were also associated with poorer OS (Table 1).[29]

It is important to consider that the number of patients was different between these clinical studies, and that some studies, including our own, might be influenced by low statistical power due to relatively small sample sizes. Nevertheless, the MSKCC prognostic factors are still valid for predicting survival in metastatic RCC in the era of targeted therapy. These results indicate that the MSKCC scores are associated with the behavior of the disease, rather than with specific forms of therapy. In addition to the factors included in the MSKCC score, the number of neutrophils, the platelet count, and the number and/or location of metastatic lesions might be independent prognostic factors in patients treated with molecular-targeted agents (Table 1).

Genetic factors affecting pharmacokinetics and pharmacodynamics. The clinical efficacy of sunitinib depends on the systemic exposure of the targeted organ to the active compounds. A recent meta-analysis of pharmacokinetic data in 443 patients treated with sunitinib showed that higher plasma levels of sunitinib and of its active metabolite SU12662 were associated with prolonged PFS and OS.[30] Orally-administered sunitinib is absorbed by the intestinal mucosa and metabolized in the liver. The primary metabolite, N-de-ethylated metabolite SU12662, reaches plasma concentrations that are similar to the parent compound sunitinib and has biochemically-equivalent activity to sunitinib, but its half-life is prolonged.[13,30] The efflux transporters and the cytochrome P450 3A (CYP3A) family play a role in the absorption and metabolism of the drug.[13,30] The active metabolite and the parent compound are multitargeted TKI that inhibit PDGFR-α and PDGFR-β; VEGFR-1, VEGFR-2, and VEGFR-3; stem cell factor receptor c-KIT; Fms-like tyrosine kinase 3 receptor (Flt-3); and the glial cell line-derived neutrophic factor receptor.[13] The efflux transporters also regulate the cytoplasmic concentration of these agents.[13] Therefore, the efficacy of sunitinib can be influenced by multiple genes encoding efflux transporters, metabolizing enzymes, and targeted tyrosine kinases. Figure 2 describes the processes and enzymes/proteins involved in sunitinib activity, and the genes relevant to sunitinib response and/or toxicity are

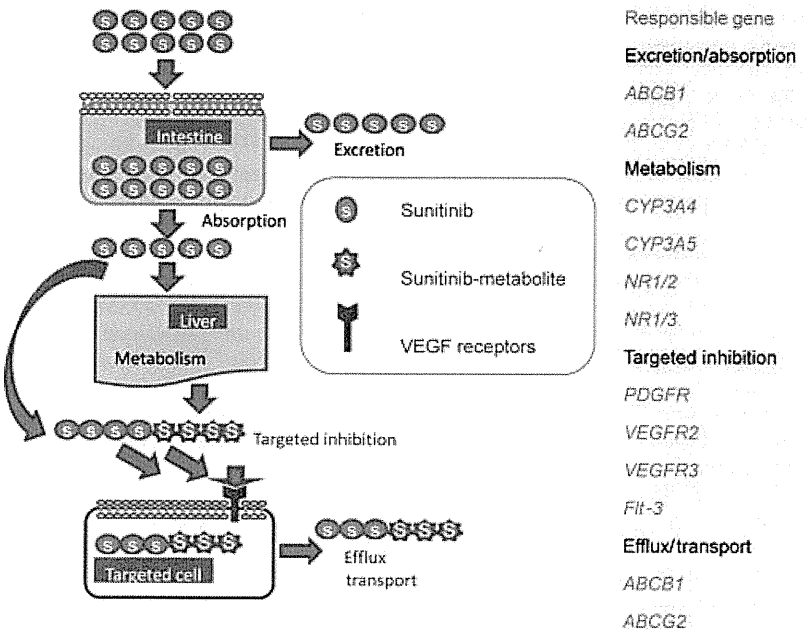

Fig. 2. Genetic prognostic factors in metastatic renal cell cancer patients treated with sunitinib. Orally-administered sunitinib is absorbed from the intestinal mucosa and metabolized in the liver to an active metabolite. Efflux transporters and the cytochrome P450 (CYP)3A family contribute to sunitinib absorption and metabolism. Metabolized active form and the parent compound inhibit platelet-derived growth factor receptor (PDGFR)-α and PDGFR-β, and vascular endothelial growth factor receptor (VEGFR)-1, VEGFR-2 and VEGFR-3. Therefore, pharmacokinetic and pharmacodynamic variables influence the efficacy of sunitinib. ABCB1, ATP-binding cassette transporter protein member B1; ABCG2, ATP-binding cassette transporter protein member G2; CYP3A4, cytochrome P450 3A4; CYP3A5, cytochrome P450 3A5; Flt-3, Fms-like tyrosine kinase receptor-3; NR1I2, nuclear receptor subfamily 1, group I, member 2; NR1I3, nuclear receptor subfamily 1, group I, member 3; PDGFR, platelet-derived growth factor receptor; VEGFR, vascular endothelial growth factor receptor.

Table 2. Genes relevant to sunitinib response and/or toxicity

Factors	Genotype	rs no.	Description	References
ABCB1	TTT haplotype*	rs1045642, rs1128503, rs2032582	Increased risk of hand–foot syndrome	32
ABCB1	TCG haplotype*	rs1045642, rs1128503, rs2032582	Improved progression-free survival	33
ABCG2	421C>A	rs2231142	High concentration, increased risk of adverse events, such as hypertension and facial acne	39
ABCG2	TT haplotype†	rs2622604	Any toxicity >grade 2	32
CYP3A5	6986 A>G	rs776746	Improved progression-free survival	33
Flt-3	738 C>T	rs1933437	Increased risk of leukopenia and thrombocytopenia	42
NR1I3	Absence of a CAT haplotype‡	rs2307424, rs2307418, rs4073054	Improved progression-free survival	33
NR1I3	Absence of a CAG haplotype‡	rs2307424, rs2307418, rs4073054	Increased risk of leukopenia	32
PDGFR-α	Homozygous of GCGT§	rs35597368, rs1800810, rs1800813, rs1800812	Decreased overall survival	33
VEGFR-2	1718 A>T	rs1870377	Improved overall survival	33
VEGFR-2	1191 T>C	rs2305948	Any toxicity >grade 2	32

Description of haplotypes: **ABCB1 3435C/T, 1236C/T,* and *2677G/T*; †*ABCG2 15622C/T* and *1143C/T*; ‡*NR1I3 5719C/T, 7738A/C,* and *7837T/G*; §*PDGFR-α 1580T/C –1171C/G -735G/A* and *-573G/T*. ABC, ATP-binding cassette; CYP3A5, cytochrome P450 3A5; Flt-3, Fms-like tyrosine kinase receptor-3; PDGFR, platelet-derived growth factor receptor; VEGFR, vascular endothelial growth factor receptor.

summarized in Table 2. To achieve personalized medicine, the complete understanding of sunitinib pharmacogenomics and the molecular profile of each individual patient are necessary.[14]

Absorption, excretion, and efflux of sunitinib. Upon oral administration, sunitinib is absorbed from the gastrointestinal tract in a process regulated by efflux transporters.[30] The ATP-binding cassette (ABC) transporter proteins, particularly multidrug resistance-1//ABC member B1 (ABCB1), formerly known as P-glycoprotein, multidrug resistance-associated protein-1/ABCC1, and breast cancer resistance protein/ABCG2, formerly known as mitoxantrone-resistant protein, mediate absorption and/or excretion through the intestinal wall and regulate the efflux of a wide variety of anticancer drugs from target cells (Fig. 2). These transporter proteins might be involved in the efficacy of sunitinib and in the resistance to sunitinib.

The T genotype in *ABCB1 3435C/T*, which is associated with higher exposure to drugs transported by ABCB1 via decreased mRNA stability, and the consequent decreased expression of *ABCB1* transporter,[31] might be a key factor in ABCB1-mediated sunitinib transport. A multicenter pharmacogenetic

doi: 10.1111/j.1349-7006.2011.02054.x

association study revealed that the *ABCB1 TTT* haplotype in *3435C/T*, *1236C/T*, and *2677G/T* was related to hand–foot syndrome (HFS).[32] In addition, van der Veldt reported that the presence of a *TCG* copy in the same *ABCB1* haplotype was a significant predictor of improved PFS.[33] Interestingly, in a report describing accelerated CYP3A4-mediated drug metabolism in *Abcb1* knockout mice, Schuetz *et al.*[34]suggested that decreased ABCB1 expression activates enzymes involved in drug absorption or disposition.

Several studies reported higher sunitinib affinity for ABCG2 than ABCB1.[35,36] Shukla *et al.*[35] demonstrated that sunitinib stimulates ATP hydrolysis by both transporters in a concentration-dependent manner, and that the affinity for ABCG2 (IC_{50}: 1.33 μM) is higher than that for ABCB1 (IC_{50}: 14.2 μM). Kawahara *et al.* analyzed the kinetics of sunitinib inhibition on ABCG2- and ABCB1-mediated transport. The authors showed that sunitinib acts as a competitive inhibitor of the transporter function of ABCG2 and ABCB1, and that sunitinib has higher affinity for ABCG2 than ABCB1.[36] A previous genetic analysis revealed that, among single nucleotide polymorphism (SNP) in the *ABCG2* gene, *ABCG2 421C/A* is the most common mutant allele in the Japanese population and in other Asian populations (>30%), and that it is associated with low ABCG2 protein expression. The authors also showed that this variable is rare in African Americans (<5%) and Caucasians (<10%).[37,38] This finding might explain the higher incidence of hematological adverse events in Asian patients. Indeed, a report suggested that the homozygous variant of *ABCG2 421C/A* might be involved in the elevated exposure to sunitinib and severe toxicity observed in a patient with RCC.[39] In addition, recent pharmacogenetic analyses revealed that two *ABCG2* gene polymorphisms (*–15622C/T* and *1143C/T*) were strongly associated with sunitinib-induced toxicity in patients.[32] Thus, *ABCG2* genetic variants might lead to increased systemic exposure to sunitinib, resulting in dose-limiting toxicities.

Metabolism. Sunitinib is metabolized in the liver, primarily by the CYP3A4 enzyme. No functional polymorphisms of *CYP3A4* have been identified. The CYP3A5 enzyme metabolizes several TKI, including erlotinib, gefitinib, and imatinib, and might be a key determinant in the interindividual differences observed in CYP3A-mediated drug metabolism.[40] In addition, the expression of CYP3A4 and CYP3A5 is regulated by the ligand-activated nuclear receptors NR1I2 (nuclear receptor subfamily 1, group I, member 2 or pregnane X receptor [PXR]) and NR1I3 (nuclear receptor subfamily 1, group I, member 3 or constitutive androstane receptor [CAR]).[41]

The *CYP3A5* gene, which is 83% homologous to *CYP3A4*, has a functional polymorphism, *6986A/G*, in intron3. The variant G allele creates a cryptic acceptor splice site and transcribes variant mRNA with an excess 131-bp fragment between exons 3 and 4.[31] The CYP3A5 protein translated from the variant mRNA is truncated at a premature stop codon, resulting in a reduced amount of complete CYP3A5 protein. van der Veldt *et al.*[33] reported that the A allele of *6986A/G* in the *CYP3A5* gene, which creates the *CYP3A5* expressor phenotype, is a predictive factor for prolonged PFS. The prolonged PFS observed in patients with the expressor phenotype might be caused by greater metabolism of sunitinib, and thereby increased levels of the active metabolite, which has a longer half-life than the parent compound.[33]

In addition, a relationship has been reported between the absence of the *CAG* haplotype in the *NR1I3* gene, which encodes the CAR, and an increased risk for leukopenia.[32] Nuclear receptor NR1I3 plays an important role in the regulation of multiple drug detoxification genes, such as *CYP3A4*.[41] Another study also extracted the polymorphic variants of *NR1I2* and *NR1I3*, identifying them as predictive factors for PFS and OS in sunitinib-treated metastatic RCC patients.[33]

Pharmacodynamics and targeted inhibition. Besides pharmacokinetic factors, pharmacodynamic factors might affect the efficacy and toxicity of sunitinib. In RCC, the major therapeutic effect of sunitinib is thought to be the inhibition of the VEGFR on tumor-associated endothelium, leading to reduced tumor angiogenesis.[13,30] In addition, the inhibition of the PDGFR might increase the anti-angiogenic effects of sunitinib by targeting pericytes, which protect endothelial cells from apoptosis.[13,30]

The presence of the A allele of the *1718T/A VEGFR-2* polymorphism and the presence of two *GCGT* copies of the *1580T/C*, *–1171C/G*, *–735G/A*, and *–573G/T* polymorphisms in PDGFR-α are associated with decreased OS and prolonged OS, respectively.[33] However, these polymorphisms are not significantly associated with prolonged PFS. These findings suggest that polymorphisms in *VEGFR-2* and *PDGFR-α* might be associated with the nature of the disease, and might therefore be prognostic instead of predictive. The presence of the T allele of the *VEGFR-2 1191C/T* polymorphism is related to the development of any toxicity higher than grade 2, including fatigue, thrombocytopenia, and hypertension.[32] Polymorphisms are also predictive for the development of coronary heart disease due to the lower binding efficiency of VEGF to the polymorphic VEGFR-2.[33] The polymorphic receptor might therefore be involved in sunitinib-induced cardiac toxicity and the development of hypertension.

In addition, the association between the Flt-3 *738T/C* polymorphism and a reduced risk of leukopenia was reported.[32] The protective effect of the Flt-3 *738C* allele against sunitinib-induced thrombocytopenia was confirmed by van Erp *et al.*[42] Therefore, the Flt-3 *738C/T* polymorphism plays a role in the variability of sunitinib-induced bone marrow toxicity.

Circulating biomarkers. Considering the pharmacological effect and biological activity of sunitinib, one should consider measuring the plasma levels of the soluble ligands (including the VEGF family and placental growth factor; PlGF), the specific ligands of VEGFR-1, and the soluble form of the receptors, which include soluble VEGFR (sVEGFR)-2 and sVEGFR-3. If the systemic exposure to sunitinib is a key factor in the predictive value, the less competitive ligands, which include VEGF and PDGF, might be associated with greater efficacy of sunitinib. Indeed, the basal levels and the fold changes of these specific ligands and their receptors have been reported as potential biomarkers of sunitinib.

The mechanisms underlying sunitinib-induced alterations in the levels of these growth factors and of their soluble receptors have not yet been elucidated. Sunitinib-induced angiogenesis inhibition might increase VEGF and PlGF as a positive feedback (Fig. 3). However, combining sunitinib with the soluble forms of the receptors might cause precipitation or degradation and decrease their levels. Rini *et al.* reported that the plasma levels of VEGF-A and PlGF in patients treated with sunitinib increased significantly after 28 days by 2.8-fold (range: 0.4- to 13.6-fold) and 3.9-fold (range: 0.8- to 20.4-fold) over baseline, respectively, whereas the mean sVEGFR-3 levels decreased by 37.6%.[43] DePrimo *et al.*[44] also reported that at the end of cycle 1 (day 28), VEGF and PlGF levels increased greater than threefold (relative to baseline) in 24 of 54 (44%) and 22 of 55 (40%) cases, respectively ($P < 0.001$). In contrast, sVEGFR-2 levels decreased by at least 20% in all patients, resulting in a 30% decrease in 50 of 55 (91%) patients ($P < 0.001$) during cycle 1, while sVEGFR-3 levels decreased ≥30% in 48 of 55 cases (87%) and ≥20% in all but two cases.[44] These levels tended to return to near baseline after 2 weeks of treatment, indicating that these effects were dependent on drug exposure. In addition to the soluble proteins, bone marrow-derived circulating endothelial progenitors (CEP) and circulating endothelial cells (CEC) increase when angiogenesis is required. Gruenwald

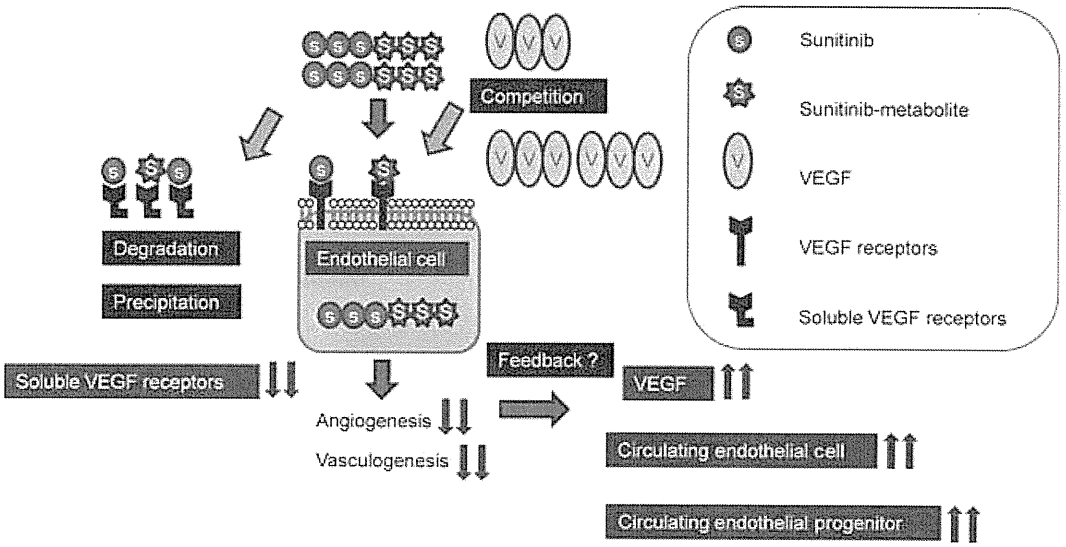

Fig. 3. Circulating soluble proteins and endothelial cells as prognostic factors in metastatic renal cell cancer patients treated with sunitinib. Mechanisms underlying sunitinib-induced alterations in the levels of these growth factors and of their soluble receptors have not yet been elucidated. Sunitinib-induced angiogenesis inhibition might increase vascular endothelial growth factor (VEGF) and circulating endothelial cells and progenitor cells as a positive feedback. In contrast, combining sunitinib with the soluble forms of the receptors might cause precipitation or degradation, and decrease their levels. VEGF, vascular endothelial growth factor.

et al. reported that CEC values in metastatic RCC patients are significantly higher than in healthy individuals (mean value: 49 ± 44 CEC/mL *vs* 8 ± 8 CEC/mL, $P = 0.0001$).[45] In this study, during the first course of sunitinib, the CEC of the patients increased from 49 ± 44 CEC/mL at baseline to 84 ± 59 CEC/mL after 14 days ($P = 0.0331$) and 89 ± 63 CEC/mL after 28 days ($P = 0.0159$) of treatment.[45] The CEC levels declined during the subsequent treatment-off period to baseline levels and below (range: 19–58 CEC/mL).[45] Figure 3 describes circulating soluble proteins and CEC/CEP as candidate biomarkers for sunitinib efficacy.

Rini *et al.* first reported that baseline sVEGFR-3 and VEGF-C levels might be prognostic factors for PFS, as well as predictive factors for objective response. Patients with lower levels than median baseline values (sVEGFR-3, 47 000 pg/mL; VEGF-C, 722.1 pg/mL) had longer PFS than patients with greater levels than the median.[43] The lack of correlation between VEGF-A or PlGF levels and PFS could be due to the fact that the study consisted of bevacizumab-refractory patients. The predictive value of baseline serum VEGF-A was reported by other groups. Porta *et al.*[46] investigated the association between sunitinib-treatment outcome and baseline serum VEGF-A levels in 85 patients treated with sunitinib, among whom 60 had a pure clear-cell RCC, whereas 12 had a predominantly clear-cell mixed histology, and 13 had a pure non-clear-cell histology. In this study, patients with increased baseline VEGF-A had a significantly decreased PFS period (odds ratio: 2.14, 95% confidence interval [CI]: 1.324–3.459).[46] This study reported that patients with increased VEGF-A have a median PFS of 4.7 months (95% CI: 2.8–8.3), whereas patients with non-elevated VEGF-A have a median PFS of 11.2 months (95% CI: 6.5–15).[46]

The fold changes in these angiogenesis-associated proteins could also be a potential biomarker of sunitinib. DePrimo *et al.*[44] reported that significantly larger changes in VEGF, sVEGFR-2, and sVEGFR-3 levels were observed in patients exhibiting objective tumor response, compared to those exhibiting stable disease or disease progression ($P < 0.05$). In addition, total drug trough, sunitinib levels, and SU12662 levels correlated modestly with the change in mean sVEGFR-2 and sVEGFR-3 plasma levels relative to baseline by linear regression analysis.[44]

Endothelial progenitors and CEC also play an integral part in tumor angiogenesis, and might be suitable predictive and prognostic biomarkers for treatment with angiogenesis inhibitors. Gruenwald *et al.*[45] reported that in patients with PFS above the median value, CEC values increased significantly from baseline (mean value: 40 ± 41 CEC/mL to 111 ± 61, $P = 0.0109$) at day 28, whereas in patients with PFS below the median value, the increase remained insignificant (mean value: 53 ± 45 CEC/mL to 69 ± 61, $P = 0.1848$). Farace *et al.*[47] reported that although baseline CEC values were not associated with PFS or OS, baseline circulating progenitor cell values were associated with PFS ($P = 0.01$) and OS ($P = 0.006$) in patients treated with TKI. In addition, changes in circulating progenitor cell values between days 1 and 14 were also associated with PFS ($P = 0.03$).[47]

Both circulating soluble proteins and endothelial cells have recently been measured in various clinical trials as potential biomarkers of the response to anti-angiogenesis agents, and research to further assess their utility is ongoing.

Others. The known adverse effects of sunitinib include HFS, diarrhea, stomatitis, hypertension, fatigue, and hypothyroidism. If adverse effects depend on the degree of systemic exposure to sunitinib, on which clinical efficacy also depends, adverse effects might be potential predictors of sunitinib efficacy in metastatic RCC patients. Correlations have been reported between clinical response and hypertension, and hypothyroidism and HFS.[48–50] Figure 4 shows the correlation between the worst adverse effects and the best clinical response in RCC patients. Rixe *et al.* retrospectively analyzed the putative correlation between sunitinib activity and adverse effects in patients ($n = 32$) with metastatic RCC. The pattern of toxicity was compared between responders and non-responders.[48] The appearance or worsening of hypertension (grade 2 or above) was found to be the single independent predictor of improved clinical response (odds ratio: 2.33, 95% CI: 1.69–3.22, $P = 0.009$) by multivariate analysis using logistic regression. By univariate analysis, a higher response rate was observed in patients with stomatitis ($P = 0.015$), fatigue ($P = 0.019$), hypertension

doi: 10.1111/j.1349-7006.2011.02054.x

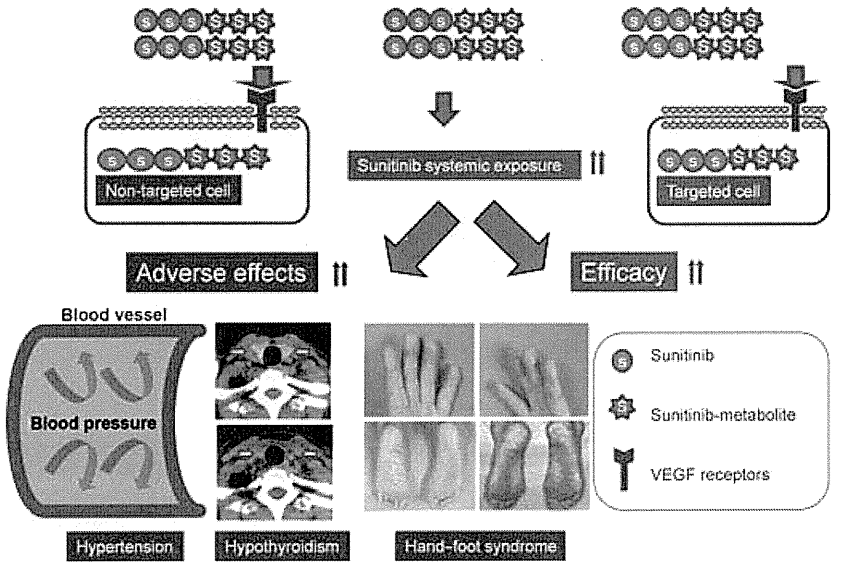

Fig. 4. Adverse effects as candidate biomarkers of favorable efficacy for sunitinib. Correlations between sunitinib efficacy and adverse effects, including hypertension, hypothyroidism, and hand–foot syndrome, were reported.

($P = 0.02$), and testicular erythema ($P = 0.04$), as well as hair depigmentation ($P = 0.042$).[48]

The possibility that the occurrence of hypothyroidism might affect the outcome of patients with metastatic RCC was prospectively investigated in consecutive patients who were to receive treatment with sunitinib or sorafenib.[49] Assessment included serum levels of thyroid-stimulating hormone (TSH), tri-iodothyronine (T3), and thyroxine (T4). Among these patients, subclinical hypothyroidism, defined as an increase in TSH above the ULN (>3.77 μM/mL), with normal T3 and T4 levels, was evident in five patients at baseline and occurred in 30 patients (36.1%) within the first 2 months after treatment initiation.[49] There was a statistically-significant correlation between the occurrence of subclinical hypothyroidism during treatment and the ORR (hypothyroid patients *vs* euthyroid patients: 28.3% *vs* 3.3%, respectively, $P < 0.001$).[49] Moreover, a multivariate analysis identified the development of subclinical hypothyroidism as an independent predictor of survival (hazard ratio: 0.31, $P = 0.014$).[49] Therefore, Schmidinger *et al.*[49] concluded that hypothyroidism might serve as a marker of favorable treatment outcome in metastatic RCC patients.

Very recently, at a meeting of the American Society of Clinical Oncology–Genitourinary, a report of a retrospective investigation of the correlation between HFS and sunitinib antitumor efficacy was presented.[50] In this study of 770 patients included in the analysis (after cycle 1, day 1), 179 (23%) developed HFS (all grades included), and 591 (77%) did not develop HFS. The median PFS (14.3 *vs* 8.3 months, $P < 0.0001$), OS (38.2 *vs* 18.9 months, $P < 0.0001$), and ORR (66.5% *vs* 31.8%, $P < 0.0001$) were significantly higher in the group with HFS than in the group without HFS.[49] Moreover, a multivariate analysis also demonstrated that treatment-emergent HFS remained a significant independent predictor of survival benefit ($P = 0.001$ and $P < 0.001$ for PFS and OS, respectively) after adjusting for other significant independent prognostic markers, including MSKCC factors.[50]

We have also found initial tumor size to be a good predictor of tumor reduction. We retrospectively analyzed 139 metastatic lesions, 16 primary lesions, 86 sunitinib-treated lesions, and 69 sorafenib-treated lesions in 54 patients with metastatic RCC.[51] A linear, moderate-to-strong association between initial tumor size and tumor size reduction rate was demonstrated (correlation coefficient: −0.441, $P < 0.001$). When these tumors were divided into two groups, according to threshold value (23.95 mm), which was determined by receiver-operating characteristic curve analysis, the smaller tumors demonstrated a significantly greater size reduction than the larger tumors by Mann–Whitney *U*-test ($P < 0.001$).[51] We believe that this simple observation constitutes useful information for physicians who treat metastatic RCC.

Biomarkers of Other Targeted Agents

In the present study, we provide a brief overview of biomarkers for other targeted agents used in the treatment of metastatic RCC. Several studies, which were introduced in this review, included patients treated with other angiogenesis inhibitors, such as sorafenib, bevacizumab, pazopanib, and axitinib,[25,26,47,49,51] and suggested that most factors might be possible universal biomarkers for these agents. Other studies investigated the association between efficacy and genetic characteristics, soluble plasma biomarkers, and clinical symptoms.

In a phase-III clinical trial of pazopanib in RCC, predictive genetic markers were explored.[52] Xu *et al.* reported that three polymorphisms in *IL8* and *HIF1A*, and five polymorphisms in *HIF1A*, *NR1I2*, and *VEGFA*, showed a nominally significant association with PFS and response rate (RR), respectively.[52] From these results, they concluded that pharmacodynamic factors might predict treatment responses to pazopanib monotherapy in patients with RCC.[52]

Plasma proteins (VEGF, soluble VEGFR-2, carbonic anhydrase IX, tissue inhibitor of metalloproteinase-1 [TIMP-1], and Ras p21) were analyzed to identify prognostic biomarkers or indicators of response to sorafenib in patients enrolled in the phase-III clinical trial Treatment Approaches in Renal Cancer Global Evaluation Trial.[53] In this study, the reciprocal changes that were also observed in VEGF and sVEGFR-2 levels following sorafenib treatment were similar to those observed with sunitinib.[53] In addition, a multivariate analysis, which included ECOG PS, the MSKCC score, and the potential biomarkers, demonstrated that the elevated plasma level of TIMP-1, which inhibits most of the matrix metalloproteinases, was an independent, poor prognostic factor.[53] Although further investigation is necessary, TIMP-1 should be

an important candidate as a potential biomarker for anti-angiogenesis therapy.[53]

Finally, some studies have suggested that hypertension is a predictive biomarker of efficacy in patients receiving targeted agents. Rini *et al.*[54] reported the final results of a phase-III trial of bevacizumab plus IFN-α versus IFN-α monotherapy in patients with metastatic RCC. In this study, patients who developed hypertension on bevacizumab plus IFN-α had a significantly improved PFS and OS versus patients without hypertension.[54] Similarly, axitinib efficacy was also reported to correlate with diastolic blood pressure. From five phase-II multicenter trials of axitinib in multiple solid tumors, including metastatic RCC, Rini *et al.* reported that the median OS (25.8 *vs* 14.9 months) and median PFS (10.2 *vs* 7.1 months) were greater in patients who developed hypertension.[55]

Conclusion

In this review, we introduced the current candidate biomarkers of sunitinib therapy. Regarding the clinical factors, the MSKCC prognostic factors seem to be valid predictors of survival in metastatic RCC, as summarized in Table 1. Host genetic factors associated with efflux transporters, metabolizing enzymes, and targeted tyrosine kinases modify the efficacy and the toxicity of sunitinib (Fig. 2). Both circulating soluble proteins and cells, which include VEGFR and their ligands, and the CEC/CEP, have been considered as potential candidate biomarkers of the response to anti-angiogenesis agents, and research to further assess their utility is ongoing (Fig. 3). Finally, we introduced severe, adverse effects as candidate biomarkers of favorable efficacy (Fig. 4). Among the targeted agents, sunitinib is an attractive clinical tool, and biomarkers of sunitinib efficacy are desirable. An important caveat is that, to date, almost all of these studies have been retrospective. Although further implementation in prospective studies is necessary, if validated, these biomarkers can be utilized to measure therapeutic response and design treatment strategies for metastatic RCC.

Acknowledgments

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

Disclosure Statement

Kiyohiko Hatake obtained research funding from Takeda Pharmaceutical Co. Ltd., Chugai Pharmaceutical Co. Ltd., and Kyowa Hakko Kirin Co. Ltd.

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Prognostic Impact of C-reactive Protein for Determining Overall Survival of Patients With Castration-resistant Prostate Cancer Treated With Docetaxel

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OBJECTIVE To verify the prognostic impact of C-reactive protein (CRP) for patients with castration-resistant prostate cancer (CRPC) treated with docetaxel in a single institution.

METHODS A group of 80 consecutive patients with CRPC were treated with docetaxel in our institution from January 2005 to May 2010. The patients received 75 mg/m^2 of docetaxel intravenously every 3 weeks. The prognostic value of all covariables, including CRP, was assessed using the Cox proportional hazard model. Risk stratification for overall survival was described from the results of the multivariable analysis.

RESULTS The median survival period for all patients was 14.5 months. The multivariable analysis showed that CRP and hemoglobin levels were independent prognostic factors for overall survival. Based on the presence of an elevated CRP concentration and/or a low hemoglobin level, all patients were stratified into 3 risk groups: those with no risk factors (low-risk group), those with 1 risk factor (intermediate-risk group), and those with 2 risk factors (high-risk group). The overall survival curves were clearly tiered according to the risk groups, with the 1-year overall survival rates being 86.3%, 60.5%, and 23.0% for the low-, intermediate-, and high-risk groups, respectively (P <.001).

CONCLUSION CRP is an independent prognostic factor for overall survival of patients with CRPC treated with docetaxel. Risk stratification based on CRP and hemoglobin could be helpful for estimating the overall survival. UROLOGY 78: 1131–1135, 2011. © 2011 Elsevier Inc.

Docetaxel is the first chemotherapeutic agent to demonstrate a survival benefit in patients with castration-resistant prostate cancer (CRPC),[1,2] yet the efficacy of docetaxel varies by patient. Because docetaxel is a cytotoxic agent, eventual adverse effects should not be ignored. In this regard, identification of prognostic factors would be an essential step in designing a therapeutic strategy for patients with CRPC being treated with docetaxel. It has been shown that pain, Gleason score, Eastern Cooperative Oncology Group performance status (ECOG PS), presence of visceral metastases, hemoglobin, albumin, and alkaline phosphatase (ALP) are prognostic factors for overall survival,[3-6] and several prognostic algorithms have also been proposed.[4,5,7,8]

Recently, the presence of a systemic inflammatory response that is measured by an acute-phase reactant has been recognized to be associated with a poor prognosis in various advanced cancers. C-reactive protein (CRP), which is a representative acute-phase reactant, has been shown to be 1 such significant prognostic factor.[9-11] We have also reported that CRP is an independent prognostic factor for patients with renal cell carcinoma and urothelial carcinoma of the upper urinary tract and bladder.[12-14] For patients with CRPC, 2 studies have previously reported that CRP is an independent prognostic factor.[15,16]

The aim of this study is to verify the prognostic impact of CRP for overall survival for patients with CRPC treated with docetaxel.

MATERIAL AND METHODS

Patients

A group of 80 consecutive patients with CRPC were treated with docetaxel at our institution from January 2005 to May

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Submitted: March 29, 2011, accepted (with revisions): July 29, 2011

0090-4295/11/$36.00
doi:10.1016/j.urology.2011.07.1416