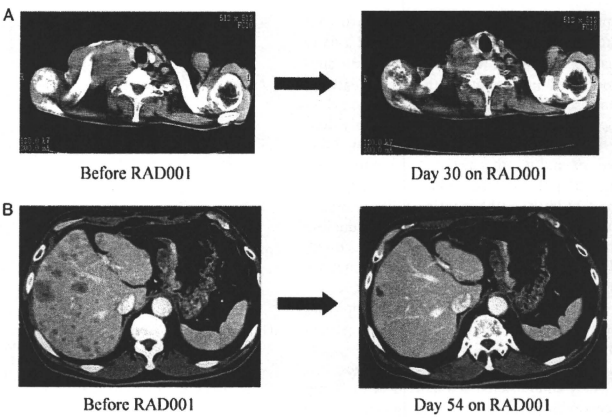

Figure 2. Computed tomography images of tumor response to RAD001 treatment. (A) Shrinkage of metastases in supraclavicular lymph nodes in a patient with esophageal cancer. (B) Shrinkage of liver metastases in a patient with gastric cancer.

10 mg/day. After one cycle of RAD001 treatment, computed tomography revealed that lymph nodes with metastases in the right supraclavicular region had shrunk markedly (Fig. 2A). A 64-year-old male with gastric adenocarcinoma and liver metastases who had undergone four prior chemotherapy regimens showed a partial response to RAD001 that persisted for >4 months at the dose of 10 mg/day (Fig. 2B).

DISCUSSION

Evidence implicating the phosphatidylinositol 3-kinase–Akt–mTOR signaling pathway in the pathogenesis of a variety of malignancies has prompted the development of therapeutic strategies to modulate this pathway. RAD001 is an oral inhibitor of the mTOR pathway, and we have now performed a dose-escalation Phase I study of this drug in Japanese patients with advanced solid tumors in order to evaluate its safety and pharmacokinetics. Therapy with RAD001 at oral doses of up to 10 mg once daily was relatively well tolerated in the study subjects. Indeed, the safety and tolerability of RAD001 in the Japanese patients were similar to those observed in previous studies with larger populations of Caucasian patients, for whom the most common drug-related toxicities included rash, stomatitis and fatigue. Previous studies have reported that patients receiving RAD001 manifested hyperglycemia and hyperlipidemia, probably as a result of inhibition of mTOR-regulated glucose and lipid metabolism (16,18,19). Grade 3 hyperglycemia was observed in one patient treated with 10 mg/day, whereas hyperlipidemia was not observed in our study. One patient in our study developed pneumonitis of Grade 2, with this condition having previously been identified as a potential class-related toxicity for mTOR inhibitors that should be monitored in clinical trials with these agents (16–20). However, the condition of pneumonitis in our study was reversible after discontinuation of RAD001 treatment. The pharmacokinetic profile of RAD001 in Japanese patients was also similar to that in Caucasian patients. RAD001 was absorbed rapidly, with the C_{max} being achieved as early as 1–2 h after oral administration. A recent Phase I study of RAD001 performed in Europe and the USA showed that the mean ($\pm$ SD) C_{max} in patients with advanced cancer was 32 ± 9 and 61 ± 17 ng/ml at daily doses of 5 and 10 mg, respectively, with a mean AUC_τ of 238 ± 77 and 514 ± 231 ng h/ml, respectively (16). These results for Caucasian patients are similar to those obtained here with Japanese patients, especially for the dose level of 10 mg/day (C_{max} of 65.9 ± 1.40 ng/ml and AUC_τ of 711 ± 113 ng h/ml. Given the limited number of patients in both studies, these results suggest that there are no substantial differences in the pharmacokinetics of RAD001 between the two populations. RAD001 has already undergone extensive clinical testing in the setting of renal and cardiac transplantation (21,22). Our present data are also supported by observations with 673 renal transplant patients who received RAD001 (23). This large cohort included 80% Caucasian patients and 2.5% patients of Asian origin with no significant differences in clearance of RAD001 being apparent between the Asian and Caucasian patients. The data from this study, combined with those from previous studies, suggest that the pharmacokinetic and safety data for RAD001 obtained in larger clinical trials with Caucasian patients are likely applicable to the Japanese population.

Although response was not a primary outcome of our study, two of three patients treated with RAD001 at a daily dose of 10 mg manifested marked tumor shrinkage. This anti-tumor activity occurred in patients with esophageal and gastric cancer. One esophagogastric cancer patient also exhibited a partial response to RAD001 treatment at a daily dose of 5 mg in a previous Phase I study (16). The likelihood that these findings will extend to other patients is supported by recent studies suggesting that defects in the mTOR signaling pathway are important in the pathogenesis of these cancers. mTOR is an upstream regulator of hypoxia-inducible factor-1α, which is a key mediator of gastric cancer growth (24). Pre-clinical studies have shown that the mTOR inhibitor rapamycin inhibits the growth of human gastric adenocarcinoma cell lines, gastric cancer, gastrointestinal tumors, and the development of peritoneal carcinomatosis from gastric cancer *in vitro* or *in vivo* (24–27).

In conclusion, the results of our Phase I study suggest that RAD001 can be safely administered at a daily dose of 10 mg to Japanese patients with advanced solid malignancies. The pharmacokinetic characteristics of RAD001 in Japanese patients did not appear to differ from those previously observed in Caucasian patients. The safety profile and potential broad-spectrum efficacy of RAD001 thus warrant additional clinical evaluation of this new agent.

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Conflict of interest statement

The authors Katsutoshi Kurei and Ken Kobayashi are employed by Novartis Pharma.

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Disturbance of the Growth Hormone–Insulin-like Growth Factor-1 Axis Associated with Poor Performance Status in Patients with Solid Tumors

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Objective: Hormonal imbalance characterized by excessive production of growth hormone (GH) and a low circulating concentration of insulin-like growth factor (IGF)-1 has been demonstrated in individuals with various serious conditions. However, little is known about changes in the GH–IGF-1 axis in cancer patients.
Methods: We prospectively examined the circulating levels of several hormones in 58 patients with solid tumors who were classified according to Eastern Cooperative Oncology Group performance status (PS): PS 0–1, $n = 15$; PS 2, $n = 15$; PS 3, $n = 15$; and PS 4, $n = 13$. The relations of hormone concentrations, with a focus on the GH–IGF-1 system, to PS were evaluated by Spearman's rank correlation test and regression analysis.
Results: The circulating levels of IGF-1, IGF-binding protein-3 and thyroid hormones (total T_3 and T_4) were inversely correlated with PS score. The concentration of GH was increased irrespective of PS but not statistically significant. The ratio of IGF-I to GH was inversely correlated with PS. The levels of GH and IGF-1 in all patients were also inversely correlated.
Conclusions: The present study suggests that the GH–IGF-1 axis is disturbed in patients with cancer.

Key words: growth hormone – insulin-like growth factor-1 – performance status

INTRODUCTION

Medical oncology has made substantial advances with the development of new treatment strategies based on a better understanding of cancer biology. Despite such progress, however, a large proportion of individuals with advanced cancer still experience a fatal outcome (1).

Performance status (PS) refers to the level of activity that cancer patients are capable of achieving and is an important prognostic factor independent of the anatomic extent or histological characteristics of cancer (2). After cancer diagnosis, patients will be exposed to the detrimental consequences not only of the cancer itself but also of anticancer treatment. Most patients with advanced cancer thus exhibit a deterioration in PS at some point during the course of their disease. Such a decreased PS is associated with a substantial impairment in quality of life, reduced responsiveness to anticancer therapies and increased mortality. To date, however, an effective treatment for the cancer-related deterioration in PS has not been developed, largely as a result of its multifactorial pathogenesis. New insights into the underlying pathophysiological mechanisms are likely to provide a basis for the development of effective therapeutic strategies to improve the PS of cancer patients.

Hormonal aberrations characterized by excessive production of growth hormone (GH) and a low circulating concentration of insulin-like growth factor (IGF)-1 have been

detected in patients with diverse conditions including sepsis, burns, renal failure, AIDS and anorexia nervosa as well as in individuals who have undergone surgery (3,4). Such perturbation of the GH–IGF-1 system may contribute adversely to the condition of critically ill patients, and treatments to correct the hormonal imbalance, by administration of GH or IGF-1, have been explored (4,5). Although circulating GH levels have also been found to be increased in individuals with various types of cancers, including those of the colon, lung, breast, liver and endometrium as well as lymphoma (6–12), the influence of cancer on the GH–IGF-1 axis has not been defined. We have now prospectively examined the circulating levels of several hormones in cancer patients with different PS scores and have investigated the relation of changes in hormonal profile, with a focus on the GH–IGF-1 system, to PS.

PATIENTS AND METHODS

Patients with histologically proven cancer were eligible for the study. Other inclusion criteria were an age of at least 20 years and a projected life expectancy of at least 1 month. The main exclusion criteria were blood malignancies or use of corticosteroids. The study subjects were sequentially enrolled in each institution and divided into four groups on the basis of Eastern Cooperative Oncology Group (ECOG) PS score (0–1, 2, 3 or 4), with a targeted accrual of 15 patients in each group. The number of patients enrolled in each group was counted by the patient registration office and feedback to each institution to enroll planned number of patients. Written informed consent was obtained from all patients, and the study protocol was approved by the institutional ethics committee of each of the participating institutions.

Blood samples were once collected for each patient in the early morning before the subjects had had breakfast and after they had fasted overnight or in the morning excluding 1 h after breakfast and 1 h before lunch. This was planned to avoid possible peaks of GH value in the circadian rhythm. Serum and plasma samples were obtained by centrifugation and stored at $-20^{\circ}C$ until assay. Serum GH and IGF-I levels were determined by solid-phase radioimmunoassay and immune radiometric assay, respectively. Serum triiodothyronine (total T_3), thyroxine (total T_4) and thyroid-stimulating hormone (TSH) levels were determined by electro chemiluminescent immunoassay. Serum concentrations of IGF-binding protein-3 (IGFBP-3) and thyroxine-binding globulin (TBG) were measured by competitive radioimmunoassay. All assays were performed in a blinded manner in the outside laboratory. Other laboratory variables such as total protein, albumin, cholesterol, triglyceride, C-reactive protein, creatinine and hemoglobin as well as markers of liver function were measured in routine hospital tests. Height, weight, body mass index (BMI) and food intake were also recorded for all patients. Primary endpoint of this study was defined as the relation between serum GH levels, IGF-I levels and PS.

Data are presented as means ± SD, and Spearman's rank correlation test was applied to assess the correlation between two variables. A P value of <0.05 was considered statistically significant.

RESULTS

Patient Characteristics

A total of 58 patients (34 men and 24 women) were enrolled in the study at five centers in Japan between January 2005 and March 2006. Median age at enrollment was 64 years (range, 28–81 years). The most frequent principal diagnoses were lung cancer (33%, $n = 19$), gastric cancer (22%, $n = 13$) and colorectal cancer (19%, $n = 11$). The numbers of patients in each PS group at study entry were 15, 15, 15 and 13 for PS 0–1, 2, 3 and 4, respectively. The baseline clinical characteristics of the patients according to the PS group are shown in Table 1. Complete blood test data were available for all patients.

Food Intake, BMI and Laboratory Variables

The Spearman test revealed that PS score was inversely correlated with weight ($r = -0.54$, $P < 0.001$), BMI ($r = -0.53$, $P < 0.001$) and food intake ($r = -0.73$, $P < 0.001$) (Table 2). Inverse correlations were also apparent between PS and circulating levels of total protein ($r = -0.59$, $P < 0.001$), albumin ($r = -0.66$, $P < 0.001$), total cholesterol ($r = -0.33$, $P = 0.014$), choline esterase ($r = -0.61$, $P < 0.001$) and hemoglobin ($r = -0.37$, $P = 0.004$). PS also tended to be positively correlated with levels of alkaline phosphatase ($r = 0.23$) and lactate dehydrogenase ($r = 0.09$), but these relations did not achieve statistical significance. Significant positive correlations were detected between PS and the concentration of C-reactive protein ($r = 0.59$, $P < 0.001$) and the number of white blood cells ($r = 0.42$, $P = 0.001$).

Hormone Levels

The plasma concentration of GH was not significantly correlated with PS ($r = 0.15$, $P = 0.25$), whereas that of IGF-1 was inversely correlated with PS ($r = -0.44$, $P = 0.001$) (Table 3). An inverse correlation was also apparent between PS and the concentration of IGFBP-3 ($r = -0.39$, $P = 0.002$), the major carrier protein for IGF-1 in the circulation. The concentration of GH was inversely correlated with that of IGF-1 ($r = -0.314$, $P = 0.018$). Whereas TSH level was not correlated with PS ($r = 0.04$, $P = 0.76$), the concentrations of total T_3 (-0.57, $P < 0.001$), total T_4 (-0.38, $P = 0.003$) and TBG (-0.44, $P = 0.001$) were inversely correlated with PS. The ratio of IGF-I to GH (IGF-I/GH), a

combined indicator of GH and IGF-I, also showed correlation with PS ($r = 0.262$, $P = 0.049$) (Table 4).

Table 1. Patient characteristics

	Performance status			
	0–1	2	3	4
Assessable patients	15	15	15	13
Median age (range)	64 (49–73)	66 (50–81)	60 (28–77)	69 (54–81)
Sex (male/female)	11/4	7/8	9/6	7/6
Principal diagnosis				
Lung cancer	9	5	3	2
Gastric cancer	1	3	4	5
Colorectal cancer	2	5	4	0
Esophageal cancer	0	1	1	1
Pancreatic cancer	1	0	0	2
Breast cancer	0	0	1	1
Sarcoma	1	1	0	0
Renal cancer	1	0	0	0
Adenoid cystic cancer	0	0	1	0
Biliary tract cancer	0	0	1	0
Head and neck cancer	0	0	0	1
Cervical cancer	0	0	0	1

DISCUSSION

In this prospective evaluation of hormonal status in cancer patients, we have shown that the circulating levels of thyroid hormones (T_3 and T_4) and of components of the IGF system (IGF-1 and IGFBP-3) were inversely correlated with PS score. Given that the GH concentration also tended to be increased in patients with a high PS score, our results are indicative of an imbalance between GH and the IGF system in such patients.

Increased interpulse levels of GH have been described in critically ill patients including those with several types of cancer (4,13–15). Fasting levels of GH were also found to be significantly greater in patients with colon cancer than in control subjects (2.9 ± 3.1 versus 0.5 ± 0.2 ng/ml) (11). Our data now show a similarly high plasma concentration of GH (3.0 ± 3.7 ng/ml) in cancer patients irrespective of PS, although we did not determine values for matched controls.

Most circulating IGF-1 and IGFBP-3 are synthesized in the liver, where expression of each is increased by GH (Fig. 1). IGF-1 has a long half-life in plasma (up to 12 h), and its circulating level is highly correlated with that of GH. IGFBP-3 binds >95% of plasma IGF-1 and influences cell proliferation by controlling the access of IGF-1 to IGF receptors (16,17). In most instances, the circulating level of IGFBP-3 has been found to correlate with that of IGF-1 and is thought to reflect the status of IGF-1 in plasma. Our prospective data now show that the circulating

Table 2. Laboratory variables stratified by performance status

	Performance status				*P* value
	0–1	2	3	4	
Height (cm)	162 ± 7	158 ± 10	163 ± 9	156 ± 8	NS
Weight (kg)	58 ± 10	53 ± 15	49 ± 10	40 ± 4	<0.001
BMI (kg/m^2)	22 ± 3	21 ± 4	19 ± 4	16 ± 2	<0.001
Food intake (%)	82 ± 25	62 ± 27	27 ± 29	15 ± 19	<0.001
TP (g/dl)	7.1 ± 0.4	6.5 ± 0.4	6.2 ± 0.7	5.8 ± 0.9	<0.001
Albumin (g/dl)	3.9 ± 0.3	3.4 ± 0.5	3.0 ± 0.6	2.4 ± 0.7	<0.001
TC (mg/dl)	180 ± 32	186 ± 53	169 ± 48	125 ± 54	0.014
TG (mg/dl)	126 ± 76	113 ± 51	122 ± 80	88 ± 27	NS
ChE (IU/l)	258 ± 54	178 ± 77	174 ± 82	105 ± 48	<0.001
ALP (IU/l)	450 ± 353	480 ± 344	540 ± 446	741 ± 477	NS
LDH (IU/l)	250 ± 103	256 ± 119	323 ± 265	371 ± 434	NS
Cre (mg/dl)	0.7 ± 0.2	0.6 ± 0.2	0.9 ± 1.2	0.7 ± 0.5	NS
CRP (mg/dl)	0.8 ± 1.0	2.1 ± 2.1	4.6 ± 5.2	10.6 ± 10.5	<0.001
WBC ($10^3/\mu$l)	5.9 ± 1.9	5.3 ± 2.4	8.3 ± 4.8	11.7 ± 6.7	0.001
Hb (g/dl)	12.0 ± 1.6	10.3 ± 1.4	11.5 ± 2.4	9.3 ± 1.7	0.004
Platelets ($10^4/\mu$l)	27.9 ± 7.7	30.6 ± 22.7	25.6 ± 9.5	29.9 ± 12.6	NS

Data are means $\pm$ SD. *P* values were determined by Spearman's rank correlation test. NS, not significant; BMI, body mass index; TP, total protein; TC, total cholesterol; TG, triglyceride; ChE, choline esterase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; Cre, creatinine; CRP, C-reactive protein; WBC, white blood cells; Hb, hemoglobin.

Table 3. Circulating hormone levels according to performance status

	Performance status				*P* value
	0–1	2	3	4	
GH (ng/ml)	2.5 ± 2.4	2.5 ± 3.1	3.1 ± 3.7	4.1 ± 5.5	NS
IGF-1 (ng/ml)	149 ± 49	96 ± 60	135 ± 102	64 ± 42	0.001
IGFBP-3 (μg/ml)	1.9 ± 0.5	1.9 ± 0.8	1.9 ± 0.8	1.0 ± 0.6	0.002
TSH (μIU/ml)	2.6 ± 1.9	2.3 ± 1.8	2.2 ± 1.0	2.5 ± 1.6	NS
Total T_3 (ng/ml)	1.1 ± 0.3	0.9 ± 0.2	0.9 ± 0.2	0.7 ± 0.2	<0.001
Total T_4 (μg/dl)	9.8 ± 1.8	9.8 ± 1.9	9.6 ± 1.6	7.1 ± 2.4	0.003
TBG (μg/ml)	22.3 ± 3.9	24.4 ± 7.0	20.8 ± 4.3	15.7 ± 4.3	0.001

Data are means ± SD. *P* values were determined by Spearman's rank correlation test. GH, growth hormone; IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor-binding protein-3; TSH, thyroid-stimulating hormone; TBG, thyroxine-binding globulin.

Table 4. Ratio of IGF-1 to GH according to performance status

	Performance status				*P* value
	0–1	2	3	4	
IGF-1/GH (ng/ml)	366 ± 645	201 ± 405	185 ± 229	62 ± 98	0.049

Data are means ± SD. *P* values were determined by regression analysis.

concentration of IGF-1 and IGFBP-3 was negatively correlated with PS score but GH did not show clear correlation with PS score. The ratio of IGF-I/GH also showed correlation with PS. These results thus suggest that the relation between GH secretion and circulating IGF-1 levels is disturbed in cancer patients.

Under normal conditions, GH secreted by the pituitary gland induces hepatic IGF-1 production, which in turn exerts feedback suppression of GH secretion (Fig. 1). Acquired GH resistance characterized by the combination of high levels of GH and low levels of IGF-1 has been demonstrated to varying extents in patients with a wide range of conditions including sepsis, trauma, burns, renal failure and AIDS (3,4,18,19). The primary defect in acquired GH resistance is a reduction in IGF-1 concentration which then leads to increased GH concentration; however, low levels of IGF-1 are not improved, despite increased or normal levels of GH (Fig. 1) (3,20). In the present study, a significant inverse correlation was apparent between circulating GH and IGF-1 levels in the entire cohort, consistent with the pattern of acquired GH resistance.

It remains unclear, however, whether the disturbance of the GH–IGF-1 axis is merely a non-specific consequence of cancer or whether it contributes adversely to the complex pathophysiology of cancer. Nutritional state is known to affect the function of the GH–IGF-1 axis. Acute dietary restriction and chronic malnutrition, especially accompanied by severe protein deficiency, have been shown to lead to

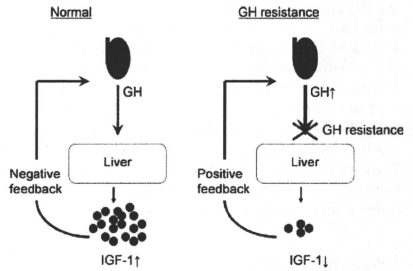

Figure 1. Diagram of the growth hormone (GH) and insulin-like growth factor I (IGF-I) axis in healthy persons (left) and those with acquired GH resistance. Under normal conditions, GH secreted by the pituitary gland stimulates production of IGF-1 by liver. IGF-1 exerts feedback suppression. The primary defect in acquired GH resistance is a reduction in IGF-1 concentration which then leads to increased GH concentration; however, low levels of IGF-1 are not improved, despite increased or normal levels of GH.

increased levels of GH and reduced levels of IGF-1 (21,22). In the present study, the circulating concentration of IGF-1 was significantly correlated with BMI ($r = 0.46$, $P < 0.001$), albumin level ($r = 0.41$, $P = 0.002$) and total protein level ($r = 0.36$, $P = 0.007$). The circulating level of GH was negatively correlated with BMI ($r = -0.40$, $P = 0.003$) but was not related to total protein and albumin levels. Patients with lung cancer were previously shown to have a reduced IGF-1 concentration and an increased GH pulse frequency before the development of malnutrition (23). Furthermore, acquired GH resistance in cachectic patients with colorectal cancer has been proposed not to be an adaptation to malnutrition but to be caused by the tumor itself (24). Together, these various observations suggest that although inadequate nutrition is likely to contribute to the altered GH/IGF-I axis in cancer patients, other factors also play a role. It has been shown that in rat hepatocytes in primary culture cytokines, interleukin-1β

and tumor necrosis factor-α inhibit GH-stimulated IGF-1 synthesis at least partly due to suppression of hepatic GH receptor synthesis (25). Given the significant positive correlation between PS and C-reactive protein observed in the present study, inflammatory cytokine deregulation in cancer patients can participate in the development of acquired GH resistance. Further studies to investigate the mechanism for acquired GH resistance in cancer patients are warranted.

A wide range of conditions sharing the common feature of catabolism exhibit a similar pattern of disturbance of the GH–IGF-1 axis characterized by high GH and low IGF-1 levels (4). Treatment to reverse this defect by restoring IGF-1 levels through administration of GH has been shown to result in improvement in metabolic parameters and to provide clinical benefit in well-defined groups of patients, such as those with AIDS or anorexia nervosa (4,26). On the other hand, a small pilot study with 10 terminally ill cancer patients showed that GH administration for 3 days had limited effects on metabolic parameters (23). Given that the various conditions associated with acquired GH resistance have different pathophysiological mechanisms, GH administration in cancer patients may not necessarily be clinically beneficial.

In conclusion, the results of the present study show that the GH–IGF-1 system is disturbed in cancer patients and that this anomaly may play a role in the deterioration of PS. Therapeutic strategies for correcting this hormonal imbalance merit investigation. Such approaches may alleviate the cachexia and malaise associated with cancer.

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Conflict of interest statement

None declared.

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MINIREVIEW

Epidermal growth factor receptor in relation to tumor development: EGFR-targeted anticancer therapy

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Keywords
epidermal growth factor receptor (*EGFR*) mutation; *KRAS* mutation; monoclonal antibodies; tyrosine kinase inhibitor

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The discovery that signaling by the epidermal growth factor receptor (EGFR) plays a key role in tumorigenesis prompted efforts to target this receptor in anticancer therapy. Two different types of EGFR-targeted therapeutic agents were subsequently developed: mAbs, such as cetuximab and panitumumab, which target the extracellular domain of the receptor, thereby inhibiting ligand-dependent EGFR signal transduction; and small-molecule tyrosine kinase inhibitors, such as gefitinib and erlotinib, which target the intracellular tyrosine kinase domain of the EGFR. Furthermore, recent clinical and laboratory studies have identified molecular markers that have the potential to improve the clinical effectiveness of EGFR-targeted therapies. This minireview summarizes the emerging role of molecular profiling in guiding the clinical use of anti-EGFR therapeutic agents.

KRAS mutations and sensitivity to therapy with mAb to epidermal growth factor receptor in colorectal cancer

Cetuximab is a chimeric mouse–human mAb that targets the extracellular domain of the epidermal growth factor receptor (EGFR) and thereby blocks downstream signal transduction via the phosphatidylinositol 3-kinase/Akt and Ras/Raf/mitogen-activated protein kinase pathways (Fig. 1). Because it is an antibody (IgG1 isotype), cetuximab may also induce antibody-dependent cell-mediated cytotoxicity, although the clinical relevance of antibody-dependent cell-mediated cytotoxicity with regard to the antitumor efficacy of cetuximab is likely to be relatively low [1].

Cetuximab exhibits single-agent activity against metastatic colorectal cancer (mCRC) refractory to previous chemotherapies [2]. An analysis of 80 patients with mCRC, (who had previously undergone treatment) enrolled in a study of cetuximab monotherapy found a mutation rate of 38% for the proto-oncogene *KRAS* in tumor specimens and discovered that such mutations were associated with resistance to cetuximab, showing an overall response rate of 0 versus 10% for mutation-positive and mutation-negative patients, respectively [3]. More recently, a trial comparing cetuximab + best supportive care (BSC) with BSC alone in 394 patients with mCRC after failure of prespecified chemotherapy found a *KRAS* mutation rate of 69% [4]. Analysis of the cetuximab + BSC arm (n = 198) of the trial, however, revealed that only 1.2% of the *KRAS* mutation-positive patients (n =

Abbreviations
BSC, best supportive care; CML, chronic myeloid leukemia; EGFR, epidermal growth factor receptor; mCRC, metastatic colorectal cancer; NSCLC, non-small cell lung cancer; OS, overall survival; PFS, progression-free survival; TKI, tyrosine kinase inhibitor.

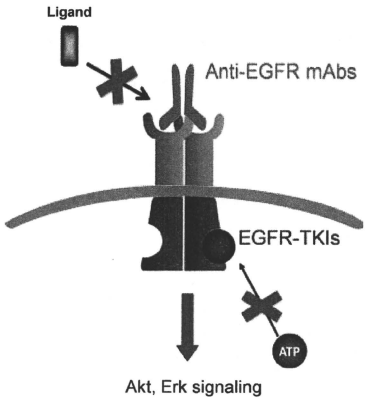

Fig. 1. Two different types of EGFR-targeted agents. mAbs target the extracellular domain of the receptor, and small-molecule TKIs target the intracellular tyrosine kinase domain of the EGFR.

81), compared with 12.8% of patients with wild-type *KRAS* (n = 117), responded to cetuximab monotherapy (Table 1). Furthermore, *KRAS* mutations were significantly associated with a shorter progression-free survival (PFS) (7.2 versus 14.8 weeks) and a shorter overall survival (OS) (4.5 versus 9.5 months) among the cetuximab-treated patients (Table 1). No survival benefit was observed in patients whose tumors harbored wild-type *KRAS* compared with those whose tumors were positive for mutant *KRAS* in the BSC-only arm (OS of 4.8 versus 4.6 months, respectively), revealing a lack of prognostic value for *KRAS* status (Table 1). These data thus indicate that the prolonged survival of patients with tumors harboring wild-type *KRAS* was a result of the benefit from cetuximab monotherapy rather than of a more favorable prognosis for the subset of patients treated with cetuximab + BSC.

Similar findings, in terms of clinical efficacy among patients with tumors harboring wild-type *KRAS*, were obtained in a retrospective analysis of a trial of panitumumab in patients with mCRC [5]. Panitumumab, a fully human mAb targeted to the extracellular domain of EGFR, is of the IgG2 isotype, and its antitumor effects are probably attributable to inhibition of EGFR signaling rather than to antibody-dependent cell-mediated cytotoxicity. The *KRAS* status was assessed in 92% (n = 427) of tumor samples from patients enrolled in the phase III registration trial of panitumumab versus BSC, and *KRAS* mutations were detected in 43% of the tested tumors. Furthermore, patients whose tumors harbored wild-type *KRAS* exhibited a 17% response rate in the panitumumab-monotherapy arm, whereas those with *KRAS* mutation–positive tumors failed to respond to panitumumab (Table 1). The median PFS time was significantly longer in panitumumab-treated patients with wild-type *KRAS* than in those with mutant *KRAS* (12.3 versus 7.4 weeks) (Table 1). The median OS time in panitumumab-treated patients with wild-type *KRAS* was also longer than that in those with mutant *KRAS* (8.1 versus 4.9 months) (Table 1). On the basis of these results, the European Medicines Agency approved the use of panitumumab only for mCRC patients with tumors possessing wild-type *KRAS*. This was the first approval of an agent for mCRC that was based on patient-specific molecular profiling, opening a new vista for genotype-directed therapy in this disease.

KRAS mutation as a mechanism of resistance to EGFR-targeted therapy

The KRAS protein is localized to the inner surface of the cell membrane. The binding of ligand to EGFR induces receptor dimerization and consequent conformational changes that result in activation of the intrinsic tyrosine kinase, receptor autophosphorylation and a transient activation of RAS GTPases (Fig. 2). Activated RAS targets various downstream effectors to exert pleiotropic cellular effects. *KRAS* is the most frequently mutated oncogene in several types of human cancer. These mutations, most of which are located in codons 12 and 13, occur in up to 40% of patients with mCRC [6]. Activating mutations of *KRAS* result in activation of the mitogen-activated protein kinase

Table 1. Activity of therapy with monoclonal anti-EGFR in patients with mCRC, based on the *KRAS* mutation status. MT, mutant; RR, response rate; WT, wild-type.

			RR (%)		PFS (weeks)		OS (months)	
Authors	Agent	n	WT	MT	WT	MT	WT	MT
Karapetis *et al.* [4]	Cetuximab	198	12.8	1.2	14.8	7.2	9.5	4.5
Amado *et al.* [5]	Panitumumab	208	17	0	12.3	7.4	8.1	4.9

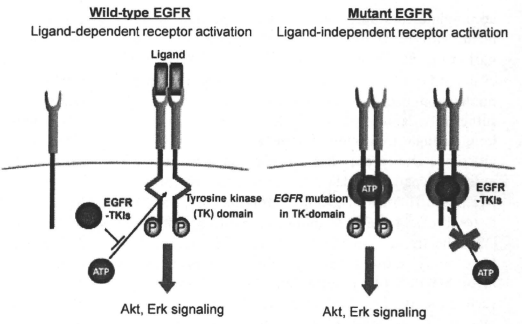

Fig. 2. In the wild-type EGFR, ligand binding to EGFR leads to receptor dimerization, autophosphorylation and activation of downstream signaling pathways. Compared with wild-type EGFR, mutant receptors preferentially induce ligand-independent dimerization and activate downstream signaling pathways. *EGFR* mutations result in repositioning of critical residues surrounding the ATP-binding cleft of the tyrosine kinase domain of the receptor and thereby stabilize the interaction with EGF-TKIs.

signaling cascade, independently of EGFR activation. Mutation of *KRAS* thus bypasses the need for ligand binding to EGFR and results in constitutive activation of signaling downstream of the receptor, which, in turn, promotes cell proliferation and metastasis as well as inhibiting apoptosis. These effects of *KRAS* mutation support continued cancer cell survival, even in the presence of upstream EGFR inhibition [7,8].

EGFR mutations and sensitivity to EGFR-tyrosine kinase inhibitor therapy in non–small cell lung cancer

Imatinib was designed to compete with ATP at the ATP-binding site within the tyrosine kinase domain of ABL, which is activated as a result of the chromosomal translocation that gives rise to the *BCR–ABL* fusion gene in chronic myeloid leukemia (CML). The marked success of imatinib in the treatment of CML provided compelling evidence for the effectiveness of small-molecule tyrosine kinase inhibitors (TKIs) and triggered the development of this class of agents for targeting growth factor receptors frequently expressed in epithelial cancers [9]. Two such inhibitors of the tyrosine kinase activity of EGFR (EGFR-TKIs), gefitinib and erlotinib, compete with ATP for binding to the tyrosine kinase pocket of the receptor, thereby inhibiting receptor tyrosine kinase activity and EGFR signaling pathways (Fig. 1). Early clinical studies showed that a subset of patients with non-small cell lung cancer (NSCLC) experienced a rapid, pronounced and durable response to single-agent therapy with EGFR-TKIs. Subsequent retrospective analysis of clinical data consistently demonstrated that a clinical response to these agents is more common in women than in men, in Japanese people than in individuals from Europe or the USA, in patients with adenocarcinoma than in those with other histological subtypes of cancer, and in individuals who have never smoked than in those with a history of smoking [10]. These clinical observations paved the way for translational research that aimed to identify, at the molecular level, patients who might benefit from such therapy. In 2004, three groups in the USA made the landmark observation that NSCLC patients who experienced a dramatic response to gefitinib or erlotinib commonly harbored somatic mutations of the drug's target, EGFR [11–13]. Indeed, *EGFR* mutations are present more frequently in women, in individuals of East Asian ethnicity, in patients with adenocarcinoma, and in never-smokers, the same groups identified clinically as most likely to respond to treatment with EGFR-TKIs.

Several prospective clinical trials of gefitinib or erlotinib for treatment of NSCLC patients with *EGFR* mutations have been performed to date, revealing radiographic response rates from 55 to 91% [14–21] (Table 2). These values are much higher than those historically observed with standard cytotoxic chemotherapy for advanced NSCLC. As the data

Table 2. Prospective study of EGFR-TKI monotherapy for NSCLC patients with *EGFR* mutations. RR, response rate.

Authors	Agent	*n*	RR (%)
Inoue *et al.*[14]	Gefitinib	16	75
Asahina *et al.* [15]	Gefitinib	16	75
Sutani *et al.* [16]	Gefitinib	27	78
Yoshida *et al.* [17]	Gefitinib	21	91
Sunaga *et al.* [18]	Gefitinib	19	76
Tamura *et al.* [19]	Gefitinib	28	75
Sequest *et al.* [20]	Gefitinib	34	55
Sugio *et al.* [21]	Gefitinib	19	63

accumulate, an improvement in OS, conferred by treatment with these drugs, is also expected in patients harboring *EGFR* mutations. It was not possible to evaluate OS in most of the clinical trials at the time of publication because the number of patients was not sufficiently large and the follow-up period was not long enough to obtain precise estimates of survival outcome. Our group has recently analyzed updated individual patient data from seven Japanese prospective phase II trials of gefitinib monotherapy, including a total of 148 *EGFR* mutation–positive individuals [22]. The Iressa Combined Analysis of Mutation Positives study showed that gefitinib confers a highly favorable PFS (9.7 months) and OS (24.3 months) in such patients. The median survival time of approximately 2 years, achieved in patients with *EGFR* mutation-positive NSCLC by treatment with EGFR-TKIs, supports the notion that this group of patients constitutes a clinically distinct population. The substantial clinical benefits of treatment with EGFR-TKIs in *EGFR* mutation-positive NSCLC patients raise the question of whether first-line treatment with EGFR-TKIs might be more beneficial than standard cytotoxic chemotherapy in this genotype-defined population. In the Iressa Combined Analysis of Mutation Positives study, we performed an exploratory comparison between gefitinib and systemic chemotherapy in the first-line setting. We found that first-line gefitinib treatment yielded a significantly longer PFS than did systemic chemotherapy in *EGFR* mutation-positive NSCLC patients, supporting the use of gefitinib as an initial therapy in this patient population. This finding is consistent with a subset analysis of a recently completed randomized phase III study, known as the Iressa Pan-Asia Study, which showed that first-line treatment with gefitinib significantly improved the PFS of *EGFR* mutation-positive patients with advanced NSCLC compared to treatment with carboplatin and paclitaxel. We are currently performing phase III randomized studies comparing platinum-based chemotherapy with gefitinib in chemotherapy-naïve NSCLC patients with *EGFR* mutations. Such ongoing phase III clinical trials will help to determine whether gefitinib monotherapy becomes the standard of care for *EGFR* mutation-positive NSCLC.

EGFR mutation as a mechanism underlying sensitivity to therapy with EGFR-TKIs

The discovery of *EGFR* mutations has led not only to the identification of a molecular predictor of sensitivity to EGFR-TKIs but also to examination of the biological effects of such mutations on EGFR function. Deletions in exon 19, and a point mutation (L858R) in exon 21, are the most common *EGFR* mutations as well as the most extensively evaluated to date. Initial studies, based on transient transfection of various cell types with vectors encoding wild-type or mutant versions of EGFR, showed that the extent of activation of mutant receptors by EGF is more pronounced and sustained than is that of the wild-type receptor [11]. Subsequently, NSCLC cell lines with exon-19 deletions or the L858R point mutation were identified, and the *EGFR* mutations were found to confer ligand-independent activation of EGFR [23]. We also found that the constitutive activation of endogenous mutant EGFR is attributable to the ability of the receptor to undergo ligand-independent dimerization (Fig. 2) [23]. Introduction of the two most common EGFR mutants into transgenic mice was recently shown to result in the formation of lung adenocarcinomas, demonstrating that expression of these constitutively activated forms of EGFR is sufficient for transformation and required for maintenance of these tumors [24]. These various observations indicate that *EGFR* mutation-positive tumors are dependent on, or 'addicted' to, EGFR signaling for their growth and survival. Similar addiction is evident in *BCR/ABL*-positive CML and in *KIT* mutation-positive gastrointestinal stromal tumors, both of which are highly sensitive to imatinib. Exposure of *EGFR* mutation-positive NSCLC tumors to EGFR-TKIs thus results in EGFR signaling pathways being turned off and the cancer cells undergoing apoptosis. Moreover, *EGFR* mutations result in repositioning of critical residues surrounding the ATP-binding cleft of the tyrosine kinase domain of the receptor and thereby stabilize the interaction with EGF-TKIs, leading to an increase of $\sim$ 100-fold in sensitivity to inhibition by EGFR-TKIs compared with that of the wild-type receptor (Fig. 2) [11,25]. These factors combine to render *EGFR* mutation-positive NSCLC more sensitive to EGFR-TKIs.

Molecular mechanisms associated with acquired resistance to therapy with EGFR-TKIs

Despite the great benefits of EGFR-TKIs in the treatment of NSCLC associated with *EGFR* mutations, most, if not all, patients ultimately develop resistance to these drugs. The first mechanism to be discovered of such acquired resistance is a secondary mutation, T790M, in the *EGFR* [26]. To date, this mutation has been found in $\sim$ 50% of NSCLC tumors from patients who developed acquired resistance to EGFR-TKIs.

The position of the T790M mutation within the EGFR is analogous to the positions of mutations in other tyrosine kinases known to result in resistance to imatinib (T315I in *ABL*, T764I in *PDGFRA* and T670I in *KIT*) [27–29]. The conserved threonine residues in these different kinases are located near the kinase active site and appear to be critical for the binding of ATP and the corresponding TKIs. Structural modeling suggests that the T790M mutation of *EGFR* creates steric hindrance that prevents EGFR-TKIs from interacting with the ATP-binding pocket of the receptor. Furthermore, biochemical analysis showed that, in cells expressing both T790M mutant and wild-type forms of EGFR, EGFR-TKIs are not able to inhibit the phosphorylation of either type of the receptor.

The T790M mutation of *EGFR* was initially thought to occur during treatment with EGFR-TKIs, given that it was initially identified only in tumor specimens from a patient with NSCLC who relapsed after 24 months of complete remission despite continued gefitinib therapy [26]. However, subsequent development of a highly sensitive detection method, mutant-enriched PCR analysis, and its application to detect the T790M mutation in 280 NSCLC tumor specimens obtained from patients before treatment with EGFR-TKIs, revealed the presence of the mutation in a small proportion of tumor cells in 10 (3.6%) of these specimens [30]. Similarly, a minor proportion of cells harboring a *BCR/ABL* mutation associated with imatinib resistance was detected in a patient with CML before treatment with this drug; the proportion of mutant cells was later found to have increased after treatment onset and the development of resistance [31]. These observations suggest that a small fraction of NSCLC tumor cells may harbor the T790M mutation of *EGFR* before treatment with EGFR-TKIs and that these cells come to predominate as a result of their selective proliferation during such treatment, resulting in the development of clinical resistance.

NSCLC tumors that acquire resistance to gefitinib or erlotinib as a result of the *EGFR* T790M mutation remain dependent on EGFR signaling for their growth and survival. Alternative strategies for inhibiting the activity of the mutant receptors may thus be able to overcome the acquired resistance to EGFR-TKIs. This possibility has prompted the development of second-generation irreversible EGFR-TKIs. These agents are also ATP mimetics, similarly to the reversible EGFR-TKIs gefitinib and erlotinib, but they covalently bind cysteine 797 at the edge of the ATP-binding cleft of the EGFR [32]. Some irreversible EGFR-TKIs have been shown to inhibit EGFR phosphorylation, as well as the growth of NSCLC cell lines harboring the T790M mutation of *EGFR* [32,33]. Future clinical trials of these irreversible EGFR-TKIs in NSCLC patients with the *EGFR* T790M mutation are warranted.

Amplification of the gene for the receptor tyrosine kinase MET has also recently been identified as a mechanism of EGFR-TKI resistance, being detected in 22% of tumor samples from NSCLC patients with *EGFR* mutations who acquired gefitinib resistance [34]. *MET* amplification confers EGFR-TKI resistance by activating ERBB3 signaling in an EGFR-independent manner. This redundant activation of ERBB3 permits the cells to transmit the same downstream signaling in the presence of EGFR-TKIs. Exposure of EGFR-TKI-resistant NSCLC cells with *MET* amplification to MET-TKI or EGFR-TKI alone did not inhibit cell growth or survival signaling, given that both EGFR and MET signaling were found to be activated and to be mediated by ERBB3 (also known as HER3) in these cells. However, the combination of both types of TKI overcame resistance to EGFR-TKIs, attributable to *MET* amplification.

The *EGFR* T790M mutation and *MET* amplification account for ~ 70% of all known causes of acquired resistance to EGFR-TKIs in NSCLC, indicating that other mechanisms of resistance await discovery. It is therefore important to continue to study preclinical models, with regard to which the collection of tumor specimens and establishment of cell lines from patients who have developed EGFR-TKI resistance is key.

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PHASE I STUDIES

Phase I clinical and pharmacokinetic study of sorafenib in combination with carboplatin and paclitaxel in patients with advanced non–small cell lung cancer

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Summary *Objectives* Unsatisfactory efficacy of current treatments for advanced lung cancer has prompted the search for new therapies, with sorafenib, a multikinase inhibitor, being one candidate drug. This phase I trial was conducted to evaluate drug safety and pharmacokinetics as well as tumor response of sorafenib in combination with paclitaxel and carboplatin in patients with advanced non-small cell lung cancer (NSCLC). *Methods* Eligible patients received paclitaxel (200 mg/m^2) and carboplatin (area under the curve [AUC]of 6 mg min mL^{-1}) on day 1 and sorafenib (400 mg, twice daily) on days 2 through 19 of a 21-day cycle. *Results* Four of the initial six patients (cohort 1) experienced dose-limiting toxicities (DLTs), resulting in amendment of the treatment protocol. An additional seven patients (cohort 2) were enrolled, two of whom developed DLTs. DLTs included erythema multiforme, hand-foot skin reaction, and elevated plasma alanine aminotransferase in cohort 1 as well as gastrointestinal perforation at a site of metastasis and pneumonia in cohort 2. Most adverse events were manageable. One complete and six partial responses were observed among the 12 evaluable patients. Coadministration of the three drugs had no impact on their respective pharmacokinetics. *Conclusion* The present study confirmed that sorafenib at 400 mg once daily in combination with carboplatin AUC 5 mg min mL^{-1} and paclitaxel 200 mg/m^2 is feasible in Japanese patients with advanced NSCLC. The results of this study also showed that this combination therapy had encouraging antitumor activity and was not associated with relevant pharmacokinetic interaction in Japanese NSCLC patients.

Keywords Carboplatin · Lung cancer · Paclitaxel · Pharmacokinetics · Safety · Sorafenib

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Introduction

Non–small cell lung cancer (NSCLC) accounts for ~75% of all lung cancers and is the most common cause of cancer-related deaths worldwide [1]. Individuals with metastatic NSCLC are candidates for palliative systemic chemotherapy that confers only a limited survival benefit [2, 3]. The dismal outlook for patients with advanced NSCLC who receive currently available therapies has prompted a search for new, more effective chemotherapeutic agents and combination regimens. Target-based therapies are therefore being pursued as potential treatment alternatives.

Sorafenib (BAY 43-9006; Nexavar; Bayer HealthCare, Montville, NJ; Onyx Pharmaceuticals, Emeryville, CA), is an oral multikinase inhibitor that inhibits Raf serine-threonine kinases and several receptor tyrosine kinases

that function in tumor growth and angiogenesis [4]. The Ras-Raf-MEK-ERK signaling pathway plays a pivotal role in the regulation of tumor cell growth by relaying signals from the cell surface to the nucleus, with the components of this pathway, including Raf, thus representing potential targets for anticancer treatment [5, 6]. Sorafenib also targets the vascular endothelial growth factor (VEGF) receptors VEGFR-2 and VEGFR-3 as well as platelet-derived growth factor receptor–β (PDGFR-β), the ligands for which (VEGF and PDGF) are pro-angiogenic factors essential for tumor growth and metastasis [4]. Sorafenib has recently been approved for treatment of advanced renal cell carcinoma and hepatocellular carcinoma in the United States, Europe, and several other countries. Furthermore, sorafenib is currently undergoing clinical evaluation for a variety of additional cancers, including NSCLC.

Although several phase I clinical trials of sorafenib alone or in combination with other drugs have been conducted [7–19], no such phase I study for a specific type of lung cancer has been performed. The aim of the present phase I study was to evaluate the safety and pharmacokinetics of sorafenib in combination with carboplatin and paclitaxel in patients with advanced NSCLC.

Patients and methods

Patient selection

Eligible patients were 18 years of age or older with unresectable NSCLC, as confirmed histologically or cytologically, and with a life expectancy of at least 12 weeks. They were required to be naïve to chemotherapy and to have an Eastern Cooperative Oncology Group performance status of 0 or 1. The eligibility criteria also included adequate bone marrow, hepatic, and renal function as well as normal blood coagulation parameters. Individuals were excluded if they had previous or concurrent cancer distinct in primary site or histology from NSCLC or any cancer curatively treated >3 years prior to study entry; clinically active or significant cardiovascular disease; human immunodeficiency virus infection, chronic hepatitis B or C, or other serious infections; a seizure disorder requiring medication; a history of organ allograft, substance abuse, or medical, psychological, or social conditions that might interfere with participation in the study; or allergy to the study treatment. Pregnant or breast-feeding patients were also excluded. All patients received information regarding the nature and purpose of the study, and they provided written informed consent in accordance with institutional guidelines. The study protocol was approved by the Institutional Review Board of Kinki University Hospital.

Study design

The study was designed as a single-center, open-label, non–placebo-controlled phase I trial to define the safety, tolerability, pharmacokinetics, and tumor response profile of sorafenib administered according to a dosing schedule of 18 days on and 3 days off and in combination with paclitaxel and carboplatin chemotherapy in chemonaïve patients with advanced NSCLC. The other phase I trial of sorafenib in combination with paclitaxel and carboplatin had already confirmed the safety of sorafenib 400 mg twice daily in combination with paclitaxel at 225 mg/m^2 and carboplatin at area under the curve [AUC] of 6 mg min mL^{-1} in a dose-escalation manner [16]. Based on this result, the starting doses of the present study were decided as follows; Paclitaxel (200 mg/m^2, infused over 3 h) and carboplatin (AUC 6 mg min mL^{-1} during infusion for 30 min) were administered consecutively on day 1, and sorafenib (400 mg, twice daily) was administered for 18 days starting on day 2. There was a concern that sorafenib may inhibit cytochrome P450 enzymes responsible for the clearance of paclitaxel. Based on this possible pharmacokinetic interaction and antagonistic effects, sorafenib administration was discontinued for two days (days 20 and 21) before the next administration of paclitaxel in both the present study and the other phase I trial [16]. This treatment cycle was repeated every 21 days until unacceptable toxicity, tumor progression, or death occurred. Carboplatin-paclitaxel chemotherapy was not allowed to exceed six cycles, after which sorafenib administration could continue until the occurrence of intolerable toxicity, disease progression. If fewer than two of the first six patients experienced dose limiting toxicity (DLT) in the first cycle, the dose level was to be recommended for subsequent clinical trials and an additional six patients were to be enrolled to the cohort.

Patient evaluation

All observations pertinent to the safety of sorafenib were recorded, including results of physical examinations, vital signs, adverse events, use of concomitant medications, and laboratory test data. Patients were routinely monitored for adverse events, which were recorded with severity and relation to study medication according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. Assessment of the chest and abdomen for tumors was performed radiologically (computed tomography or magnetic resonance imaging) according to the Response Evaluation Criteria in Solid Tumors (RECIST) [20]. The same radiological method was performed to maintain consistency of evaluation. Patients for whom antitumor efficacy (complete or partial response)

was observed or who had stable disease were continuously treated according to the study protocol. Measurements were repeated in patients with a complete or partial response at a time more than 4 weeks after the response criteria were first met in order to confirm tumor response according to RECIST.

Pharmacokinetics

To investigate the effect of paclitaxel-carboplatin on the pharmacokinetics of sorafenib, we collected blood samples on days 2 and 19 of treatment cycle 1 for cohort 1 and determined the plasma concentration of sorafenib. On both days, samples were collected at 0 h (pre–morning dose of sorafenib); at 0.5, 1, 3, 6, and 12 h (pre–evening dose); and at 24 h (pre–morning dose on day 3). After dosing on day 19, additional samples were collected at 48 and 72 h (before infusion of paclitaxel in cycle 2). The evening dose of sorafenib was not administered on day 19 of cycle 1 for the purpose of pharmacokinetic sampling. As a result of amendment to the treatment protocol for cohort 2, a modified schedule of blood sampling was adopted. For determination of the plasma concentration of sorafenib, blood samples were collected at the same time points in cycle 2 as in cycle 1, with the exception that the blood sample obtained at 12 h after the morning administration of sorafenib on day 2 was collected before the evening dose on day 2 in cohort 2. The concentration of sorafenib in plasma samples was determined with the use of a validated high-performance liquid chromatography–tandem mass spectrometry (LC-MS/MS) assay.

To investigate the effects of sorafenib on the pharmacokinetics of paclitaxel and carboplatin, we collected blood samples on day 1 of cycle 1 for cohort 1 and determined the plasma concentrations of carboplatin, paclitaxel, and the paclitaxel metabolite 6-hydroxy-paclitaxel. Samples were collected at 0 h, 1.5 h (during paclitaxel infusion), 3 h (within 5 min before completion of paclitaxel infusion), 3.5 h (within 5 min before completion of carboplatin infusion), as well as 4, 5, 7, 11, 24, and 48 h. The amended treatment protocol for cohort 2 was accommodated by collection of blood samples immediately before, 1.5 h after the start of, within 5 min before completion of, as well as 0.5, 1, 2, 4, 8, 21, and 45 h after completion of paclitaxel infusion on day 1 of cycles 1, 2, and 3 for paclitaxel, and immediately before, within 5 min before completion of, as well as 0.5, 1, 3, 7, 20, 31, and 44 h after completion of carboplatin infusion on day 1 of cycles 1, 2, and 3 for carboplatin. The plasma concentrations of free (unbound) platinum derived from carboplatin, of paclitaxel, and of 6-hydroxy-paclitaxel were measured with the use of atomic absorption spectrophotometry and were validated by LC-MS/MS assays.

Pharmacokinetic parameters, including the AUC, maximum concentration (C_{max}), and elimination half-life ($t_{1/2}$), for sorafenib, paclitaxel, and carboplatin were calculated by noncompartment analysis as previously described [17].

Results

Patient demographics

A total of 13 chemonaïve patients with advanced NSCLC was enrolled in the study, six in cohort 1 and seven in cohort 2. The baseline demographics for all patients are shown in Table 1. Histological diagnosis revealed that the most common histology was adenocarcinoma (eight patients, or 61.5%), followed by large cell carcinoma and squamous cell carcinoma (each with two patients, or 15.4%).

DLT

Table 2 summarizes the dosing regimens for evaluated cohorts together with DLTs. The first six patients enrolled in cohort 1 were treated with 400 mg of sorafenib twice daily (days 2 to 19) combined with paclitaxel at 200 mg/m^2 and carboplatin at an AUC of 6 mg min mL^{-1} (30-min infusion). Four of these six patients experienced DLTs during the first cycle of treatment (two with erythema

Table 1 Patient demographics

	No. of patients
Total enrolled	13
Cohort 1	6
Cohort 2	7
Age (years)	
Median	66
Range	41–76
Sex	
Male	9
Female	4
ECOG performance status	
0	4
1	9
Disease stage	
IV	13
Histology	
Adenocarcinoma	8
Large cell carcinoma	2
Squamous cell carcinoma	2
Undifferentiated carcinoma	1

ECOG Eastern Cooperative Oncology Group

Table 2 Observed DLTs according to dose level

Cohort	Paclitaxel (mg/m^2)	Carboplatin ($mgminmL^{-1}$)	Sorafenib (mg)	No. of patients	No. of patients with DLTs	DLTs
1	200	6	400 twice daily	6	4	Erythema multiforme, grade 3 ($n=2$) Hand-foot skin reaction, grade 3 ($n=1$) ALT elevation, grade 3 ($n=1$)
2 (cycle 1)	200	5	400 once daily	7	0	None
2 (cycle 2)	200	5	400 twice daily	7	2	Perforation, GI, small bowel NOS, grade 3 ($n=1$) Infection-lung (pneumonia) of grade 3 with neutrophil of grade 4 ($n=1$)

DLTs dose-limiting toxicities, *ALT* alanine aminotransferase, *GI* gastrointestinal, *NOS* not otherwise specified

multiforme of grade 3, one with a hand and foot skin reaction of grade 3, and one with elevation of plasma alanine aminotransferase [ALT] of grade 3). One of the patients diagnosed with erythema multiforme developed a rash of grade 1 on the arms, thigh, and hip on day 5; by day 15, the rash had spread to the entire body with development of pruritus (grade 3), and histopathologic analysis of skin biopsy specimens revealed superficial dermal vasodilation as well as perivascular lymphocyte and plasma cell infiltration, consistent with erythema multiforme (Fig. 1a, b). The second patient also developed a localized rash of grade 1 that appeared in the right lower part of the abdomen on day 5 and had spread to the entire body with the development of a high fever on day 12; histopathologic analysis of skin biopsy specimens again supported a diagnosis of erythema multiforme. Both patients responded well to steroid therapy and improved.

Given that the incidence of DLT at the adopted dose level exceeded that predefined for the maximum tolerated dose, a modified dose level consisting of 400 mg of sorafenib once daily (days 2 to 19) combined with paclitaxel at 200 mg/m^2 and carboplatin at an AUC of 5 mg min mL^{-1} (60-min infusion) was evaluated for the seven additional patients of cohort 2. None of these seven patients experienced DLT during cycle 1. Intrapatient escalation of sorafenib dose was allowed if the patient did not experience DLT in cycle 1 of cohort 2; the dose of sorafenib was thus increased to 400 mg twice daily from day 2 to day 19 in subsequent courses. Among the seven patients who received sorafenib at 400 mg twice daily combined with paclitaxel (200 mg/m^2) and carboplatin (AUC of 5 mg min mL^{-1}), two individuals developed DLT: one a perforation of the small bowel of grade 4 and one pneumonia of grade 3. The patient with gastrointestinal perforation, who had metastases in the left adrenal gland and small intestine, developed abdominal pain, fever, and peritonitis 26 days after initiation of sorafenib at 400 mg twice daily and required emergency surgery. He recovered after surgery, and pathological examination of the surgical specimen confirmed the presence of tumor cells at the site of perforation. Given the marked tumor response of the patient on radiographic examination, the perforation event was likely associated with the antitumor effect of the study treatment.

Safety

All 13 enrolled patients were evaluable for safety analysis. Treatment-emergent adverse events (Table 3) occurred in all patients, the most common being hematologic or dermatologic in nature, sensory neuropathy, anorexia, and nausea. Neutropenia of grade 4 occurred in nine (69%) patients (four in cohort 1 and five in cohort 2). Hand-foot skin reaction occurred in five patients (three in cohort 1 and two in cohort 2), hypertension in four patients (two in cohort 1 and two in cohort 2), elevated plasma lipase in four patients (three in cohort 1 and one in cohort 2), and erythema multiforme in three patients (two in cohort 1 and one in cohort 2).

Antitumor activity

Tumor response was evaluated in 12 of the 13 patients (Fig. 2), with the remaining patient in cohort 2 not being available for assessment of such response. One patient in cohort 1 had a confirmed complete response, and six patients (three in each cohort) had a confirmed partial response; the overall response rate was thus 58% (95% confidence interval of 28 to 85%). Five patients, two in cohort 1 and three in cohort 2, had stable disease. Cavitation of lung lesions was observed in one patient (Fig. 1c, d). The median time to disease progression was 5.7 months (95% confidence interval of 4.3 to 20.1 months).

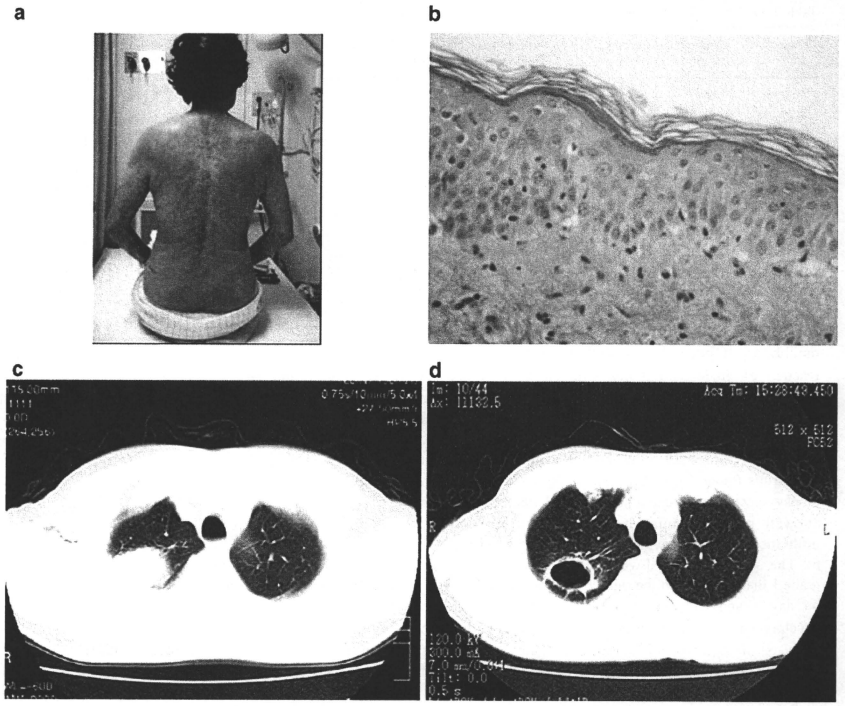

Fig. 1 Development of erythema multiforme and tumor cavitation in patients with advanced NSCLC treated with sorafenib in combination with carboplatin-paclitaxel. **a** A rash, initially localized to the arms, thigh, and hip, spread to the entire body. **b** Hematoxylin-eosin staining of a skin lesion from the patient shown in (**a**) revealed infiltration of inflammatory cells, mostly lymphocytes, around superficial dermal blood vessels and the epidermal-dermal junction. Liquefaction degeneration in basal epidermal layers and cavernous transformation in part of the epidermal squamous cell layer were also observed. **c**, **d** Computed tomography revealed a solid tumor without cavitation in the right lung of a patient at baseline (**c**), whereas the same tumor showed marked central cavitation on day 19 of cycle 1 (**d**)

Pharmacokinetics

Pharmacokinetic analysis for sorafenib in the presence of paclitaxel and carboplatin (Table 4) was based on the patients in cohort 1 (cycle 1) and cohort 2 (cycles 1 and 2) after administration of a single dose (day 2) or multiple doses (day 19). The increases in mean C_{max} from days 2 to 19 were consistent with those in mean AUC_{0-12}, likely reflecting the long mean $t_{1/2}$ (20.4 to 26.8 h on day 19). In cohort 2, the increases in the mean values of AUC_{0-12} and C_{max} in cycle 2 (400 mg, twice daily) compared with those in cycle 1 (400 mg, once daily) were consistent with the increase in sorafenib dosing. At steady state, after multiple administrations of sorafenib at 400 mg twice daily together with paclitaxel and carboplatin, the mean values of AUC_{0-12} and C_{max} in cohort 1 (cycle 1, day 19) were 31.3 mg h L^{-1} and 4.6 mg/L, respectively, and those in cohort 2 (cycle 2, day 19) were 39.1 mg h L^{-1} and 5.9 mg/L, respectively.

Given that treatment was discontinued after cycle 1 in four of the six patients in cohort 1, the effects of multiple doses of sorafenib on the pharmacokinetics of paclitaxel and carboplatin were evaluated in cohort 2. Pharmacokinetic analysis for paclitaxel and carboplatin was performed during cycle 1 before sorafenib administration and during cycles 2 and 3 after sorafenib administration (Table 4). Small increases in the mean AUC and C_{max} values for